MEASUREMENT OF WATER POLLUTION DETERMINING THE SOURCES AND CHANGES OF MICROBIAL CONTAMINATION AND IMPACT ON FOOD SAFETY FROM FARMING TO RETAIL LEVEL FOR FRESH VEGETABLES

Report to the
Water Research Commission

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EXECUTIVE SUMMARY

Background and motivation

Most water requirements in South Africa are met through surface water sources (rivers, dams and streams) (CSIR, 2010). Due to low rainfall levels in the country, most regions do not have adequate water supplies for domestic or industrial use (CSIR, 2010). Further, the quality of this scarce resource is often compromised due to ineffective municipal wastewater management systems which often result in direct discharge into rivers causing large scale pollution, sewage from informal settlements that lack adequate sanitation facilities and waste from intensive animal production systems, industrial companies, hospitals or the mining sector (Adesifoye and Okoh, 2017; Verlicchi and Grillini, 2020; Teklahaimanot *et al.*, 2014).

In South Africa, irrigated agriculture is the main user of surface and groundwater, with an estimated 53% to 61% of all available water sources used for irrigation purposes (Bonthuys, 2018). Thus the current demand for clean water in the horticultural industry far exceeds current available, potable water sources. In many parts of the country, river water is used without any purification step due to cost of treatment. This, due to high pollution levels, increases the potential risk of crop contamination and constitute a food safety risk. Previous studies found high levels of faecal contamination indicator bacteria (coliforms and *Escherichia coli*), and zoonotic microorganisms such as Shigatoxin-producing *E. coli* O157:H7 (STEC), non-O157:H7 shigatoxin-producing *E. coli*, *Salmonella spp*. and other emerging or important waterborne or foodborne human pathogens (Diodati *et al.*, 2015). The use of polluted water sources for irrigation purposes may pose a risk of transferring these pathogens onto crops, especially those which undergo minimal postharvest processing and are usually consumed raw (Uyttendaele *et al.*, 2015).

To date, it is well established that fresh produce may be contaminated with human pathogens at various points along the farm to market supply chain. Potential points of contamination can be at a pre-harvest level through irrigation water, pesticide sprays using polluted surface water, faeces due to animal husbandry practices or wildlife and birds, untreated manure used as fertiliser, or, contaminated soil and dust (Althaus *et al.,* 2012). At the post-harvest level produce can be contaminated through receival baths, washwater or pesticide spray applications using

non potable water sources. Dirty hands, crates or bins, conveyor belts and other contact surfaces may further contribute to product cross contamination (Althaus *et al.,* 2012).

Worldwide an increase in the number of foodborne disease outbreaks have been linked with the increased demand and consumption of fresh fruit and vegetables. During 2008, the World Health Organization (WHO) and Food and Agriculture Organization (FAO) prioritised fresh produce as the highest food safety risk in terms of microbial hazards associated with the product (FAO/WHO, 2008). The factors considered included outbreak history, potential for contamination, number and severity of disease outbreaks and impacts on trade and the national economy (Denis *et al.*, 2016). Produce with the highest risk was leafy vegetables and leafy herbs followed by berries, greens onions, melons and sprouted seeds and tomatoes.

Worldwide governing bodies, fresh produce industries and food processing companies realised the urgency of implementing control measures to ensure the microbiological safety of food products. Subsequently, the need for commodity-specific supply-chain management systems and guidelines, based on scientific data (including natural microbial levels and pathogen presence/absence) was identified (Luning *et al.*, 2011). In Canada, for example, targeted surveys of the microbiological quality of fresh produce at the retail level formed part of a Food Safety Action Plan (FSAP). Further, the negative impact of foodborne disease outbreaks on a country is well known as the widely publicised *E. coli* O104:H4 outbreak in the European Union in May 2011. The initial assumption was cucumbers and tomatoes from Spanish origin which resulted in 225 million Euros loss per week for the Spanish vegetable producers. Notwithstanding, the origin was finally tracked to an Egyptian company producing sprouts. This case study highlighted the economic impact of these outbreaks and the importance of rapid, accurate diagnostic test methods (http://www.bbc.co.uk/news/world-europe-13683270, accessed 15 June 2015).

Further to this, the increasing use of antimicrobials in the healthcare system and intensive livestock farming have led to increased levels of antibiotic resistance microbial populations in the environment, thus exerting selection pressures and inducing the transfer of antibiotic resistance genes to potential human pathogenic bacteria (Manyi-Loh e*t al.,* 2018). By polluting strategic resources, the risk to the consumer increases having a more severe impact on human health, the environment and food security. Contaminated environmental resources has been reported to play an important role in the increased prevalence and dissemination of potential human pathogenic bacteria resistant to multiple antibiotics (Larsson *et al.*, 2018). Extended

spectrum β-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae have been identified as serious and urgent health threats (CDC, 2019). These pathogens have been identified in the health care system, the agroecosystem including wastewater, irrigation water, soil, vegetable crops and animal husbandry (Ebomah and Okoh, 2020; Njage and Buys, 2014; Richter *et al.*, 2019; Richter *et al.*, 2020).

In the WRC project K5/1773//4 the potential link between microbiological quality of irrigation water and "at-harvest" fresh produce was investigated by testing for indicator and potential pathogenic organisms. Subsequently, WRC project K5/1875//4 was solicited to continue the investigation into the potential link between irrigation water and fresh produce (fruit and vegetables) up to, as well as after harvest from the farm to the market. A clear link was established between contaminants isolated from contaminated irrigation water and the associated fresh produce. This project focused on determining the microbiological safety, influence of processing, occurrence and characteristics of multidrug resistant human pathogenic bacteria from fresh vegetables (whole, minimally processed and RTE), most often consumed in the Tshwane and Cape Town Metropoles. It was envisaged that results of this study will contribute to establishing a crop-specific knowledge base which will inform the microbiological guidelines for fresh vegetables (whole, minimally processed and ready-to-eat (RTE) vegetables produced commercially and on small-scale farms as well as at the point-of-sale (formal and informal traders). In addition, we are developing a policy brief in collaboration with our research collaborators and the WRC with a view to engage with government at the national, regional and municipal level to develop and implement mitigation strategies to ensure food safety. The microbiological hazards analysis, mapping of prevalence of multidrug resistant human pathogenic bacteria in the water/plant-food-public health interface will enable South African regulatory bodies to anticipate changes in the environment and respond appropriately. This will impact human, plant, animal and environmental health as part of a *One Health solution* and will contribute to the National Antimicrobial Resistance Strategy Framework. Governmental departments, academia and policy-makers should work together closely to develop and implement measures to mitigate the risk associated with AR.

Project aims

The main aim of this project was to determine the link between water pollution and crop contamination and to determine sources of product microbial contamination and assess the

impact on food safety from farming to retail for selected fresh vegetable supply chains. More specific objectives, which have all been achieved, included the following:

1. To select and motivate for fresh, minimally processed and fresh-cut ready-to-eat (RTE) vegetable produce to be analysed at stages of packhousing, processing and retailing at selected formal and informal markets in the Tshwane and Cape Town Metros.
2. To measure microbial contamination of fresh, minimally processed, fresh-cut and/or ready-to-eat packaged vegetables in selected fresh produce supply chains, from the farming packhouse and processing stages up to selected formal retailers.
3. To measure microbial contamination of selected fresh vegetables at selected informal traders/retailers.
4. To determine the microbiological quality of fresh vegetable produce from the farming stage (excluding field irrigation), to the packhousing, processing and retailing stages at selected formal and informal food supply chains, in order to identify potential contamination points.
5. To determine the prevalence and to characterise potential human pathogenic bacteria in the selected fresh, minimally processed and ready-to-eat vegetables sold at the selected formal and informal retailers and food supply chains

Research Findings

Aim 1: To select and motivate for fresh, minimally processed and fresh-cut ready-to-eat vegetable produce to be analysed at stages of packhousing, processing and retailing at selected formal and informal markets in the Tshwane and Cape Town Metros.

A literature review on the content of a potential fresh vegetable basket from formal and informal supply chains was conducted. Topics covered in this literature review therefore included i) formal supply chains (commercial retailers, national fresh produce markets and farmers markets); ii) informal supply chains (street vendors including mobile vendor trolleys); iii) food safety and food quality in fresh produce supply chains, and iv) basic vegetable basket components. Additionally, information on consumption data (popularity, consumed raw or cooked) and general producer/consumer food safety knowledge, attitudes and practices (KAP) were obtained through conducting questionnaire surveys. Questionnaire A was an online questionnaire testing the South African consumers' food safety knowledge, attitudes and practices, fresh vegetables consumed in Tshwane and Cape Town Metropoles (Appendix III,

Supplementary information) in collaboration with Consulta (a private company specialising in conducting surveys). Questionnaire B was an interviewed questionnaire to determine the South African consumers buying fresh produce from informal trading green grocers/street vendors: food safety knowledge, attitudes and practices, vegetables consumed (Appendix III, Supplementary information). Questionnaire C was an interviewed Questionnaire focusing on "South African street trading green grocers' food safety knowledge, attitudes and practices, fruit and vegetables sold" (Appendix III, Supplementary information).

The final list of fresh produce selected included fresh vegetables which have been identified as high risk crops by the CDC based on the number of foodborne disease outbreaks associated with the produce, results from the literature review as well as the consumption data (popularity, consumed raw or cooked) information from the questionnaire surveys in the formal and informal sector. This included whole, minimally processed and RTE leafy green vegetables (lettuce and spinach), African leaf greens (morogo), tomatoes, cucumbers, green beans, broccoli and carrots. The sampling sites included commercial vegetable supply chains (n=6), farmers markets (n=2), informal vendors (mobile trolley vendors, green grocers) (n=32) and small-scale farmers (n=8) and associated vendors. Selected sampling sites and fresh vegetables in the Tshwane Metropole and Cape Town Metropole in the formal and informal markets are summarised in Table 5 and 6 respectively under experimental procedures in Chapter 3. Ethical clearance was obtained for this project funded by the Water Research Commission (K5/2706//4): Ethical clearance number EC 180 327-182.

Aims 2 to 5: Results of the microbiological quality, contamination points, prevalence and characteristics of human pathogenic bacteria in formal and informal supply chains were assessed. Aim 5 of the solicited project specifically excluded sampling and analysis of irrigation water. However, the cost of the analysis of irrigation water and at harvest fresh produce was covered by grant funding from the "Partnerships for Enhanced Engagement in Research (PEER)-funded project, a USAID/DST initiative focusing on characterising and tracking antimicrobial resistance in the water-plant-food public health interphase" (PEER cycle 5, Grant 48). These results are included in the final integrated report, since determining the microbiological quality of irrigation water sources used in commercial and small-scale farming and comparison thereof is critical as far as identifying contamination points in fresh produce supply chains are concerned.

Fresh vegetables at the point-of-sale (formal and informal retailers) in the Tshwane Metropole.

Vegetable samples, including spinach, tomato, lettuce, cucumber and green beans, were purchased from retailers, street traders, trolley vendors and farmers' markets. The *E. coli* counts on the fresh produce ranged from zero to unacceptably high according to the previous NDOH guidelines currently under revision (< 2.3 log CFU/g) (NDOH, 2000). The hygiene indicator bacteria counts were mostly not significantly different between formal and informal markets, with exceptions noted on occasion. When compared to international standards, 70-98% of the vegetables had satisfactory (≤ 100 CFU/g) *E. coli* counts. *E. coli* was isolated from 14.86% of the *vegetable* samples, predominantly from leafy green vegetables, while none of the isolates harboured diarrheagenic virulence genes. However a high percentage (40.30%) of the isolates were multidrug-resistant (MDR). No *Salmonella* spp. nor *L. monocytogenes* were isolated from any of the samples tested.

Microbiological analysis showed that 17.4% of the vegetable samples were contaminated with ESBL/AmpC-producing *Enterobacteriaceae* which predominantly included *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter asburiae*, and *Klebsiella pneumoniae.* These microorganisms are known to represent increased risks related to environmental integrity, food safety and human health. Multidrug resistance was observed in 96.1% of isolates tested, while resistance to aminoglycoside, chloramphenicol, and tetracycline was most prevalent. The presence of β-lactamase genes were confirmed in 75.3% isolates from all vegetable types, mainly in *E. coli*, *Enterobacter* spp., and *Serratia* spp. isolates. CTX-M group 9 was the dominant ESBL type, while EBC was the most prevalent plasmidic type AmpC β-lactamase. This is the first report of the presence of MDR ESBL/AmpC-producing *Enterobacteriaceae* in raw vegetables sold at selected retailers in Gauteng Province, South Africa.

Commercially produced leafy green vegetables from five selected supply chains in Gauteng Province and North West Province supplying retailers in Tshwane Metropole.

Water samples (river, dam, irrigation pivot point and washwater), spinach/lettuce samples were collected from the farm, the processing facilities and a retail and microbial analysis showed that the *E. coli* levels in river water used for irrigation in supply chain one were acceptable according to the DWAF (1996) guidelines of < 1000 CFU/100 ml, while the *E. coli* numbers were lower in water from the holding dam and the irrigation pivot point. *However, where river water was directly applied via overhead irrigation, E. coli was enumerated from the spinach samples, contact surfaces and from the water (river, irrigation pivot point and washwater)*

throughout the supply chain. The *E. coli* counts in borehole water and holding dams ranged from zero to unacceptably high on occasion (DWAF, 1996), while water from the irrigation pivot point met the guideline requirements. The *E. coli* levels on spinach and from supply chains 1-5 from the farm, though processing an at retail ranged from zero to counts above the acceptable limits according to the Department of Health (NDOH) guidelines for ready to eat fresh fruit and vegetables, currently under revision (*E. coli* CFU 0/g) (NDOH, 2000). Similarly the coliform counts exceeded the maximum limit of 2.3 log CFU/g allowed in the national guidelines.

Multidrug resistance was observed in 43.8-50.9% *E. coli* isolates from the three supply chains, with a higher percentage from supply chains where river water was used for irrigation. This included resistance to aminoglycosides, cephalosporins, penicillins, tetracycline, sulphonamides, chloramphenicol and carbapenems. Overall, a greater percentage of resistance phenotypes were from water *E. coli* isolates, followed by isolates from the spinach/lettuce vegetables and contact surfaces. A clear link was established between irrigation water and spinach/lettuce samples at different points of production, processing and retail in all five commercial supply chains. Virulence genes (*eae* and *stx 1*) were detected in three *E. coli* isolates only, while none of none of the diarrheagenic virulence genes tested for were detected. Neither *Salmonella* spp. nor *Listeria monocytogenes* were detected in the leafy greens (spinach and lettuce) samples from the commercial supply chains from the farm to the point-of-sale. However, *Salmonella* spp. and *Listeria monocytogenes* were detected in approximately 3-5% of all irrigation water samples.

Microbiological analysis showed that between 14.58% and 17.6% of the samples were contaminated with ESBL/AmpC-producing Enterobacteriaceae which predominantly included *Serratia fonticola, Escherichia coli* and *Klebsiella pneumonia.* Interestingly, 25% of the ESBL/AmpC-producing isolates were from retail spinach samples, while only 12.5% were obtained from spinach sampled in the field and throughout the processing facility. Multidrug resistance was observed in 98% of isolates tested. CTX-M Group 1 was the dominant ESBL type, followed by TEM and SHV; whilst the CIT-type was the only plasmid-mediated AmpC genetic determinant detected in the spinach supply chains. Class 1 and 3 Integrons aiding the dissemination of antimicrobial resistance between microorganisms were detected in 79.17% of the isolates. This is the first report on the prevalence of ESBL/AmpC-producing Enterobacteriaceae isolated throughout commercial spinach production systems harbouring class 1 and/or class 3 integrons in Gauteng Province, South Africa.

Fresh produce from informal vendors in the Tshwane Metropole.

Apples, carrots, cabbage, spinach and tomatoes, were sourced from informal street vendors (SV) in selected areas (Mamelodi, Atteridgeville) of Gauteng Province, South Africa. All samples had coliform counts above the acceptable limits according to the previous NDOH guidelines currently under revision (< 2.3 log CFU/g). Similarly *Enterobacteriaceae* counts exceeded the acceptable limits (< 4 log CFU/g), according the Public Health Protection Agency guidelines in the United Kingdom (UK) for ready to eat foods. No viable *L. monocytogenes* was detected, one sample was positive for *Salmonella* spp. and ESBL-*Enterobacteriaceae* was detected from 18.4% with diversity including *Enterobacter* spp. *Rahnella* spp., *Proteus* spp., *Serratia* spp., *Citrobacter* spp. and *Hafnia alvei.*

Escherichia coli was enumerated from 22% of the samples and detected from 17.6% of the samples after enrichment. The majority (85.71%) of *E. coli*, isolated from spinach, apples, carrots, cabbage and tomatoes, were multidrug resistant. Resistance to Aminoglycoside, Cephalosporin, Penicillin and Chloramphenicol antibiotic classes were most prevalent. Antibiotic resistance genes detected included bla_{TEM}, *tetA* gene, *tetB, tetL*, *sulI, sulII*), *aadA1a* and *strAB*. A single isolate was found to harbour the *eae* virulence factor. Moreover, *E. coli* isolates were grouped into the intra-intestinal infectious phylogenetic group E (28.57%), the rare group C (26.79%), the generalist group B1 (21.43%) and the human commensal group A (16.07%) using ERIC-PCR DNA fingerprinting analysis.

Fresh produce, including traditional African leafy greens, from small-scale farms and associated vendors supplied in the Tshwane Metropole.

Fresh produce (spinach, chinensis and rape) and water used for irrigation were collected from primary production and retail from small-scale farms in two regions of South Africa. An assessment of the microbial status of small-scale farming systems in production of leafy green vegetables in South Africa was conducted. Total coliform counts exceeded the maximum limit of 2.3 log CFU/g allowed by the previous NDOH guidelines for ready to eat fresh fruit and vegetables (NDOH, 2000). *Listeria* spp. were detected in irrigation water only. *Escherichia coli* isolates isolated included an Enterotoxigenic *E. coli* (ETEC) strain, Enteroaggregative *E. coli* (EAEC) strain and Enteropathogenic *E. coli* (EPEC) strain. Total coliform counts ranged from an average log 0.06-4.51 CFU/100 ml in water samples, an average log 2.43-3.74 CFU/g in soil, and an average log 1.63-5.57 CFU/g in fresh produce.

Twenty-one (4.93%) of fresh produce and environmental samples were contaminated with *Salmonella* spp., including 17.00% irrigation water samples and 1.38% leafy green vegetable samples, mainly from only one growing region. Seven different *Salmonella* spp. serotypes were identified; *Salmonella* IIIb 42 or II 42 subspecies, *Salmonella* Enteritidis and *Salmonella* II 42:z29 or *Salmonella* Djama, *Salmonella* Havana and *Salmonella* Typhimurium were the most prevalent. All the isolates carried *hilA, invA, ssrA, sipA, pipD*, *misL* and *stn* virulence genes. Furthermore, 3.8% of isolates carried at least 15 virulence genes tested for. Analysis of the isolates showed that 92.45% were multidrug resistant. Repetitive PCR analyses, demonstrated high diversity amongst the *Salmonella* spp. from mainly one farm, with isolates from fresh produce and water in the same cluster clearly showing a link between the irrigation water and the fresh produce analysed.

Commercial fresh vegetable processing/packaging facility in Philippi in the Western Cape Province.

Fresh produce types: broccoli coleslaw (broccoli stems, carrots and cabbage) and lettuce samples were collected at different processing points from a packhouse Philippi, Western Cape South Africa, and from retailers. The untreated/unprocessed samples had high microbial counts which were then reduced to significantly lower levels after peeling and washing in a chlorine (150-200 ppm) solution. An increase in microbial counts to levels significantly higher than on the treated samples was observed in shredded samples and bagged mix coleslaw samples. Mixed coleslaw bags sampled from the retailer had significantly higher microbial levels than mixed coleslaw from the same batch sampled at the packhouse directly after packaging. The microbial levels of lettuce decreased gradually throughout processing. No *Salmonella* or Shiga-toxin-producing *E. coli* (STEC) were detected. Overall, 89% of the presumptive ESBL-producing organism isolates were identified as *Enterobacter cloacae* (64%) *Klebsiella oxytoca* (18%), *E. coli* (7%). ESBL genes were detected in 14% of the isolates tested. This included *Enterobacter cloacae* carrying $bla_{\text{CTX-M}}$ and bla_{TEM}, *Klebsiella oxytoca* carrying $bla_{\text{CTX-M}}$ and *E. coli* carrying $bla_{\text{TEM.}}$ Multidrug resistance against Ampicillin, Gentamicin, Tetracycline, Ciprofloxacin, and Chloramphenicol was observed in 6% of the ESBL-producing Enterobacteriaceae.

Informal supply chain fresh produce sampling and microbiological analysis in the Cape Town Metropole.

Fresh produce (lettuce, cabbage, spinach, tomatoes, green beans and green peppers) was sampled at five selected informal vendors in the Cape Town metropole. The general hygiene

counts for all sites were well over the advised coliform limits according to the Department of Health. No *Salmonella* or *L. monocytogenes* was detected in any of the fresh produce. The presence of *E. coli* occurred in sporadic cases indicating evidence of poor handling practices at the informal vendors. The prevalence of ESBL-producing Enterobacteriaceae was relatively low with 4% of the fresh produce sampled that tested positive for these bacteria. Most of the phenotypically confirmed ESBL-producing Enterobacteriaceae had at least one of the ESBL genes of interest, while one isolate harboured three ESBL encoding genes, including *bla* $_{\text{TEM}}$, *bla* $_{\text{CTX-M}}$ and *bla* $_{\text{SHV}}$. All ESBL-producing Enterobacteriaceae were multidrug resistant as well. Taking all the evidence into consideration, it is clear that post-harvest handling of fresh produce can be improved. In this study, the presence of ESBL-producing Enterobacteriaceae on fresh produce has been confirmed in samples sold at informal markets in the Cape Town Metropolitan area.

General conclusions

- The results from this study confirmed the need for scientific knowledge-based microbiological quality and safety guidelines for whole, minimally processed and RTE fresh produce from formal and informal supply chains, including small-scale farms.
- Most of the fresh vegetable samples analysed in this study had average coliform counts above the acceptable limit of 2.3 log CFU/g as stipulated in the Department of Health (NDOH, 2000) guidelines, currently under revision. These guidelines were used as a reference point in the absence of national guidelines.
- Hygiene indicator bacteria counts were often lower further down the supply chain.
- However, hygiene indicator bacteria counts of RTE packaged coleslaw were higher following processing which indicates that the results are dependent on the specific crop and post-harvest processing steps.
- Some countries do not include coliform counts in the guidelines for microbiological testing of RTE foods, since coliform counts are naturally high and part of the microflora. This should be taken into considered during the revision process of the South African guidelines. Alternatively the maximum limits for the coliform counts should be informed by scientific results to date.
- *Escherichia coli* counts varied at selected sites and produce ranging between 0 up to 5 log CFU/g (high counts mostly at informal vendors and farmers markets).
- Naturally, coliform and Enterobacteriaceae counts of vegetables are often >4 log CFU/g.

- The use of indicator organisms alone is not enough to indicate the microbiological safety status of fresh produce, the agricultural production environment and in processing facilities. The presence of foodborne pathogens such as shigatoxin-producing *E. coli* (i.e. *E. coli* O157:H7), Salmonella and *Listeria monocytogenes*.
- More than 40% of *E. coli* isolates from fresh produce and associated irrigation water fresh produce were multidrug resistant.
- Clinically important ESBL/AmpC-producing Enterobacteriaceae included *E. coli, Klebsiella pneumoniae* and *Enterobacter* spp.
- Multidrug resistance was observed in 80-98% of ESBL/AmpC-producing Enterobacteriaceae isolates from water and irrigated fresh vegetables.
- High prevalence of ESBL & Amp C genetic determinants a concern, since there is a risk of transfer to commensal bacteria.

Recommendations for future research

- Development of more realistic, fit-for-purpose guidelines for irrigation and processing water suitable for the specific scenarios and unique challenges encountered in the formal and informal sectors based on sound scientific evidence.
- Guidelines for irrigation water need to be expanded to include not only hygiene indicator microorganisms (coliforms, *E. coli*) that have traditionally been used, but other members of for example the Enterobacteriaceae family, i.e. *Salmonella* spp. and shigatoxin-producing *E. coli* should also be considered.
- There is a national need to map out the potential contributors to the growing antimicrobial resistance (AMR) problems, i.e. sewage plants, the mining sector, animal husbandry, etc.
- AMR surveillance in agroecosystems and fresh produce should be expanded to generate reliable, comparable and locally relevant information which can be used to develop and implement mitigation strategies.
- The assessment of potential human health risks posed by pathogens that have acquired resistance to antimicrobial drugs is a new application of risk assessment that is closely related to microbiological risk assessment.
- Establishing and maintaining a national central database of antimicrobial resistance surveillance data is imperative to characterise the potential risk with a view to develop and implement risk mitigation strategies.

- The occurrence and characterisation of chemical pollutants should also be investigated, since resistance to chemicals exists in the agricultural environments.
- It is important to include Plant Health as part of a holistic One Health approach to address environmental, human and animal health issues.
- Establishment of a One Health Ethics Committee is needed to enhance the ethical clearance process.
- Governmental departments, academia and policy makers should work together closely to develop an action plan and implement measures to mitigate the microbiological quality, safety and antimicrobial resistance prevalence risk in fresh produce supply chains.
- In order to make an impact on policy, working with government at all three levels, national, provincial and local is necessary.
- Link isolates/info between different sectors/stakeholders. Results from antimicrobial resistance studies (ARBs, ARGs, whole genome sequencing of isolates, resistomes in water, fresh produce) should be linked with reference labs.
- Establish of core training including food safety training, training on practices and improved hygiene in fresh produce supply chains.
- Summarise the existing information in a simplified way to communicate this with municipalities/government. The summary document can be used to engage with relevant stakeholders at municipal level to show the status of rivers and why people are getting ill with a view to developing and implementing mitigation strategies.
- Communicating with all the relevant role-players in the fresh produce supply chains including the public, farmers, retailers (formal and informal), municipalities, advisory boards and policy makers.
- Governmental departments, academia and policy makers should work together closely to develop and implement measures to mitigate the risk associated with antimicrobial resistance.

Innovative aspects of the project

Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis was initially developed for the clinical setting and is now used in the agricultural landscape for identification of potential human pathogenic bacteria in the water-plant-food-public health interface.

The methodologies have been optimised and we have shared the information with our colleagues, including Stellenbosch University as well as the University of Fort Hare and they all used the MALDI-TOF for isolate identification.

This also made the results between the groups more comparable since the same methodology was used for isolate identification and characterisation.

The combination of viable counts, AMR profiles (phenotypic and genotypic) and using rapid MALDI-TOF analysis for isolate identification to identify and characterise the potential risk in formal and informal supply chains generated a knowledge base for hazard identification and characterisation. The methodology can easily be duplicated in other laboratories for developing and implementing risk mitigation strategies.

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Reference group members were as follows:

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AmpC	β-lactamase
AMR	Antimicrobial resistance
ANOVA	Analysis of variance
BPW	Buffered peptone water
CDC	Center for Disease Control and Prevention
CFS	Centre for Food Safety
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standards Institute
CSIR	Council for Scientific and Industrial Research
DAFF	Department of Agriculture Forestry and Fisheries
DDST	Double disk synergy diffusion test
DWAF	Department of Water Affairs
EAEC	Enteroaggregative *Escherichia coli*
ECCP	European Commission & Consumer Protection Directorate-General
ECDC	European Centre for Disease Prevention and Control
EE	Enterobacteriaceae Enrichment broth
EHEC	Entero-haemorrhagic *Escherichia coli*
EIEC	Enteroinvasive *Escherichia coli*
EMB	Eosin methylene blue
EPEC	Entero-pathogenic *Escherichia coli*
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus
ESBL	Extended Spectrum Beta-Lactamase
ETEC	Enterotoxigenic *E. coli*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FAO	Food and Agriculture Organization of the United Nations
FSAP	Food Safety Action Plan
G.A.P	Good agricultural practices
GDP	Gross domestic product
HPA	Health Protection Agency
IDTT	Industrial Development Think Tank
MALDI-TOF	Matrix assisted laser desorption ionization time of flight mass spectrometry
MARI	Multiple-antibiotic-resistance index

MDR	Multidrug resistant
MPN	Most probable number
NDOH	South African Department of Health
NSW	New South Wales Food Authority
RTE	ready-to-eat
STATSSA	Statistic South Africa
STEC	Shiga toxin-producing *Escherichia coli*
TSB	Tryptone soy broth
USA	United States of America
VRBG	Violet Red Bile Glucose
WHO	World Health Organization
XLD	Xylose lysine deoxycholate

LIST OF UNITS

%	percentage
× *g*	centrifugal force
°C	degrees Celsius
β	beta
µg	microgram
µl	microlitre
e.g.	for example
i.e.	that is
L	litre
ml	millilitre
no.	number
PCR	polymerase chain reaction
rpm	revolutions per minute

CHAPTER 1

INTRODUCTION

1.1 Background, aims and rationale of the research

Most water requirements in South Africa are met through surface water sources (rivers, dams and streams) (CSIR, 2010). Due to low rainfall levels in the country, most regions do not have adequate water supplies for domestic or industrial use (CSIR, 2010). Further, the quality of this scarce resource is often compromised due to ineffective municipal wastewater management systems which often result in direct discharge into rivers causing large scale pollution, sewage from informal settlements that lack adequate sanitation facilities and waste from intensive animal production systems, industrial companies, hospitals or the mining sector (Adesifoye and Okoh, 2017; Verlicchi and Grillini, 2020; Teklahaimanot *et al.*, 2014).

In South Africa, irrigated agriculture is the main user of surface and groundwater, with an estimated 53% to 61% of all available water sources used for irrigation purposes (Bonthuys, 2018). The current demand for clean water in the horticultural industry thus far exceeds current available, potable water sources. In many parts of the country, river water is used without any purification step due to cost of treatment. This due to high pollution levels increases the potential risk of crop contamination and constitute a food safety risk. Previous studies found high levels of faecal contamination indicator bacteria (coliforms and *Escherichia coli*), and zoonotic microorganisms such as Shigatoxin-producing *E. coli* O157:H7 (STEC), non-O157:H7 shigatoxin-producing *E. coli*, *Salmonella spp*. and other emerging or important waterborne or foodborne human pathogens (Diodati *et al.*, 2015). The use of polluted water sources for irrigation purposes may pose a risk of transferring these pathogens onto crops, especially those which undergo minimal postharvest processing and are usually consumed raw (Uyttendaele *et al.*, 2015).

To date, it is well established that fresh produce may be contaminated with human pathogens at various points along the farm to market supply chain. Potential points of contamination can be at a pre-harvest level through irrigation water, pesticide sprays using polluted surface water, faeces due to animal husbandry practices or wildlife and birds, untreated manure used as fertiliser, or, contaminated soil and dust (Althaus *et al.,* 2012). At the post-harvest level produce

can be contaminated through receival baths, washwater or pesticide spray applications using non potable water sources. Dirty hands, crates or bins, conveyor belts and other contact surfaces may further contribute to product cross contamination (Althaus *et al.,* 2012).

Worldwide an increase in the number of foodborne disease outbreaks have been linked with the increased demand and consumption of fresh fruit and vegetables. During 2008, the World Health Organization (WHO) and Food and Agriculture Organization (FAO) prioritised fresh produce as the highest food safety risk in terms of microbial hazards associated with the product (FAO/WHO, 2008). The factors considered included outbreak history, potential for contamination, number and severity of disease outbreaks and impacts on trade and the national economy (Denis *et al.*, 2016). Produce with the highest risk was leafy vegetables and leafy herbs followed by berries, greens onions, melons and sprouted seeds and tomatoes.

Worldwide governing bodies, fresh produce industries and food processing companies realised the urgency of implementing control measures to ensure the microbiological safety of food products. Subsequently, the need for commodity-specific supply-chain management systems and guidelines, based on scientific data (including natural microbial levels and pathogen presence/absence) was identified (Luning *et al.*, 2011). In Canada, for example, targeted surveys of the microbiological quality of fresh produce at the retail level formed part of a Food Safety Action Plan (FSAP). Further, the negative impact of foodborne disease outbreaks on a country is well known as the widely publicised *E. coli* O104:H4 outbreak in the European Union in May 2011. The initial assumption was cucumbers and tomatoes from Spanish origin which resulted in 225 million Euros loss per week for the Spanish vegetable producers. Notwithstanding the origin was finally tracked to an Egyptian company producing sprouts. This case study highlighted the economic impact of these outbreaks and the importance of rapid, accurate diagnostic test methods (http://www.bbc.co.uk/news/world-europe-13683270, accessed 15 June 2015).

Further to this, the increasing use of antimicrobials in the healthcare system and intensive livestock farming have led to increased levels of antibiotic resistance microbial populations in the environment, thus exerting selection pressures and inducing the transfer of antibiotic resistance genes to potential human pathogenic bacteria (Manyi-Loh et al., 2018). By polluting strategic resources the risk to the consumer increases having a more severe impact on human health, the environment and food security. Contaminated environmental resources has been reported to play an important role in the increased prevalence and dissemination of potential

human pathogenic bacteria resistant to multiple antibiotics (Larsson *et al.*, 2018). Extended spectrum β-lactamase (ESBL) and carbapenemase-producing Enterobacteriaceae have been identified as serious and urgent health threats (CDC, 2019). These pathogens have been identified in the health care system, the agroecosystem including wastewater, irrigation water, soil, vegetable crops and animal husbandry (Ebomah and Okoh, 2020; Njage and Buys, 2014; Richter *et al.*, 2019; Richter *et al.*, 2020).

In the WRC project K5/1773//4 the potential link between microbiological quality of irrigation water and "at-harvest" fresh produce was investigated by testing for indicator and potential pathogenic organisms. Subsequently, WRC project K5/1875//4 was solicited to continue the investigation into the potential link between irrigation water and fresh produce (fruit and vegetables) up to, at as well as after harvest from the farm to the market. A clear link was established between contaminants isolated from contaminated irrigation water and the associated fresh produce. This project focused on determining the microbiological safety, influence of processing, occurrence and characteristics of multidrug resistant human pathogenic bacteria from fresh vegetables (whole, minimally processed and RTE) most often consumed in the Tshwane and Cape Town Metropoles. It was envisaged that results of this study will contribute to establishing a crop-specific knowledge base which will inform the microbiological guidelines for fresh vegetables (whole, minimally processed and ready-to-eat (RTE) vegetables produced commercially and on small-scale farms as well as at the point-of-sale (formal and informal traders). In addition, we are developing a policy brief d in collaboration with our research collaborators and the WRC with a view to engage with government at the national, regional and municipal level to develop and implement mitigation strategies to ensure food safety. The microbiological hazards analysis, mapping of prevalence of multidrug resistant human pathogenic bacteria in the water, plant-food-public health interface will enable South African regulatory bodies to anticipate changes in the environment and respond appropriately. This will impact human, plant, animal and environmental health as part of a One Health solution and will contribute to the National Antimicrobial Resistance Strategy Framework. Governmental departments, academia and policy makers should work together closely to develop and implement measures to mitigate the risk associated with AR.

The main aim of this project was to determine the link between water pollution and crop contamination and to determine sources of produce microbial contamination and assess the impact on food safety from farming to retail for selected fresh vegetable supply chains. More specifically objectives included:

1. To select and motivate for fresh, minimally processed and fresh-cut ready-to-eat (RTE) vegetable produce to be analysed at stages of packhousing, processing and retailing at selected formal and informal markets in the Tshwane and Cape Town Metros.
2. To measure microbial contamination of fresh, minimally processed, fresh-cut and/or ready-to-eat packaged vegetables in selected fresh produce supply chains, from the farming packhouse and processing stages up to selected formal retailers.
3. To measure microbial contamination of selected fresh vegetables at selected informal traders/retailers.
4. To determine the microbiological quality of fresh vegetable produce from the farming stage (excluding field irrigation), to the packhousing, processing and retailing stages at selected formal and informal food supply chains, in order to identify potential contamination points.
5. To determine the prevalence and to characterise potential human pathogenic bacteria in the selected fresh, minimally processed and ready-to-eat vegetables sold at the selected formal and informal retailers and food supply chain.

In order to achieve the first objective of this research project a review of the type of fresh, minimally processed and ready-to-eat vegetables in a potential food basket available from formal retailers and informal vendors was conducted (Chapter 2). Details of typical formal and informal supply chains in South Africa, food safety and quality in the supply chains and finally basic vegetable basket components were summarised in this review. In order to compile a final list of fresh produce selected for sampling and microbiological analysis purposes questionnaire surveys were also conducted. The surveys aimed to determine general producer/consumer (formal and informal) food safety knowledge, attitudes and practices (KAP), consumption data of vegetable popularity and whether the produce is typically consumed raw, cooked or both. Sampling sites and fresh produce selected for microbiological evaluation, questionnaire surveys of consumers (formal and informal) and street trading vendors in both the Tshwane and Cape Town Metropoles were summarised in Chapter 3, section 3.2. Detailed descriptions of formal and informal supply chain sampling sites selected for analysis in the Tshwane Metropole and Cape Town Metropole were included in sections 3.3 and 3.5 respectively. In addition, experimental procedures followed to determine the microbiological quality and safety, occurrence, identities and characteristics of potential human pathogenic isolates from the collected samples (water, soil, fresh produce, contact surfaces) were described in Chapter 3. Results obtained for the consumer Questionnaire surveys, sampling and analysis of samples

collected were summarised in Chapter 4. General conclusions and recommendations for future research were included in Chapter 5.

1.2 References

ADEGOKE AA, FALEYE AC, SINGH G and STENSTRÖM TA (2016). Antibiotic resistant superbugs: assessment of the interrelationship of occurrence in clinical settings and environmental niches. Molecules **22**(1), 29.

ADESIFOYE MA and OKOH AI (2017). Ecological and public health implications of the discharge of multidrug-resistant bacteria and physicochemical contaminants from treated wastewater effluents in the Eastern Cape, South Africa. Water **9**, 562.

ALTHAUS D, HOFER E, CORTI S, JULMI A and STEPHAN R (2012). Bacteriological survey of ready-to-eat lettuce, fresh-cut fruit, and sprouts collected from the Swiss Market. Journal of Food Protection **75**, 1338-1341.

BONTHUYS J (2018). Irrigation water use. The Water Wheel July/August 2018.

COUNCIL FOR SCIENTIFIC AND INDUSTRIAL RESEARCH (2010. A CSIR perspective on water in South Africa-2010. Council for Scientific and Industrial Research, CSIR Report No. CSIR/NRE/PW/IR/2011/0012/A [ISBN: 978-0-7988-5595-2].

DENIS N, ZHANG H, LEROUX A, TRUDER R and BIETLOT H (2016). Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. Food Control **67**, 225-234.

DEPARTMENT OF HEALTH (NDOH) (2000). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. South African Department of Health Directorate: Food Control. Available at: www.NDOH.co.za. Accessed July 10th, 2010.

DEPARTMENT OF WATER AFFAIRS (DWAF) (1996). South African Water Quality Guidelines. Volume 4. Agricultural Use: Irrigation. Second Edition. Edited by S. Holmes, CSIR Environmental Services. Pretoria: Department of Water Affairs and Forestry.

DIODATI ME, BATES AH, COOLEY MB, WALKER S, MANDRELL RE and BRANDL MT (2015). Genotypic and phenotypic similarity among shiga toxin-producing *Escherichia coli* O111 Environmental and Outbreak Strains. Foodborne Pathogens and Disease **12**, 235-243.

EBOMAH KE and OKOH AI (2020). An African perspective on the prevalence, fate and effects of carbapenem resistance genes in hospital effluents and wastewater treatment plant (WWTP) final effluents: A critical review. Heliyon **6**(5):e03899.

FAO/WHO (2008). Microbiological hazards in fresh fruit and vegetables. Retrieved from http://www.fao.org/fileadmin/templates/agns/pdf/jemra/FFV_2007_Final.pdf.

LARSSON DGJ , ANDREMONT A AND BENGTSSON-PALMEA J, BRANDTE KK, DE RODA HUSMAN AM, FAGERSTEDT P, FICK J, FLACH CF, GAZE WH, KURODA M, KVINT K, LAXMINARAYAN R, MANAIA CM, NIELSEN KM, PLANT L, PLOY MC, SEGOVIA C, SIMONET P, SMALLA K, SNAPE J, TOPP E, VAN HENGEL AJ, VERNER-JEFFREYS DW, VIRTA MPJ, WELLINGTON EM, WERNERSSON AS (2018). Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. Environment International **117**, 132-138.

LUNING PA, JACXSENS L, ROVIRA S, OSÉS SM, UYTTENDAELE M and MARCELIS W J (2011). A concurrent diagnosis of microbiological food safety output and food safety management system performance: Cases from meat processing industries. Food Control **22**, 555-565.

MANYI-LOH C, MAMPHWELI S, MEYER E and OKOH A (2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. Molecules **23** (4), 795.

NJAGE PMK and BUYS EM (2014. Pathogenic and commensal *Escherichia coli* from irrigation water show potential in transmission of extended spectrum and AmpC β-lactamase determinants to isolates from lettuce. Microbial Biotechnology **8**, 462-473.

RICHTER L, DU PLESSIS EM, DUVENAGE S and KORSTEN L (2019). Occurrence, identification, and antimicrobial resistance profiles of extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae from fresh vegetables retailed in Gauteng Province, South Africa. Foodborne Pathogens Disease **16**, 421-427.

RICHTER L, DU PLESSIS EM, DUVENAGE S and KORSTEN L (2020). Occurrence, Phenotypic and Molecular Characterization of extended-spectrum- and ampc- β-lactamase-producing enterobacteriaceae isolated from selected commercial spinach supply chains in South Africa. Frontiers in Microbiology **11**, 638.

TEKLAHAIMANOT GC, COETZEE MAA and MOMBA MNB (2014). Faecal pollution loads in the wastewater effluents and receiving water bodies: a potential threat to the health of Sedibeng and Soshanguve communities, South Africa. Environmental Science and Pollution Research **21**, 9589-9603.

UYTTENDAELE M, JAYKUS L-A, AMOAH P, CHIODINI A, CUNLIFFE D, LIESBETH JACXSENS L, HOLVOET K, KORSTEN L, LAU M, MCCLURE P, GERTJAN MEDEMA G, SAMPERS I and JASTI PR (2015). Microbial hazards in irrigation water: Standards, norms, and testing to manage use of water in fresh produce primary production. Comprehensive Reviews in Food Science and Food Safety **14**, 336-356.

VERLICCHI P and GRILLINI V (2020). Surface water and groundwater quality in South Africa and Mozambique –Analysis of the most critical pollutants for drinking purposes and challenges in water treatment selection. Water **12**, 305.

WORLD HEALTH ORGANIZATION (2008). Microbiological hazards in fresh fruits and vegetables. Microbiological Risk assessment series (https://www.who.int/foodsafety/publications/micro/MRA_FruitVeges.pdf).

CHAPTER 2

REVIEW ON THE CONTENT OF A POTENTIAL FRESH VEGETABLE BASKET FROM FORMAL AND INFORMAL SUPPLY CHAINS

Authors Erika du Plessis and Loandi Richter

2.1 Introduction

Consumption of fruit and vegetables has increased due to the known direct human health benefits driving demand in the global supply chain, including in low-income and food insecure regions such as sub-Saharan Africa and Southern Asia (FAO, 2013; Al-kharousi *et al*., 2016). Food insecurity is a serious concern in the world especially in developing countries. In contrast, South Africa (SA) is food secure at a national level and food insecure at a household level [FAO *et al*., 2019]. In SA, the agricultural sector plays an important role in providing an affordable, convenient food source to many people and contribute to ensuring food security in the country. To illustrate this point, the value of primary agriculture production (total production during the season valued at the average basic prices received by producers) was reported to be R286,4 billion during 2019 (DAFF, 2019a). This was a decrease of 0,5% when compared to the R287 847 million during 2018, which was due to a decrease in the value of horticulture (Figure 1) (DAFF, 2019a). The gross value of animal products contributed 48,4% to the total gross value of agricultural production, while horticultural products and field crops contributed 29,7% and 21,9%, respectively during 2019 (DAFF, 2019a). The primary agricultural sector contributed an estimated 3% to the country's gross domestic product (GDP) (DAFF, 2019b). When compared to the 2018 financial year the gross income from horticultural products decreased by 2,5% from R87 362 million in 2018 to R85 174 million in 2019. Households have increasingly relied more on vegetables than fruit as a food source due to their relative affordability compared to other food groups (BFAP, 2020). The estimated gross income of fresh vegetables was reported to be R25 000 million for the 2018/2019 production cycle, which was 6.3% higher than the previous year (DAFF, 2019a).

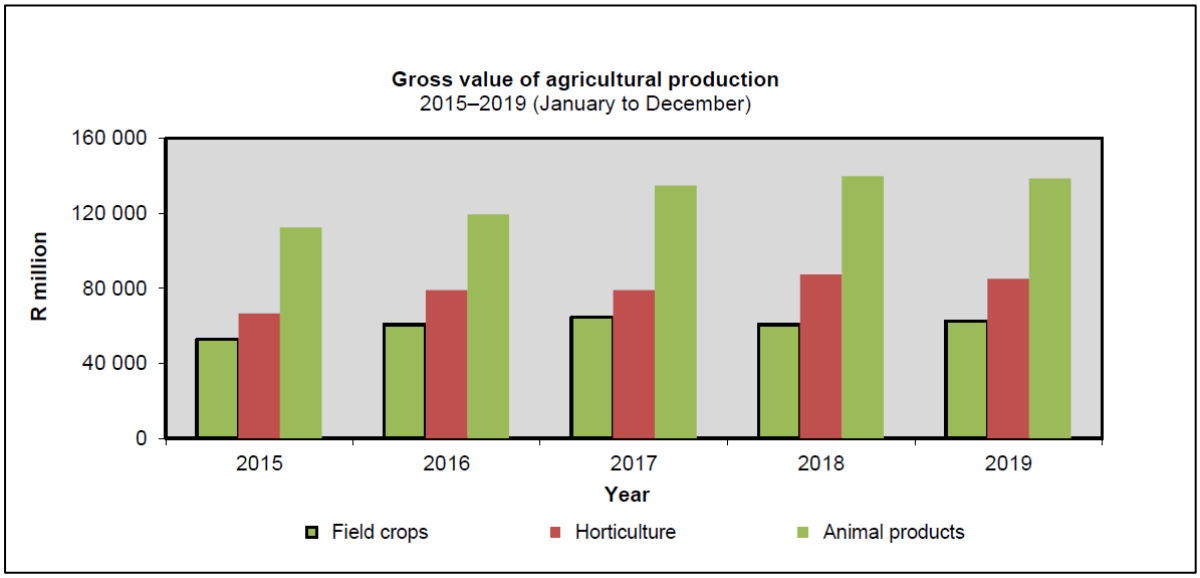

Figure 1: Gross value of agricultural production from 2015-2019 (DAFF, 2019a).

The major vegetable crops produced in South Africa included cabbages and other brassicas, carrots and turnips, cauliflower and broccoli, chillies and peppers, cucumbers and gherkins, lettuce and chicory, green peas, tomatoes and pumpkins, squash and gourds according to the recent statistics of the Food and Agriculture Organization (FAO) of the United Nations (UN) (FAOSTAT 2019) (Table 1).

Table 1 Groups of vegetable crops produced and associated production areas (hectare) in South Africa (FAOSTAT 2019)

Vegetable crop	Tonnes produced	Production area (ha)
Onions	642 081	26 149
Tomatoes	500 000	6 521
Carrots and turnips	186 227	6 400
Pumpkins, squash and gourds	170 600	15 121
Cabbages and other brassicas	144 606	2 549
Lettuce and chicory	37 621	2 462
Cucumbers and gherkins	25 133	1 675
Cauliflowers and broccoli	11 601	727
Green peas	9 317	3 704

A comparison of the tonnes of vegetables (excluding potatoes) produced over the 2016/17 to 2017/18 period was made and summarised in Table 2 (DAFF, 2018a). The total production of vegetables (excluding potatoes) increased by 1,8% from 2 984 104tons to 3037 412 tons from 2016/17 to 2017/18 (July to June). Tomato production decreased by 7,0%, while production of all other vegetable types increased.

Table 2 The production of vegetables (excluding potatoes) in South Africa for the period 2016/17 to 2017/18 (DAFF, 2018a)

Year	**2013/14**	**2014/15**	**2015/16**	**2016/17**	**2017/18**
	'1000 tons				
Tomatoes	538	547	563	632	588
Onions	619	575	687	706	709
Green mealies & sweetcorn	362	373	378	380	390
Cabbages	146	146	139	153	160
Pumpkins	245	256	254	260	264
Carrots	184	202	214	218	230
Other	593	633	630	635	696
Total	**2687**	**2832**	**2865**	**2984**	**3037**

The most important vegetable crops based on the gross value of production from June 2017 to June 2018 is compared in Figure 2 which reflect the importance of green mealies, tomatoes and onions.

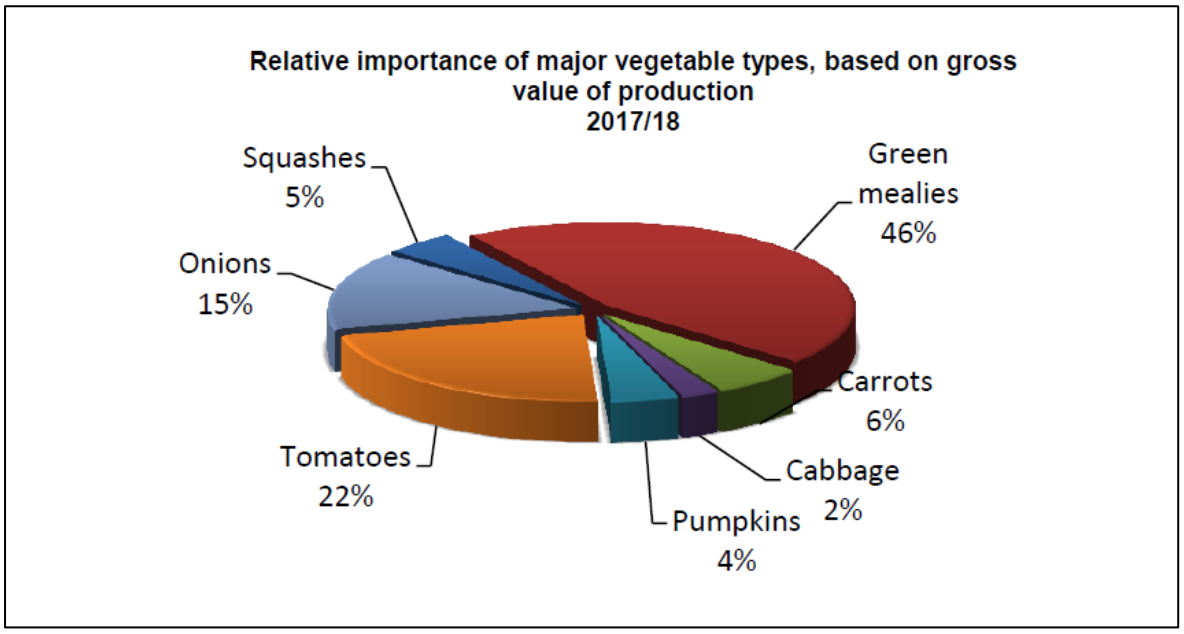

Figure 2: Importance of major vegetable types based on gross value of production (DAFF, 2018).

As far as leafy green vegetables are concerned there is no data focusing only on spinach production in the country, however a total of 2 205 hectares in SA were under lettuce and chicory production during 2018 with 33 055 tonnes produced mainly for the local market (Food and Agriculture Organisation (FAO) (FAO Stats, 2019). Although leafy green vegetables represent a small sector in SA it is still one of the major exporters in the Southern Development Community (SADC) even though it represent only 2% of local production that is exported. This compared to 46% sold through the national fresh produce markets, 42% through direct sales and own consumption, while 10% are processed (IDTT, 2018 The 2018 South African lettuce market value chain clearly shows the increase in gross value of this commodity in agriculture over the last ten years (DAFF, 2018b) (Figure 3).

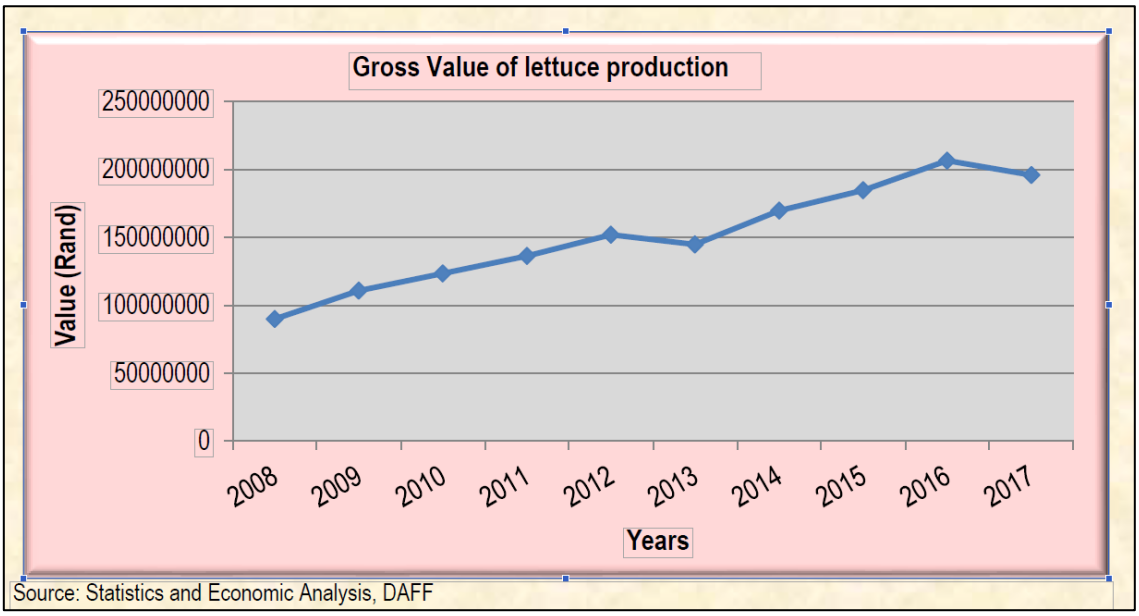

Figure 3: Gross value of lettuce production in South Africa over a ten year period (DAFF, 2018b).

2.2 Fresh produce supply chains

In SA there are various production regions which are identified according to climate, natural vegetation, soil type and establish farming practices, with processing and distribution facilities to support a well-developed distribution, storage and marketing system (Louw and Jordaan, 2016). This comprises mainly commercial and small-holder or subsistence farmers which all contributes to the economy (GreenCape, 2016). In this context the commercial sector is well organised with historical trade routes and market outlets while the small-holder farmers are often focussed on the informal sector. South Africa thus have a notable dualistic food system characterised by very well developed, highly sophisticated (formal) food distribution and marketing chains and a well-organised informal system (Louw *et al*., 2008).

Supply chain coordination plays a vital role in the production and distribution of fresh produce, especially in commercial systems, since the supplier and retailer are often far apart which requires longer transportation times and different handling centres or agents (Su *et al*., 2014). Both suppliers and traders form part of formal and informal supply chains and could potentially incur substantial losses (Su *et al*., 2014). Distribution channels of fresh produce in SA include distributed through retailers, national and informal fresh produce markets, hawkers, export channels and direct sales to wholesalers and processors (Louw and Jordaan, 2016). In a typical

fresh produce supply chain (Figure 4), processing facilities or packhouse often being responsible for the handling/cooling, minimal processing and packaging of the product (DAFF, 2015). Most packhouses/ processing facilities provide a range of final products that includes pre-packed (pillow packs) salad vegetables that contains mixtures of greens, i.e. Cos or Romaine lettuce, Betavia lettuce, Oak leaf lettuce, Butter lettuce, Red lettuce, baby spinach, broccoli, kale and/or various herbs including rocket, watercress, mizuna, Italian parsley, mint, basil, and rosemary. A web-based search of SA processing facilities indicated that other typical products include fresh whole, minimally processed or ready-to-eat (RTE) vegetables including amongst others lettuce, spinach, cabbage, broccoli, cucumbers and tomatoes.

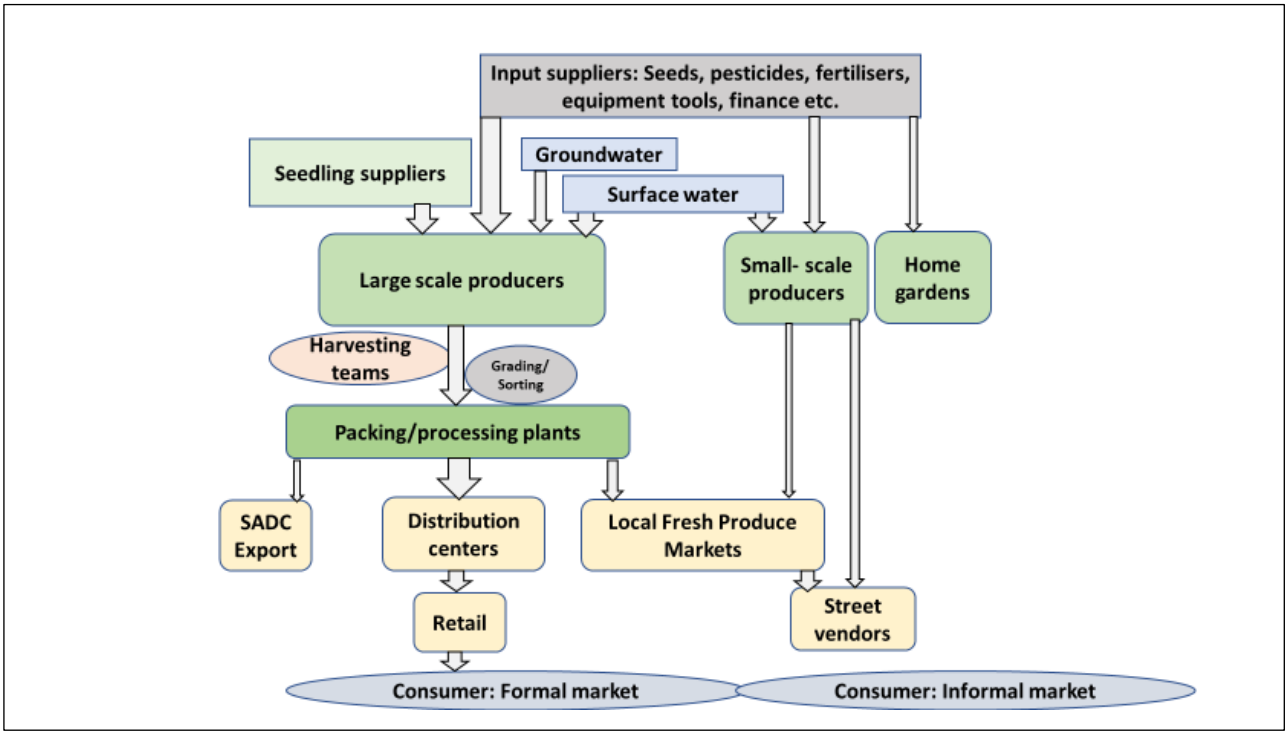

Figure 4: Typical South African fresh produce supply chain (DAFF, 2018b).

2.2.1 Formal supply chains

Retailers

In a review by Das Nair and Chisoro (2015) on trends in the supermarket industry in South Africa the increase in the number of and spread of supermarkets locally and to other African countries can be attributed to a number of factors. These are as follows: 1)increasing urbanisation, 2) increased per capita income, 3) increase in number of women working, 4) increased middle class size, 5) lower prices due to economics of scale and scope of products

on offer, and 6) modernisation of infrastructure. For example shopping malls provided space for retailer expansion not only in South Africa, but also in other countries including Zambia, Zimbabwe, and Botswana. This can be ascribed to favourable trade conditions under Southern Africa Trade Agreements (SADC) and the close proximity of these countries to Southern Africa (Das Nair and Chisoro, 2015).

The formal food retail market in South Africa is dominated by Shoprite, Woolworths, Spar and Pick n Pay. In 2013 there were a reported 920 supermarkets in South Africa, however over the next ten years the numbers increased to 3 167 (Skinner and Haysom, 2016). The largest food retailer in South Africa is Shoprite Holdings Limited which was founded in 1979 (Vorley *et al*., 2008). Shoprite was reported to have 1 581 corporate stores and approximately 40 franchise store across Africa for the 2014 financial year. The number of stores for the same time period was reported to be 1 324. They have a broad customer base and the core supermarkets including Shoprite, Checkers, Suave and OK covers the full spectrum of different income groups/living standards measures (LSM) categories.

The second largest retail chain is Pick n Pay Stores Limited that was founded in 1967 and operates in South Africa, Southern Africa, and Australia through its Franklins stores (Vorley *et al.,* 2008). This retailer caters to the middle-income class of consumers (Vorley et al., 2008). The group trades as Pick n Pay Hypermarkets, Pick n Pay Supermarkets, Pick n Pay Franchise Stores, and Pick n Pay Butcheries. They are increasingly targeting lower income consumers and subsequently acquired Boxer stores to enter this market sector (Das Nair and Chisoro, 2015).

Spar Group Limited (ZA) was formed in 1963, is the third largest retailer in South Africa and is mainly a franchise operation that focusses on smaller stores (Vorley *et al*., 2008). The group operates in South Africa, nine other African countries, Ireland and England. They also target a broad spectrum of LSMs. They trade under the following Brand names: Superspar, Spar, Kwikspar and Savemore. (Das Nair and Chisoro, 2015). They also opened forecourt convenience stores, Spar Express in collaboration with the Shell oil company.

Woolworths Holding Limited was founded in 1931 and the fourth largest retailer in the country catering for the high income sector of the population. This group contains both franchises as well as corporate-owned stores (Vorley *et al*., 2008). They have a single brand store. They also partnered with Engen to establish a number of Woolworths Foodstop stores at fuel stations.

In 2014 thy employed an estimated 38 000 people in the operations locally and in a number of African countries (Das Nair and Chisoro, 2015).

Fruit and Veg City (FVC) is the fifth largest retailer and have expanded rapidly in a short time (Das Nair and Chisoro, 2015). Their turnover has increased significantly from R 1.6 billion in 2006 to R 15 billion in 2015, with a considerably higher growth rate when compared to the other major listed retailers. There are FVC stores in South Africa, Namibia, Zambia, Zimbabwe, Mauritius, Reunion and Australia. They attribute their success to supplying different LSM groups. Currently the Food Lovers Market format caters for the more affluent population group. They aim to change all their stores catering across all LSMs to this format in future. Another entrant to the retail market is Choppies Enterprises, a retailer from Botswana with stores in South Africa and Zimbabwe and plans to enter markets in Zambia and Tanzania as well. Their current target market is low to middle income consumers (Das Nair and Chisoro, 2015).

In the past, all retailers (except Woolworths) procured their fresh produce from municipal markets, however, the key retailers now have central procuring systems in place, where fresh produce is obtained from a number of preferred suppliers as well as national fresh produce markets (NFPM) (Louw *et al.,* 2008).

National Fresh Produce Markets

In South Africa, the national fresh produce markets (NFPMs) function as commission markets with agents who trade farmer's produce on their behalf (Louw & Jordaan, 2016). The purpose of these markets are to provide equal trade opportunities for large producers and small-scale farmers. Large scale commercial farmers supply 80-90% of NFPMs fruit and vegetables, while small-scale producers contributes 10-20% of the remainder of the fresh produce volumes. However, small-scale farmers find it difficult to supply sustainable volumes of fresh produce to NFPMs. Furthermore, where access to markets is difficult, cash income from produce sales may be influenced and the quality of the product may not be of the correct standard when it eventually reaches the market influencing the income from produce sales negatively (Van der Heijden and Vink, 2010). NFPMs have various requirements for farmers, which include grading, sorting, labelling, and packaging of their produce to provide traceability (Louw and Jordaan, 2016). These requirements are legally determined by the Agricultural Product Standard Act 119 of 1990 (Louw and Jordaan, 2016).

To meet the growing demand in local fresh produce markets and consequently, the network topology of a fresh produce supply chain, successful harvest and post-harvest decisions needs to be implemented to help farmers (Besik and Nagurney, 2016). In particular, the length of a path in terms of time from the point origin to the final destination can significantly influence the quality of the fresh produce that consumers purchase and consume, giving incentive to the investigation of local food systems and associated shorter marketing channels (Besik and Nagurney, 2016).

There are nineteen fresh produce markets in South Africa (http://www.farmingportal.co.za/index.php/press-promo/item/9882-tshwane-market-south-, accessed 2017/06/21) The Johannesburg Market has a market share of 47%, while Tshwane Market's share over the 2015/16 period was reported to be 22.8% with approximately half the tonnage of fresh produce handled. Seventy five percent of the country's fresh produce supply are dealt with in the Gauteng Province Fresh Produce Markets including the Pretoria, Johannesburg, Springs and Vereeniging Markets. Informal traders are playing an increasingly prominent role in the market turnover. The markets have facilitated crop production and postharvest technology training of informal traders to assist with them with moving from the informal sector to the formal sector.

The future of the fresh produce market system in South Africa is however increasingly been reported to be jeopardised due to 1) an increase in farm-to-gate sales; 2) an increase in direct marketing to supermarket chains. If the NFPMs disappear the economically disadvantaged sellers and consumers will be affected the most. Currently 47% of vegetables, excluding potatoes, produced are traded through NFPMs. An overview of vegetable distribution channels were depicted in Figure 5.

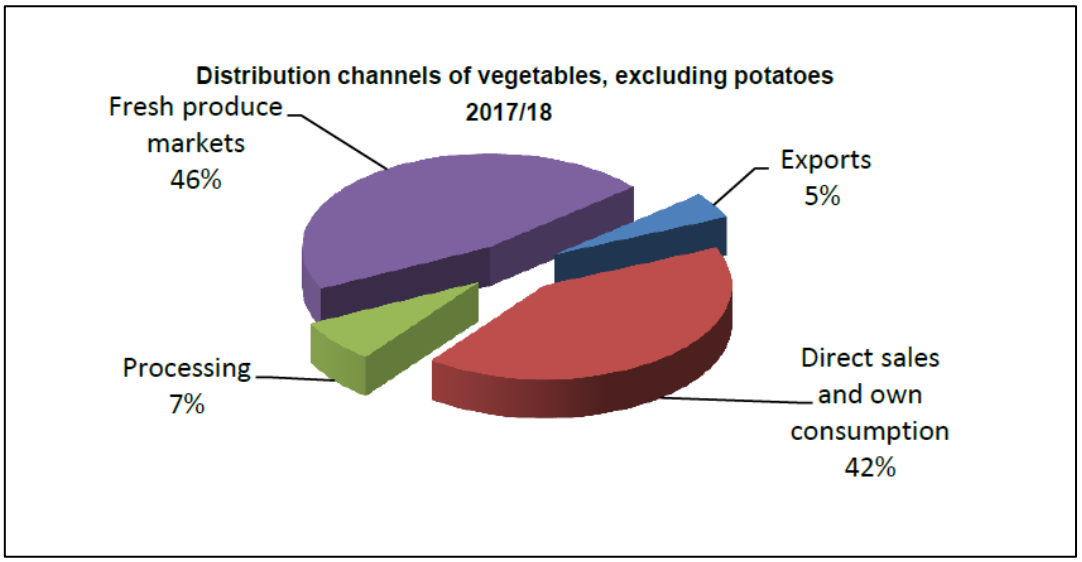

Figure 5: Schematic diagram of vegetable (excluding potatoes) distribution channels (DAFF, 2018).

2.2.2 Informal supply chains

Street vendors

According to the 2019 Quarterly labour force survey in South Africa an estimated 3,000,000 people worked in the informal sector which is slightly less than 20% of the total employment survey (StatsSA, 2019). Unregistered enterprises consist of employees who do not have a written contract of employment, do not receive basic benefits such as pensions or medical aid contributions from their employers, are not registered for income tax or form part of the informal employment sector. Informal employee incomes are often low, however the cumulative informal sector activities contribute significantly to gross domestic product (Skinner and Haysom, 2016).

People with a low socio-economic status are highly dependent on informal markets since they are located near taxi ranks, train stations and close to industries where they work (Methvin, 2015). Fresh produce including fruit and vegetables are usually packed in bags and sold by informal vendors (street trading greengrocers or from mobile trolleys) in townships and informal settlements (Figure 6) (Methvin, 2015; Charman, 2015). Residents were not less dependant on the fruit and vegetable stalls after receiving an increase in salary (Methvin, 2015).

Figure 6: Examples of informal fresh produce traders in South Africa. Photo on the left: produce sold at a farmers market in Pretoria, Gauteng; photo in the middle: produce sold at a street vendor in Thembisa, Gauteng; photo on the right: example of a mobile trolley vendor where vendors sell fresh produce n prepacked bags in Thembisa, Gauteng.

The fresh produce sold at the street vendors are bought from home gardens, local small-scale farms, National Fresh Produce Markets (NFPMs) or from formal retailers such as Makro, Shoprite, Metro Cash and Carry (Roever and Skinner, 2016). In addition, the informal traders are the main purchasers of fresh produce sold by small-scale farmers (Louw, 2008). Subsequently the growth of small farmers is of great importance, especially in developing countries like South Africa where high levels of rural poverty occur (Van der Heijden and Vink, 2010). The livelihood of small farmers depends on many factors that include reliable and sustainable access to output markets where products can be sold at a reasonable price (Van der Heijden & Vink, 2010). An advantage of informal markets is that they can source fresh produce without worrying about the high prices associated with formal supply chains (Louw, 2008).

Farmers markets

Farmers' markets are an example of shorter supply chains, represent only a small part of the fresh produce distribution network and are regarded as an excellent platform for small-scale farmers to sell their produce (Besik and Nagurney, 2016). They have increased in popularity internationally and locally, since good quality produce are sold at affordable prices (Van der Heijden and Vink, 2010; Vermeulen and Bienabe, 2007). In Cape Town small-scale producers, particularly organic farmers, have successfiully estalished box delivery services to supply heath concious consumers and deli's. Policy makers and role players in relevant government departments are increasingly recognising the importance of these markets for informal farmers to sell their fresh produce and improve their livelihoods.

2.3 Food safety and food quality in fresh produce supply chains

Increasing consumer demands have led to a substantial contribution of fresh produce to the economy as well as to the health of a country's population. Food safety and food quality are two important elements in fresh fruit and vegetable supply chains (Aung and Chang, 2014). All hazards that may make food harmful to the health of the consumer are referred to as food safety (Aung and Chang, 2014). Food safety is not negotiable and is a global issue, with a large number of people affected by contaminated food and associated diseases (Aung and Chang, 2014). The food safety responsibility is shared by producers, processors, distributors, retailers, and consumers, as food safety hazards may occur at a variety of points in the food supply chain (Aung and Chang, 2014). As a result, supply chains have evolved to implement effective food safety management to strive to bring sufficient and nutritious good quality fresh produce to the consumer (Jacxsens *et al*., 2017). In South Africa unsold fruit and vegetables from the fresh produce markets which should be disposed of are often sold illegally at reduced prices to informal retailers. The fresh-cut fruit and vegetable industry has expanded rapidly, subsequently food safety measures have to be implemented (Capozzi *et al*., 2009).

The requirements necessary to satisfy the needs and expectations of the consumer is referred to as quality (Aung and Chang, 2014). Fresh produce attributes that influence the product value to the consumer are as follows: categorising by colour, flavour (taste and aroma), texture, appearance, and the nutritional value (Aung and Chang, 2014; Besik and Nagurney, 2016). Consumers make the decision of purchase based on their sensory evaluations which consist of smell, taste, touch, hearing and sight and often tend to connect the terms "fresh", "tasty", and "good quality" to products that are being produced locally (Besik and Nagurney, 2016). Consequently, retaining the quality of fresh produce throughout the supply chain is very important to knowledgeable and discerning consumers and farmers (Besik and Nagurney, 2016). The consumers are however often unaware of the great distances the food has travelled via complex supply chains (Besik and Nagurney, 2016). Consumer trust in different fresh produce supply chains to deliver high quality and safe produce is extremely important (Aung and Chang, 2014).

Since food safety and quality in fresh produce supply chains are important and producers and retailers are held responsible, traceability systems were implemented from farm to fork (Aung and Chang, 2014). Traceability systems have a high cost implication, however the benefits gained from traceability for high-risk food such as fresh leafy vegetables outweigh the cost

(Aung and Chang, 2014). Pressures upon the food supply chain include: changing consumption patterns, climate change, and globalisation poses a challenge to the current quality assurance and control tools and methods to prevent and/or to control microbiological risks associated with fresh produce (Jacxsens *et al.,* 2017).

With an increase in fresh produce production and consumption, the associated risks of foodborne pathogen contamination also increase (Cardamone *et al*., 2015). Fresh produce contamination with human pathogens can occur throughout the pre- and postharvest stages of production and marketing. Potential sources of contamination during the preharvest production stages are soil, faeces, irrigation water, reconstituted fungicides and insecticides, dust, insects, manure, and wild or domestic animals (Althaus *et al.,* 2012). In the post-harvest phase contamination can occur during processing, handling, transportation and preparation through contaminated water or cross contamination (Yeni *et al*., 2016). The most important pathogens associated with fresh produce are pathogenic *E. coli*, *Salmonella* spp., *Yersinia* spp., *Shigella* spp., *Clostridium* spp., *Staphylococcus aureus* and *L. monocytogenes* (Berger *et al.,* 2010).

Tomatoes and leafy vegetables (lettuce, rocket, spinach) are regarded as high-risk crops due to the number and severity of foodborne disease outbreaks associated with consumption of these products (Callejón *et al*., 2015). From a microbiological safety perspective, leafy green vegetables are of greatest concern, as they are often consumed raw, or are minimally prepared and therefore have fewer barriers against microbial growth (Mritunjay and Kumar, 2015). There is an increasing demand for ready-to-eat (RTE) minimally processed vegetables due to their convenience (Kim *et al*., 2015). There is however an increased risk associated with consumption of these products, since the vegetables are often consumed raw in salads (Kim *et al*., 2015). In informal supply chains handling practices during production and packaging of the fresh product are often questionable and the street vendors typically do not have any refrigeration facilities (Methvin, 2015). As fresh produce are generally consumed as mixed salads or garnish, the identification of the source product inside the mixed food poses the first challenge for epidemiologists in outbreaks related to fresh produce (Berger *et al.,* 2010).

Highly published foodborne disease outbreaks associated with fresh produce included amongst others the 2011 *E. coli* O104:H4 outbreak, a more virulent shiga-toxin-producing strain than *E. coli* O157:H7 (http://www.bbc.co.uk/news/world-europe-13683270). The outbreak was eventually linked to a German sprout producer after first implicating Spanish cucumbers and tomatoes. More recently two outbreaks associated with *E. coli* O157:H7 contaminated fresh

produce were reported, including 1) Salinas Valley, California, Romaine lettuce *E. coli* O157:H7 outbreak 2019 – 64% female, 165 ill, 85 (52%) hospitalizations, 15 people with hemolytic uremic syndrome (HUS) and no deaths 2) Yuma, Arizona, Romaine lettuce *E. coli* O157:H7 outbreak 2018 outbreak – 210 ill and five deaths due to hemolytic uremic syndrome, a type of kidney failure. Fifteen *Salmonella* spp. outbreaks associated with cucumbers caused 1469 illnesses, 360 hospitalisations, and seven deaths in the USA from 2016-18 (CDC, 2020; https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html).

2.4 Basic vegetable basket components

Food security is defined as "when all people at all times, have physical, social and economic access to sufficient, safe and nutritious food to meet their dietary needs and preferences for an active and healthy life" (FAO, 2009). The three indicators most commonly used to measure food security/insecurity are food availability, accessibility and utilisation (Jones *et al*., 2013). According to statistics South Africa the food basket is South Africa is made up of 31 items which are put into categories; beverages, grain products, fats and oils, dairy products and eggs, fish, meat and poultry, fruits and vegetables.

However according to Schonfeldt *et al.* (2013) the poor in the country cannot afford to follow the recommended diet, even when made up of the most basic low-cost basic foods. South Africa is regarded as a middle-income country, however estimates of StatsSA (2015) reported that 53.8% of the population lives in poverty (R 779 or less per person per month, 21.7% living in extreme poverty (StatsSA, 2015). South Africa is not the only country in the world where a financial barrier to healthy eating has been reported (Faber *et al*., 2017). The food security status of a household impacts the dietary diversity. Faber *et al*. (2017) reported that there was no difference between food secure and insecure households as far as the percentage of spinach, butternut, carrots, cucumber and African leafy vegetables, consumption is concerned. African leafy vegetables mostly consumed were Amaranth spp and Bidens spinosa (blackjack) (Faber *et al*., 2010). Food insecure households preferentially consumed more cabbage, pumpkin, and sweet potato, while food secure households preferentially consumed apricot, naartjie (local name for mandarin), and watermelon (Faber *et al*., 2017). Fruit and vegetables purchased most often were summarised in Table 3.

Table 3 Vegetables and fruit purchased most often by consumers from different food security status categories

Vegetables	**Food secure** (n=125) (%)	**Food insecure** (n=273) (%)
No difference across food security status categories		
Beetroot	47.6	41.5
Butternut	71.8	74.9
Cabbage	90.3	93.4
Carrot	88.7	91.5
Green beans	70.2	62.5
Pumpkin	40.3	36.8
Spinach	61.3	59.0
Sweet potato	17.7	16.2
Tomato	91.9	91.9
Food secure households buy more		
Broccoli	20.2	7.4
Cauliflower	23.4	10.7
Cucumber	36.3	25.1
Lettuce	44.7	31.2
Fruit		
No difference across food security status categories		
Apple	98.4	95.9
Apricot	13.6	10.0
Avocado	23.4	19.6
Banana	90.4	91.9
Grape	68.0	60.9
Guava	13.6	13.0
Lemon	10.4	5.9
Mango	53.6	48.3
Naartjie (Mandarin)	25.6	20.4
Orange	58.4	57.2
Papaya	23.3	17.0
Peach	58.4	56.1
Pear	83.2	74.5
Plum	59.7	54.6
Food secure households buy		
Pineapple	22.4	14.1
Watermelon	19.4	11.2

The most recent report by the National Agricultural Marketing Council (NAMC) of South Africa available included cabbage, onions, potatoes and tomatoes vegetable in a basic food basket (NAMC, 2019). The NAMC monitors food prices and food price trends to report on factors driving commodity and food price margins (NAMC, 2019). According to Statistics South Africa, vegetables in the consumer price index (CPI) in all urban areas throughout South Africa include leaf and stem vegetables, vegetables cultivated for their fruit, root crops, non-starchy bulbs and mushrooms, dried vegetables, other preserved or processed vegetables, and

vegetables cultivated for their tuber. Although tomatoes and peas are scientifically classified as fruit, they are classified as vegetables cultivated for fruit from an economic view and in the culinary industry. The Pietermaritzburg Agency for Community Social Action (PACSA) reported in 2014 that vegetables chosen for the ideal food basket, taking cultural acceptability, nutritional value and cost into consideration, includes onion, tomato, carrot, spinach, cabbage, green pepper, and butternut (Barnard, 2014).

The choice of fresh fruit and vegetables in informal settlements are limited, the quality is compromised and the produce is often more expensive (Batterby and Peyton, 2014). Studies have indicated that low-income South Africans may be moving back to consumption of indigenous and traditional food crops for health and cultural reasons (Pereira, 2014). A study in the North West Province, South Africa has indicated that the overall variety of indigenous traditional food crops that are consumed by African families is dominated by sorghum, cowpeas, and sweet potatoes (Cloete and Idsardi, 2013). Additionally, leafy greens including amaranth leaves, pumpkin leaves, cowpea leaves, as well as wild pears, brandy bush, sweet reeds, and buffalo thorn are often consumed when seasonally available (Cloete and Idsardi, 2013).

The most common fresh produce sold by street vendors are spinach, potatoes, tomatoes, sweet corn, sweet potatoes, peas, cabbage, beans, onions, lettuce as well as other indigenous vegetables such as morogo (Mthombeni, 2013). The fresh produce that street vendors usually purchase from the FPM include: potatoes, onions and tomatoes with other vegetables and fruits such as citrus, deciduous, and subtropical fruit as well as berries, cherries, strawberries, figs, prunes, quinces and melons. In terms of fresh vegetables: carrots, green peas, cabbage, beetroot, green beans, cauliflower, pumpkins, green mealies, sweet potatoes make up the bulk of the produce (Louw, 2008). Methvin *et al*. (2015) reported that the main consumed fruits in Cape Town were; banana, apples, oranges, pears, guavas. The main consumed vegetables were potatoes, cabbage, tomatoes, spinach, onions and mixed vegetables (are sold commercially), (Methvin, 2015). The components of a potential vegetable basket for both the formal and informal supply chains based on information obtained in the literature review and from some scoping studies in formal supply chains and informal supply chains were summarised in Table 4.

Table 4 Fresh, minimally processed and ready-to-eat vegetables typically included in a vegetable basket from both the formal and informal supply chains.

Formal supply chain	Consumed (raw/cooked or both)	Informal supply chain	Consumed (raw/cooked or both)
Tomato	Raw and cooked	Tomato	Raw
Cabbage	Raw and cooked	Cabbage	Raw & cooked
Carrot	Raw and cooked	Carrot	Raw and cooked
Green beans	Cooked/ raw (not often)	Green beans	Cooked
Spinach	Raw and cooked	Spinach	Cooked
Morogo/ African leafy vegetables	Not consumed	Morogo	Cooked, very popular
Lettuce	Raw	Lettuce	Raw
Kale	Raw and cooked	Kale	Not sure how widely it is used, will determine through survey
Cucumber	Raw	Cucumber	Raw
Sweet corn/baby corn	Sweet corn cooked/ baby corn raw and cooked	Sweet corn	Cooked
Peas (snap peas)	Raw and cooked	Peas (fresh produce market)	Cooked
Onions	Raw and cooked	Onions	Mostly cooked, also raw in salad
Butternut	Cooked	Butternut	Cooked
Beetroot	Cooked (mostly), julienne slices in mixed RTE leafy salads	Beetroot	Cooked
Pumpkin	Cooked	Pumpkin	Cooked
Sweet potato	Cooked	Sweet potato	Cooked
Potatoes	Cooked	Potatoes	Cooked
Cauliflower	Raw and cooked	Cauliflower	Cooked. Not very popular
Broccoli	Raw and cooked	Broccoli	Cooked. Not very popular
Green pepper	Raw and cooked	Green pepper	Raw and cooked

2.5 References

AL-KHAROUSI ZS, GUIZANI N, AL-SADI AM, AL-BULUSHI IM and SHAHAROONA B (2016) Hiding in fresh fruits and vegetables: opportunistic pathogens may cross geographical barriers. International Journal of Microbiology **1**, 1-14.

ALTHAUS D, HOFER E, CORTI S, JULMI A and STEPHAN R (2012. Bacteriological survey of ready-to-eat lettuce, fresh-cut fruit, and sprouts collected from the Swiss Market. Journal of Food Protection **75**, 1338-1341.

AUNG MM and CHANG YS (2014). Traceability in a food supply chain: Safety and quality perspectives. Food Control **39**, 172-184.

BARNARD P (2014). The ideal food basket. The Pietermaritzburg Agency for Community Social Action (PACSA).

BERGER CN, SODHA SV, SHAW RK, GRIFFIN PM, PINK D, HAND P and FRANKEL G (2010). Fresh fruit and vegetables as vehicles for the transmission of human pathogens. Environmental Microbiology **12**, 2385-2397.

BESIK D and NAGURNEY A (2016). Quality in competitive fresh produce supply chains with application to farmers' markets. Socio-Economic Planning Sciences. Available at: http://dx.doi.org/10.1016/j.seps.2017.03.001.

BFAP Baseline (2020) Available at: https://www.bfap.co.za/?s=baseline.

CALLEJÓN RM, RODRÍGUEZ-NARANJO MI, UBEDA C, HORNEDO-ORTEGA R, GARCIA-PARRILLA MC and TRONCOSO AM (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathogens and Disease **12**(1), 32-38.

CAPOZZI V, FIOCCO D, AMODIO M, GALLONE A and SPANO G (2009). Bacterial Stressors in Minimally Processed Food. Internationa*l* Journal of Molecular Science **10**, 3076-3105.

CARDAMONE C, ALEO A, MAMMINA C, OLIVERI G and DI NOTO AM (2015). Assessment of the microbiological quality of fresh produce on sale in Sicily, Italy: preliminary results. Journal of Biological Research-Thessaloniki **22**(1), 3.

CENTRES FOR DISEASE CONTROL AND PREVENTION FOODBORNE OUTBREAKS (CDC) (2020). Available at: https://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html (accessed: 15 May 2020).

CHARMAN A (2015). Photovoice street life in Ivory Park (http://livelihoods.org.za/.

CLOETE PC and IDSARD EF (2013). Consumption of Indigenous and Traditional Food Crops : Perceptions and Realities from South Africa. Agroecology and Sustainable Food Systems **37**, 902-914.

DAS NAIR R and CHISORO-DUBE S (2015). The expansion of regional supermarket chains: Changing models of retailing and the implications for local supplier capabilities in South Africa, Botswana, Zambia, and Zimbabwe. WIDER Working Paper 2015/114. Helsinki: UNU-WIDER.

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES (DAFF) (2018a). Trends in the Agricultural Sector (https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Trends%20in%20the%20Agricultural%20Sector%202018.pdf, accessed 15/10/2020).

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES (DAFF) (2018b). Profile of the South Africa lettuce market value chain https://www.dalrrd.gov.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20Profiles/field%20crops/Lettuce%20Market%20Value%20Chain%20Profile%202018.pdf, accessed 15 October 2020).

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES DAFF (DAFF) (2019a). Economic review of the South African Agriculture. Department of Agriculture Forestry and Fisheries, Pretoria (https://www.dalrrd.gov.za/Portals/0/Statistics%20and%20Economic%20Analysis/Statistical%20Information/Economic%20Review%*202019*.pdf, accessed 12 October 2020).

DEPARTMENT OF AGRICULTURE, FORESTRY AND FISHERIES DAFF (DAFF) (2019b). South Africa Yearbook 2018/19 Agriculture, Forestry and Fisheries (https://www.gcis.gov.za/sites/default/files/docs/resourcecentre/yearbook/yb1919-3-Agriculture.pdf, accessed 12 October 2020).

FABER MA, OELOFSE PJ, VAN JAARSVELD FAM, WENHOLD and JANSEN VAN RENSBURG WS (2010). African leafy vegetables consumed by households in the Limpopo and KwaZulu-Natal provinces in South Africa. South African Journal of Clinical Nutrition **23**, 30-38.

FABER MA, FAM WENHOLD and LAURIE SM (2017). Dietary diversity and vegetable fruit consumption of households in a resource-poor peri-urban South Africa community differ by food security status, ecology of food and nutrition **56**, 62-80. DOI: 10.1080/03670244.2016.1261024

FAO (2013). Feeding the World. Food and Agricultural Organisation (http://www.fao.org/docrep/018/i3107e/i3107e03.pdf); accessed 21 July 2017.

FAO Stat (2019). Crop*s* – Food and Agricultural organisation of the United Nations. http://www.fao.org/faostat/en/#data/QC, updated March 2020, accessed 12 October 2019.

GREENCAPE (2016). Agriculture: Market Intelligence Report. GreenCape.

JACXSENS L UYTTENDAELE M, LUNING P and ALLENDE A (2017). Food safety management and risk assessment in the fresh produce supply chain. IOP Conference Series: Materials Science and Engineering **193**, 012020.

JONES AD, NGURE FM, PELTO G and YOUNG SL (2013). What are we assessing when we measure food security? A compendium and review of current metrics. Advances in Nutrition: An International Review Journal **4**, 481-505.

KIM HS, CHON JW, KIM YJ, KIM DH, KIM M and SEO KH (2015). Prevalence and characterization of extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in ready-to-eat vegetables. International Journal of Food Microbiology **207**, 83-86.

LOUW A (2008). Improved small-scale farmer access to fresh produce agri-food markets in South Africa. Regoverning markets policy brief.

LOUW A and JORDAAN D (2016). Supply chain risks and smallholder fresh produce farmers in the Gauteng province of South Africa. Southern African Business Review **20**, 286-312.

METHVIN T (2015). FOOD : LAB, Cape Town.

MRITUNJAY S and KUMAR V (2015). Fresh Farm Produce as a Source of Pathogens: A Review. Research Journal of Environmental Toxicology **9**, 59-70.

MTHOMBENI DL (2013). Impact of vegetable sales on household income of hawkers in the Limpopo province of South Africa. MSc dissertation at University of Pretoria, Pretoria.

NATIONAL AGRICULTURAL MARKETING COUNCIL (NAMC) (2019). Markets and Economic Research Centre, Food basket price monthly Issue (www.namc.co.za › food-basket-monthly-September-2019).

PEREIRA LM (2014). The Future of South Africa's Food System : What is research telling us? The South Africa Food Lab (SAFL). Available at: http://bio-economy.org.za/wp-content/uploads/2015/02/Pereira-2014-The-Future-of-South-Africas-Food-System-Executive-Summary.pdf Food Lab.

ROEVER S AND SKINNER C (2016). Street vendors and cities" Environment and Urbanisation. 28: 1-16.

SKINNER C AND HAYSOM G (2016). The informal sector's role in food security: a missing link in policy debates? (www.plaas.org.za/sites/default/files/publications-pdf/WP44%20SkinnerHaysom.pdf).

STATSSA (2019). Quarterly labour force survey (https://www.statssa.gov.za/publications/P0211/P02112ndQuarter2019.pdf).

SU J, WU J and LIU C (2014). Research on coordination of fresh produce supply chain in big market sales environment. The Scientific World Journal, 2014.

VAN DER HEIJDEN T and VINK N (2010). Good for whom? supermarkets and small farmers in South Africa –a critical review of current approaches to affectivity.

YENI F, YAVAS S, ALPAS H and SOYER Y (2016. Most Common Foodborne Pathogens and Mycotoxins on Fresh Produce: A Review of Recent Outbreaks. Critical Reviews in Food Science and Nutrition **56**, 1532-1544.

VERMEULEN H and BIENABE E (2007). What about the food 'quality turn' in South Africa? Focus on the organic movement development. Poster Paper prepared for presentation at the 105th EAAE Seminar 'International Marketing and International Trade of Quality Food Products', Bologna, Italy, March 8-10, 2007.

VORLEY B, FEARNE A and RAY D (2008). The role of fresh produce markets in South Africa, regoverning markets, Policy Brief 4. Available at : https://www.up.ac.za/media/shared/Legacy/sitefiles/file/48/2052/5_roleoffreshproducemarkets.pdf.

CHAPTER 3

EXPERIMENTAL PROCEDURES

3.1 Introduction

Sampling sites and fresh produce selected for microbiological evaluation, questionnaire surveys of consumers (formal and informal) and street trading vendors were summarised in section 3.2 below. Experimental procedures followed to collect, process and analyse samples (water, soil, fresh produce and contact surface) from formal and informal supply chains in both the Tshwane and Cape Town Metropoles were summarised in sections 3.3 to 3.5 and 3.6 to 3.8 respectively. The phenotypic and genotypic characterisation of the isolates obtained from these samples were also described in detail in these sections.

3.2 Site and fresh produce selection and proposed questionnaire/survey instrument

The final list of selected fresh produce was informed by the literature review on potential fresh vegetable basket components, CDC foodborne disease outbreak data associated with vegetables, consumption (popularity, consumed raw or cooked) information and general producer/consumer food safety knowledge, attitudes and practices (KAP) determined through conducting Questionnaire surveys. The questionnaires were developed in collaboration with Prof. G. Du Rand from the Department of Consumer and Food Sciences, University of Pretoria and Consulta, a private firm that specialises in conducting market surveys. Questionnaire A was an online testing the South African consumers' food safety knowledge, attitudes and practices, fresh vegetables consumed in Tshwane and Cape Town Metropoles (Appendix III, supplementary information) in collaboration with Consulta (a private company specialising in conducting surveys). Questionnaire B was an interviewed questionnaire and determined the South African consumers' buying fresh produce from informal trading green grocers/street vendors: food safety knowledge, attitudes and practices, vegetables consumed (Appendix III, supplementary information). Questionnaire C was an interviewed Questionnaire focusing on "South African street trading green grocers' food safety knowledge, attitudes and practices, fruit and vegetables sold" (Appendix III, supplementary information). Results of the Questionnaires were summarised in Chapter 4, section 4.1.

Leafy vegetables (spinach and lettuce) which have been identified as high risk crops based on the number and severity of foodborne disease outbreaks documented and are regularly

consumed raw in salads (WHO, 2008) were included in the final list of vegetables to be analysed. From the latest CDC foodborne disease outbreak statistics cucumbers can also be included in the high risk category CDC Foodborne Outbreak Online Database (ttps://www.cdc.gov/foodsafety/outbreaks/multistate-outbreaks/outbreaks-list.html). Crops included in the sampling plan which have not been evaluated previously in our any of our previous WRC research projects were green beans and cucumbers. The sampling sites and fresh vegetables selected in the Tshwane Metropole and Cape Town Metropole were summarised in Table 5 and 6 respectively. Ethical clearance was obtained for the project funded by the (K5/2706//4): Ethical clearance number EC 180 327-182.

Table 5 Summary of sampling sites and selected fresh vegetables for microbiological analysis in the formal and informal supply chains in the Tshwane Metropoles

Tshwane Metropole Formal Supply Chains		
Sites	**Fresh vegetables**	**Retailers**
Farmers markets selling fresh farm produce (vegetables, fruit, cheese, meat, etc.).	Cucumbers, green beans, tomatoes, lettuce, spinach.	Market clients Retailers 3, 4
Commercial leafy green (spinach and lettuce) supply chains in Gauteng Province and North West Province supplying retailers in Tshwane Metropole		
Spinach supply chain scenario 1 Commercial fresh vegetable minimal processing and packaging facility on Farm A in Gauteng Province	Spinach: whole, cut, washed, RTE pillow packs Spinach bunches: before and after wash	Retailers* 2, 3, 4
Spinach supply chain scenario 2 Commercial fresh vegetable minimal processing and packaging facility (Retailer 1) on Farm B in Gauteng Province	Baby spinach: unwashed spinach leaves in crates, after wash, RTE pillow packs Baby spinach: unwashed packed in punnets on the supplier farms	Retailer 1
Spinach supply chain scenario 3 Commercial fresh vegetable minimal processing and packaging facility (Retailer 1) on Farm C in North West Province respectively	Baby spinach: unwashed spinach leaves in crates, after wash, RTE pillow packs Baby spinach: unwashed packed in punnets on the supplier farms	Retailer 1
Spinach and lettuce supply chain scenario 4 Commercial fresh vegetable minimal processing and packaging facility supplied by Farm D in Gauteng Province	Lettuce: whole, cut, washed, RTE pillow packs, frilly leaf, cos, crisp lettuce Spinach: whole, cut, washed, RTE pillow packs	Retailers 1, 2, 3, 4

Table 5 cont.

Spinach and lettuce supply chain scenario 5		
Commercial fresh vegetable minimal processing and packaging facility on Farm E in North West Province. In winter they also send fresh produce to the fresh vegetable minimal processing and packaging facility supplied by Farm D.	Lettuce heads: whole in pillow pack Spinach: whole, stem ends cut, RTE pillow packs	Retailers 1, 2, 3, 4, 5
Tshwane Metropole Informal Supply Chains		
Street vendors/greengrocers, i.e. Atteridgeville,/Mamelodi/Marabastad	Carrots, cabbage, tomatoes, spinach	Directly to public

*Retailers 1-5 included, in no specific order, Checkers, Woolworths, Spar, Pick n Pay as well as Fruit and Veg.

Table 6 Summary of selected sampling sites and fresh vegetables collected for analysis in the formal and informal supply chains in the Cape Town Metropole

Cape Town Metropole Formal Supply Chain/s		
Sites	**Fresh vegetables**	**Retailers**
Global-GAP certified fresh produce pack store in the semi-urban Philippi area of the Cape Metropole	Broccoli/cabbage/ carrot mix & lettuce (loose, packed and RTE)	Public, street vendors and Retailers 2, 6
Lymies Fruit and Veg (Region: Delft)	Cabbage, lettuce, spinach, green beans or tomatoes	Directly to public
Corner Westgate Mall, (Mitchells Plein/Philippi)	Cabbage, lettuce, spinach, green beans or tomatoes	Directly to public
Bay 153 and 154 (Region: Rylands) OR/ AND	Cabbage, lettuce, spinach, green beans or tomatoes	Directly to public
Dicky's Fruit and Veg (Region: Gatesville)	Cabbage, lettuce, spinach, green beans or tomatoes	Directly to public

3.3 Detailed description of formal and informal supply chain sampling sites selected for analysis in the Tshwane Metropole

Potential sampling sites/processing factories where fresh vegetable produce such as lettuce, spinach, cucumbers, mixed leaf RTE pillow packs, baby vegetables are processed were identified by conducting an internet search of processing facilities focusing on the Tshwane metropole. In addition fresh produce supplier lists were obtained from the major retailer processing facilities, contact made and sites including farms and on-farm processing facilities visited subject to suppliers being willing to collaborate with us. The major retailer processing

facilities were found not to be in the Tshwane metropole, however fresh produce are typically transported and sold in the Tshwane metropole. The suppliers often have their own processing facility on farm where produce is minimally processed and packed before being transported to retailer processing facilities, where further processing takes place, i.e. cutting, washing and packing in RTE pillow packs. Two major retailer distribution centres, i.e. Freshmark (Checkers/Shoprite) DC and Woolworths DC are however in Centurion. A total of 5 processing facilities in the formal supply chain were identified for sampling and analysis from 2017/2019. Lettuce and spinach samples were collected on the farm, at packhouse receiving, through processing (after cut, after wash, after pack) and from the associated retailers.

Fresh produce markets and informal retailers including street vendors/mobile trolleys/greengrocers (Mamelodi, Marabastad, Atteridgeville) were also visited and the type of produce sold noted. In the informal supply chains produce were sampled from street trading vendors/greengrocers at selected sites in townships in the Tshwane Metropole. Vegetables selected for microbiological analysis included lettuce, spinach, green beans, cucumbers, tomatoes and African leafy greens (commonly known as morogo) at the point-of-sale from formal (farmers markets, retailers) and informal retailers (street vendors, mobile trolleys).

In the Western Cape, contact was made by Prof Sigge with the City of Cape Town's (CoCT) Environmental Health department to enlist their help in identifying sampling sites which are potential risk areas. The proposed project and work plan was submitted to the CoCT's Research Committee for approval, together with the ethical clearance from Stellenbosch University. More recently, a meeting was held the various Heads of Environmental Health of the different regions in the Cape Metropole, to specifically identify and select sampling sites for the study of microbiological quality of selected fresh produce of informal street vendors at the point of sale. The Environmental Heads selected five sites (informal vendors in different regions of the Cape Metropole). Produce included cabbage, lettuce, spinach, beans, carrots and tomatoes.

Contact was also established with a formal vegetable pack store in the Philippi area of the Cape Metropole. The pack store not only grow their own vegetables on-site, but also receive vegetables from other production areas of the Cape Metropole. The pack store sells directly to the public, street vendors and also supply the formal retail sector. They use municipal water in the pack store, but are contemplating using borehole water in future. There are, however, concerns regarding the microbiological quality of the borehole water, as higher than allowable *E. coli* levels have been detected on occasion. A large variety of vegetables (including ready-

to-eat pillow packs) are sold whole, cut, diced and washed. Vegetables include carrots, cabbage, lettuce, spinach, peppers, beans, tomatoes.

3.3.1 Fresh vegetables at the point-of-sale (formal and informal retailers)

Ten suppliers in retail and twenty in informal markets (ten street traders and ten mobile trolley vendors) as well as 13 randomly selected stalls from two farmers' markets in Gauteng Province SA were selected for sampling. In total, 545 randomly chosen vegetable samples (spinach, lettuce, cucumbers, green beans and tomatoes) were purchased between September 2017 and May 2018.

3.3.2 Commercial fresh vegetable supply chains

Five commercial fresh leafy green vegetable production supply chains were selected for sampling of fresh produce from the farm, through processing to the retailer. Contact was established two commercial processing facilities, one exclusively packaging fresh leafy vegetables for Retailer 1 and a second commercial minimal processing and packaging facility near Meyerton in Gauteng Province supplying not only retailer 1, but retailers 2, 3 and 4 as well. Fresh leafy vegetables are sourced from a number of preferred supplier farms Fresh leafy vegetables are sourced from a large number of preferred supplier farms. Three GLOBAL-GAP certified supplier farms including Farm A, Farm B and Farm C in Gauteng Province which had their own processing facilities on the farm, but also supplied the Retailer 1 minimal processing facility for further processing. An additional two farms including Farm D and Farm E supplying the second commercial processing facility were selected and lettuce and spinach were sampled throughout the supply chains from the farm to the market. Farm D does not have a processing facility, subsequently the fresh produce is transported to the processing and packaging facility where the spinach leaves and lettuce heads are washed, cut and packaged in ready-to-eat pillow packs. The produce is then transported to distribution centers in refrigerated trucks from where it is supplied to the retailer outlets. Farm E is located on a commercial GLOBAL-GAP certified farm (Farm E) in the North West Province. Leafy vegetables, including lettuce and spinach are processed and packed at the processing facility on the farm and transported in refrigerated trucks to distribution from where it is supplied to all five the major retailers in the Tshwane Metropole (1, 2, 3, 4 and 5). During winter Farm E in the North West Province supplies the processing facility when it is too cold in Gauteng Province to grow leafy green vegetables. Spinach is produced on the farm using water from a reservoir dam on the farm and overhead

irrigation pivots. In the processing/packaging facility on the farm spinach (large leaf), whole with stem ends cut, washed and sealed in bulk salad packs with 98% nitrogen are prepared. Details of the farms, their cultivation and processing practices were summarised in Table 7. Flow-diagrams of the five of leafy greens (spinach and/or lettuce) supply chains were created (Figure 7 and Figure 8).

Table 7 Comparison of the cultivation and processing practices of Farms A to E

Practice	Farm A	Farm B	Farm C	Farm D	Farm E
Certification status	Global G.A.P.	Global G.A.P.	Global G.A.P.	Global G.A.P.	Global G.A.P.
Production system	Open field	Tunnels	Tunnels	Tunnels	Open field
Irrigation water source	River, water pumped directly from river or to a storage dam	Borehole water, pumped into a storage dam	Borehole water, pumped into a storage dam	River, water pumped directly from river or to a storage dam	Canal water pumped directly into storage dam
Irrigation water storage	Uncovered storage dam	Two additional water storage dams (covered with a net) over which the source water is pumped in and circulated	Source water is pumped into another water storage dam	Dam	Dam
Irrigation method	Overhead irrigation	Overhead irrigation	Overhead irrigation	Overhead irrigation	Overhead irrigation (sprinklers)

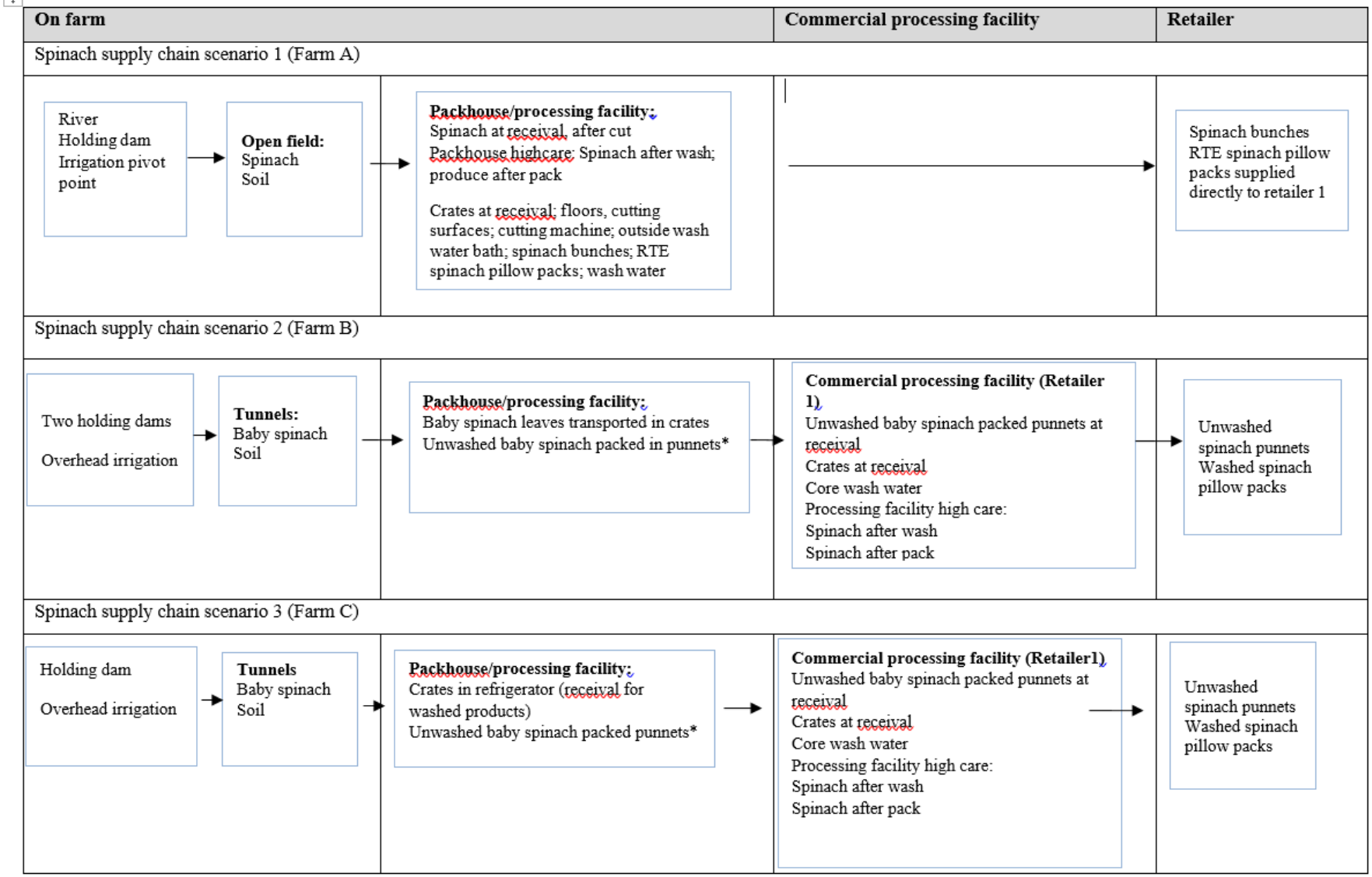

Figure 7: Flow diagrams of spinach supply chains 1-3 including the sites and points where samples were collected.

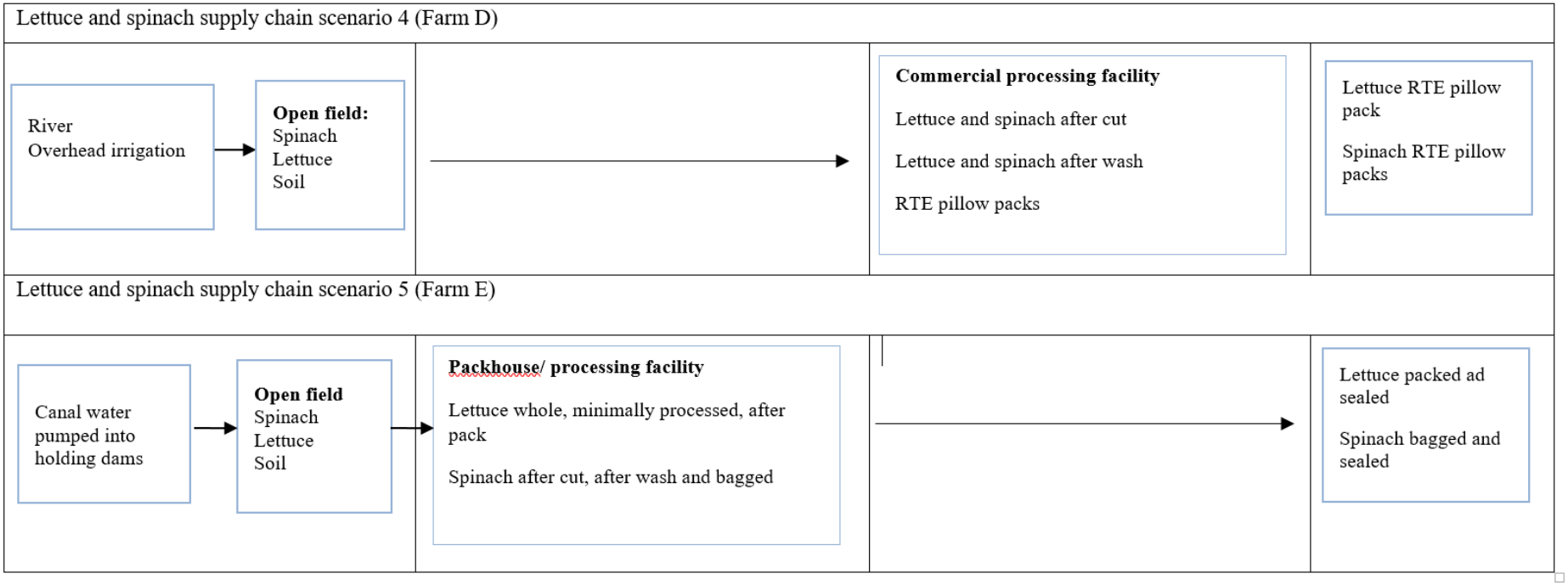

Figure 8: Flow diagrams of spinach and lettuce supply chains 4 and 5 including the sites and points where samples were collected

3.3.3 Informal vendors

Street vendors in Mamelodi and Atteridgeville

The vendors selected were in Gauteng province, which hosts over 20.4% of the South African households that reside in the informal settlements. Two sites were selected in the Tshwane Metropole; Mamelodi (Site 1) and Atteridgeville (Site 2). From each site five informal SVGGs were selected, based on the availability of products (cabbage, spinach, tomato, carrot and apples) and type of stall and proximity to large shopping centers, train station, schools, churches and taxi ranks where movement of people was high. A description of the area comprising of GPS-coordinates, temperature and relative humidity can be found in Table 8.

Table 8 Description of samples and site of street vending greengrocers in Site 1, Mamelodi

Vendor	Product	GPS coordinates	Date	Time	Temperature (°C)	Relative humidity	Rainfall (mm)
SVGG 1	Cabbage, Spinach, Tomato, Carrot and Apple	S25.698359 E28.420595	29/05/2018	10:00	21	41	0
SVGG 2	Cabbage, Spinach, Tomato, Carrot and Apple	S25,7168752 E28,334573	29/05/2018	11:00	21	41	0
SVGG 3	Cabbage, Spinach, Tomato, Carrot and Apple	25,7110391 E28,3644353	04/07/2018	09:00	10	60	0
SVGG 4	Cabbage, Spinach, Tomato, Carrot and Apple	S25,7133451 E28,3342823	27/08/2018	09:00	13	28	0
SVGG 5	Cabbage, Spinach, Tomato, Carrot and Apple	S25,7166405 E28,3414647	27/08/2018	10:00	10	28	0

Street vending greengrocer 1 (SVGG 1) The stall was located near a shopping center and was visited on the 28/05/2018 (Table 8). Behind the stall there was a rubbish dump. The apples were placed in plates. The spinach was purchased from a nearby stall, the spinach was stored in a big bin filled with water.

Street vending greengrocer 2 (SVGG 2) was located near a Denneboom mall and visited on 29/05/2018 (Table 8. The spinach was stacked, and frequently poured with water to avoid wilting. The stall was exposed to dust due to construction.

Street vending greengrocer 3 (SVGG 3) was visited on 04/07/2018 (Table 8). The spinach was stored in a metal bowl with water to keep it fresh. The cabbage was pre-cut and wrapped in clip wrap. Some fresh produce was hang on the pole in plastic bag for mixed bulk.

Street vending greengrocer 4 (SVGG 4) was visited on the 27/08/2018 (Table 8). The SVGG was located near Waltloo mall in front of a car wash and repair outlet. The spinach purchased from this market was wilted and placed too close to the floor. Fresh produce at this stall is sorted according to quality, the poor quality is given to their customers for free.

Street vending greengrocer 5 (SVGG 5). The stall was located right next to a school (Table 8). Cabbage was cut on site on a wooden material placed at least 30cm above the ground.

Table 9 Description of samples and site of street vending greengrocers in Site 2, Atteridgeville

Vendor	Product	GPS coordinates	Date	Time	Temperature (°C)	Relative humidity	Rainfall (mm)
SVGG 6	Cabbage, Spinach, Tomato, Carrot and Apple	S25,7894830 E28,0236450	18/05/17	10:00	13	47	0
SVGG 7	Cabbage, Spinach, Tomato, Carrot and Apple	S25,763167 E28,06155	23/06/17	13:00	19	18	0
SVGG 8	Cabbage, Spinach, Tomato, Carrot and Apple	S25,789438 E28,023687	03/10/17	10:00	22	52	0
SVGG 9	Cabbage, Spinach, Tomato, Carrot and Apple	S25,47103 E28,0159	01/11/17	14:30	31	18	0
SVGG 10	Cabbage, Spinach, Tomato, Carrot and Apple		01/11/17	14:30	31	18	0

Street vending greengrocer six (SVGG 6) was situated in Moshongoville (Table 9), visited on 18/05/2017 and located near a hair salon and a chicken stall where they slaughtered and cooked chickens. Fresh produce collected from this site included spinach, cabbage, carrots, tomatoes and apples (Table 9). The apples were sold loosely which was a common practice observed also in SVGG 7 and SVGG 8. The spinach was sold in bunches and stored in a bowl behind the stall.

Street vending greengrocer seven (SVGG 7) was situated in Saulsville train station (Table 9) and visited on 23/06/2017. The fresh produce was placed directly on wooden table while

others were place directly on the ground. Behind the stalls big black dustbins, these were used to store and wash the fresh produce just before the stalls are set up. Spinach was sprinkled with water to keep cool and protect it from wilting.

Street vending greengrocer Eight (SVGG 8) was visited on the 03/10/2017; the stall was in Brazzaville (Table 9) and visited on 03/10/2017. The stall was made like a house shack with the sides and the base covered with boards and metal material that were connected by thick poles. The street vendor sold fresh produce, popular snacks and some electrical appliances. Some of the fresh produce were hung on the poles while others were places directly on the floor. The tomatoes were sold loose on plates that were filled with dust.

Street vending greengrocer Nine (SVGG 9) was visited on the 01/11/2017 (Table 9). Apples, cabbage and tomatoes were sold loose with bulk apples stored in a box, tomatoes were placed in plates and the cabbage was placed on the floor.

Street vending greengrocer ten (SVGG 10) was situated in Brazzaville with samples purchased on the 01/11/2017 (Table 9). The stall was made of Zinc material as roof and cover for the sides with windows secured by security bars. The stall is located along a busy main road that is dusty and always wet with water that flows from busted pipes. The fresh produce was washed just before sampling.

3.3.4 Small-scale farms and associated vendors

Eight small-scale farms were selected in Gauteng, Mpumalanga and the North West Provinces of South Africa Table 10). Five farms (B, C, E, F and H) were located in the Brits area and the other three farms were located in the Delmas area A, D, E). Farms were selected based on the availability of morogo (especially chinensis and rape) and spinach. In addition, the farms sell these fresh produces to formal and/or informal retailers in South Africa. Farms supplying retailers in the Tshwane Metropole (Tshwane Fresh Produce Market and Atteridgeville Bakkie Vendors) included Farm B, Farm C and Farm E all located in the Brits area.

Table 10 Description of eight small-scale farm sites including fresh vegetables sampled, retailers supplied, irrigation source and method.

Sampling area	Vegetables sampled	Retailers	Farm source water	Irrigation method
Farm A	Spinach, Chinensis, Rape	Formal and informal retailers Bapsfontein	Borehole	Overhead (pivot)
Farm B	Spinach Chinensis Rape	Tshwane Market	Borehole	Overhead (pivot)
Farm C	Spinach Chinensis	Atteridgeville bakkie vendor	River	Flooding
Farm D	Spinach, Kale, Chinensis	Bapsfontein	Borehole	Overhead (sprinkler)
Farm E	Spinach, Chinensis	Tshwane Market	Borehole	Overhead (sprinkler)
Farm F	Spinach, Chinensis, Rape	Brits	Borehole	Flooding
Farm G	Spinach, Chinensis, Rape	Bapsfontein, Delmas	Borehole	Overhead (sprinkler)
Farm H	Spinach, Chinensis, Rape	Brits	Borehole	Overhead (sprinkler)

3.4 Collection and processing of fresh vegetables, water and contact surface (swab) samples from formal and informal supply chains in the Tshwane Metropole

3.4.1 Point of-sale- fresh produce sampled from farmers markets, street vendors, trolley vendors and commercial Sample collection and processing of fresh produce

Ten suppliers in retail and twenty in informal markets (ten street traders and ten mobile trolley vendors) as well as 13 stalls from two farmers' markets in Gauteng Province SA were selected for sampling. In total, 545 randomly chosen vegetable samples were purchased between September 2017 and May 2018. Depending on availability, spinach (bunches, baby leaves, or

minimally processed ready-to-eat (RTE) pillow packs) and tomatoes, from retailers, street traders, trolley vendors and farmers' markets (n=50 from each respective group), were analyzed. In addition, cucumbers (n=45), lettuce (Iceberg lettuce heads or mixed salad leaf RTE pillow packs) (n=50), and green beans (n=50) were also included from the farmers' market vendors. All samples were transported cooled and stored at 4°C until further processing within 24hrs.

Fifty grams of each spinach or lettuce sample was placed into a sterile polyethylene strainer stomacher bag containing 200 ml buffered peptone water (BPW) (3M, Johannesburg, SA) in a 1:4 weight to volume ratio. From each spinach bunch, three leaves were removed, aseptically cut, mixed, and a composite 50 g sample prepared for further analysis. Similarly, for each lettuce sample, leaves from the inner and outer layers of the lettuce heads were aseptically cut and mixed for a composite 50 g sample. A 150 g sample of tomatoes and cucumbers (composite of at least three tomatoes or cucumbers) and a 150 g sample of green beans were each placed into a sterile polyethylene stomacher bag containing 150 ml BPW in a 1:1 weight to volume ratio. Individual fresh vegetable samples was stomached for 5min at 230rpm in a Stomacher 400 circulator (Seward Ltd., London, UK).

3.4.2 Commercial supply chains

Commercial fresh vegetable/processing facilities from the farm to retail

The fresh produce replicate samples (baby spinach leaves, spinach leaves, bunches, lettuce heads, RTE pillow packs) were collected from farms in the field, at receiving and/or dispatch from the processing facilities, after cut, after wash, and after pack depending on the layout of the different farms. The final product (washed and unwashed) were also sampled at the specific retailers supplied. The following day the same baby spinach batches from the crates were sampled at receival from the Retailer 1 processing facility, after washing and packing in pillow packs as a ready-to-eat product. Unwashed spinach in the punnets and the washwater in the processing and packaging facility were also sampled, while swabs of workers' hands were also taken. Spinach and lettuce from farms where the processing facility was on the farm or in close proximity to the farm were sampled on the same day. A total number of 288 samples were collected at selected sampling points throughout the supply chains from spinach supply chain scenarios 1 to 3 from the farm to the retailer. This included soil at harvest (n=6 composite

samples); water samples at the source, irrigation point and during processing (n=72); spinach samples at harvest, during processing and at retail (n=192); and contact surface swab samples throughout production and processing of the fresh produce (n=18). All the samples were transported in cooler boxes with ice packs and processed within 24h at the University of Pretoria.

A total of 239 samples were collected comprising of lettuce (n=68), spinach (n=68), water samples (n=63) and soil samples (n=40) from the two commercial spinach and lettuce supply chains (scenario 4 and 5). Leafy greens were sampled in the field, at packhouse receival, through processing (after cut, after wash and after pack) and in the field and from the associated major retail outlets.

Three 50 g composite samples (consisting of either three lettuce heads, three baby spinach punnets (unwashed), three paper bags of baby spinach leaves sampled from the crates, three RTE-pillow packs, a spinach bunch, three lettuce heads) were prepared for each of the sampling points in the supply chains for analysis. For the large leaf spinach samples at least three leaves were used to prepare 50 g composite samples. Each sample was aseptically cut and placed into a sterile polyethylene strainer stomacher bag (Seward Ltd., London, UK) containing 200 ml 3M BPW (3M, Johannesburg) BPW in a 1:4 weight to volume ratio. Individual vegetable samples were blended for 5 min at 230 rpm in a Stomacher 400 circulator paddle blender (Seward Ltd., London).

Water (100 ml and 1 L) samples were collected in triplicate from the irrigation water sources and in the minimal processing facilities at the wash basin/washwater area. After preparing a dilution series of each of the 1 L water samples for enumeration of Enterobacteriaceae, the samples were filtered through a 0.45 µm nitrocellulose membrane (Sartorius, Johannesburg, SA), and placed into 50 ml buffered peptone water (3M BPW) (3M Food Safety, Minnesota, USA).

Soil was collected from five replicate points during harvest from the spinach production fields. A composite sample of 25 g (5 g from each replicate) were added to 225 ml 3M buffered peptone water (BPW) (3M Food Safety, Minnesota, USA).

Transystem^{TM} swabs with Amies medium (Lasec, Johannesburg) were used to collect contact surface samples. A 25cm^2 area was swabbed from crates, tables and conveyer belt surfaces respectively, in triplicate, according to the standard procedures for environmental swab sampling (Public Health England, 2014). Swabs were analysed by placing each into 9 ml BPW for the 3-4 h enrichment at 37°C prior to enrichment for presumptive ESBL/AmpC-producing Enterobacteriaceae.

3.4.3 Informal vendors

Street vendors in Mamelodi and Atteridgeville

Five fresh produce types were analysed in the study namely: apple, cabbage, carrot, spinach and tomato. The samples were collected from three townships in Gauteng. From each area five Street vending Green Grocers (SGVVs) were randomly selected based on the availability of the fresh produce. From each SVGG five fresh produce types were purchased with five subsamples per produce type to a total of 25 samples per SVGG. In total from the three areas 15 SVGGs have been visited and 375 samples were collected. A 150 g subsample of whole fresh produce (carrots, tomato and apples) consisting of equal amounts of the three units that made up the composite subsample was added to buffered peptone water (BPW) (3M, Johannesburg, South Africa) in a 1:1 weight: volume ratio (Xu *et al.*, 2015). A 1:5 weight: volume ratio was used for 50 g leafy vegetables (Xu *et al.*, 2015) also ensuring that the three units were equally represented in the composite subsample. The samples were macerated for 5 min at 230 rpm in a stomacher 400 circulator (Seward Ltd., London, UK).

3.4.4 Small-scale farmers and associated vendors

Water, soil and dark leafy green (rape, chinensis and spinach) samples were used to evaluate the presence of foodborne pathogens as well as enumeration of hygiene indicator organisms. For water samples, three subsamples (3 x 1 L and 6 x 100 ml) were collected from the water sources in the farms used to irrigate the fresh produce. Depending on the landscape of the farms, main sources of the water samples were collected from borehole inlet points, holding dams or reservoirs, pivots or sprinklers and flooding irrigation points. All the water samples were transported to the laboratory in cooler boxes with ice packs. Samples were stored at 4º C and analysed within 24h as described. After preparing a dilution series of each of the 1 L water

samples for enumeration of Enterobacteriaceae, the samples were filtered through a 0.45 µm nitrocellulose membrane (Sartorius, Johannesburg, SA), and placed into 50 ml buffered peptone water (3M BPW) (3M Food Safety, Minnesota, USA).

Rape, chinensis and spinach were sampled from each farm if and when available (if not available, Kale and Chomolia were collected). Five samples of each fresh produce crop were collected using systematic random sampling across rows in the farm field during harvest. The subsamples were made up of a composite sample of the fresh produce plants collected over the rows. The same batch of fresh produce were collected from formal and informal retailers, three subsamples of each fresh produce were purchased. Fresh produce samples were collected and transported, under sterile conditions, to the laboratory in cooler boxes with ice packs and analysed within 24h. For the large leaf spinach samples at least three leaves were used to prepare 50 g composite samples. Each sample was aseptically cut and placed into a sterile polyethylene strainer stomacher bag (Seward Ltd., London, UK) containing 200 ml 3M BPW (3M, Johannesburg) BPW in a 1:4 weight to volume ratio. Individual vegetable samples were blended for 5 min at 230 rpm in a Stomacher 400 circulator paddle blender (Seward Ltd., London).

The soil subsamples were simultaneously collected at harvest on complementary rows where the fresh produce has been sampled. The samples were collected in sterile containers and transported to the laboratory in cooler boxes with ice packs. A composite sample was then made of all five soil subsamples (5 g from each collected sample), with one sample per field. Analyses took place within 24h.

3.5 Microbiological analysis of fresh vegetables, water and contact surface (swab) samples from formal and informal supply chains

3.5.1 Enumeration of hygiene indicator bacteria and Enterobacteriaceae of fresh produce, water and contact surface samples

A tenfold dilution series of each of the 3M BPW-macerated fresh produce and 3M BPW-swab samples was prepared and plated in duplicate onto *E. coli*/coliform count plates (3M Petrifilm, 3M, St. Paul, Minnesota, USA) for hygiene indicator bacteria enumeration (coliforms, *E. coli)* following incubation for 24 at 37°C according to the manufacturer's instructions. The 100 ml

water samples collected were used for enumeration of coliforms and *E. coli* (hygiene indicator bacteria) using the most probable number (MPN) with Colilert-18 (IDEXX Laboratories Incorporated, Westbrook, ME, USA) reagents heat sealed in Quanti-Tray/2000 (IDEXX). The trays were incubated at 37° C for 24 h and inspected for chromogenic reactions and fluorescence indicating the presence of coliforms and *E. coli,* respectively. The results were recorded as log MPN *E. coli*/100 ml and log MPN coliforms/100 ml.

For Enterobacteriaceae enumeration, a 1 ml aliquot of each of 1L water samples, the 3M BPW-macerated fresh produce and 3M BPW-swab samples to prepare a tenfold dilution series). The dilutions were plated in duplicate onto VRBG (Oxoid, Basingstoke, United Kingdom Oxoid, Johannesburg, South Africa) agar plates according to SANS 21528-1:2004. The protocol and the counts were recorded as log CFU/ml of water, log CFU/g for the fresh produce and log CFU/cm^2 for the surface area samples.

3.5.2 Enrichment, detection and isolation of *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp.

Each of the prepared 3M BPW-macerated fresh produce, 3M BPW-membrane filtered 1 L water samples and 3M BPW-contact surface swab samples was incubated at 37°C for 24h. Following enrichment, *E. coli* (initially present in low numbers in water samples) were streaked (one loopful) onto Eosin methylene blue differential agar (EMB) (Merck, Darmstadt, Germany) for detection and isolation. The presence of *Salmonella* spp. was assessed using the iQ-Check Salmonella II Kit (BioRad, SA) for *Salmonella*. Once positive results were obtained, the sample was streaked onto Xylose lysine deoxycholate (XLD) agar (Biolabs, Johannesburg, SA) and *Salmonella* Brilliance agar (Oxoid, Johannesburg, South Africa). The presence of *Listeria* spp. was assessed by inoculating and incubating Fraser ½ broth plus supplement (Biorad) with the enriched BPW-sample mixtures, followed by a 25h ± 1h incubation at 30°C. Samples were then streaked onto Agar Listeria Ottavani and Agosti (ALOA) (bioMérieux, SA, France). Presumptive positive *Listeria* identities were subsequently assessed using the iQ-Check *Listeria monocytogenes* II Kit (BioRad, SA). After incubation, all colonies with different colours and morphology were isolated from the chromogenic agar and purified. Isolate identities were determined using Matrix-Assisted Laser Desorption Ionisation Time-of-Flight mass spectrometry (MALDI-TOF) (Bruker, Bremen, Germany) as described by Standing *et al.* (2013).

3.5.3 Enrichment, isolation and identification of presumptive extended-spectrum β-lactamase- and AmpC- β-lactamase-producing Enterobacteriaceae

Each of the prepared BPW-macerated fresh produce, BPW-filtered membrane water samples and BPW-contact surface swab samples were incubated for 3-4 h at 37°C after which 1 ml was added to 9 ml Enterobacteriaceae selective enrichment (EE) broth (Oxoid, Johannesburg) and incubated for 24 h at 30°C (SANS 21528-1:2004). Presumptive ESBL/AmpC-producing microorganisms were detected by streaking (10 µl) each of the enriched samples onto ChromID ESBL agar plates (bioMérieux, Midrand, SA) and incubated overnight at 30°C (Blaak *et al.*, 2014). All presumptive positive ESBL/AmpC-producing Enterobacteriaceae colonies based on colony colour, including weakly coloured colonies, on the chromogenic media were isolated and purified. Isolate identities were determined using Matrix-Assisted Laser Desorption Ionisation Time-of-Flight mass spectrometry (MALDI-TOF) (Bruker, Bremen, Germany) to the species level as described by Standing *et al.* (2013). Isolates that did not belong to the Enterobacteriaceae family were not included in further analysis.

3.5.4 Antimicrobial susceptibility testing of isolates

A selection of presumptive ESBL/AmpC-producing Enterobacteriaceae isolates, representing one unique colony color, morphology and species per sample, were selected for further study. The Kirby-Bauer disk diffusion technique was used to determine the resistance patterns of the selected strains according to the Clinical and Laboratory Standards Institute (CLSI) protocols (CLSI, 2018). All isolates were screened for ESBL production by the double-disk synergy (DDST) using cefotaxime 30 µg, cefotaxime-clavulanic acid, ceftazidime 30 µg, ceftazidime-clavulanic acid 30 µg/10 µg and cefpodoxime 10 µg, cefpodoxime-clavulanic acid 10 µg/10 µg (Mast Diagnostics, Randburg, SA). Isolates resistant to cefoxitin and cefotaxime or ceftazidime were regarded as a phenotypic indicator of AmpC production (EUCAST, 2013). Production of ESBL and AmpC enzymes were confirmed using the cefepime ESBL disc set (Cefepime 30 µg, cefepime-clavulanic acid 30 µg/10 µg) and AmpC production using the AmpC detection set (Mast Diagnostics, Randburg) (EUCAST, 2013; CLSI, 2018). According to the manufacturers' instructions *K. pneumoniae* ATCC 700603, *E. coli* NCTC 13351, and *Enterobacter cloacae* NCTC 1406 were used as positive controls and *E. coli* ATCC 25922 were included as a negative control (Mast Diagnostics).

Resistance or susceptibility of isolates were also tested using ampicillin-0 µg, augmentin-20 µg/10 µg, amoxicillin-0 µg, cotrimoxazole-1.25µg/23.75 µg, imipenem-10 µg, neomycin-10 µg, tetracycline-30 µg, gentamycin-10 µg, chloramphenicol-10 µg (Mast Diagnostics) (CLSI, 2018). Isolates resistant to three or more antimicrobial classes were regarded as multi drug resistant.

3.5.5 Detection and characterisation of β-lactamase genes and integrons

All confirmed ESBL/AmpC-producing isolates were analysed by PCR and sequencing for the presence of ESBL determinants (*bla*TEM*, bla*SHV*, bla*CTX-M*, bla*OXA) and plasmid-mediated AmpC (pAmpC) resistance genes (*bla*ACC, *bla*FOX, *bla*MOX, *bla*DHA, *bla*CIT, *bla*EBC) as well as class 1, 2, and 3 integrons (*IntI*1, *IntI*2, *IntI*3). Single colonies of each isolate were cultured aerobically under shaking conditions at 200 rpm in tryptone soy broth (MERCK, Johannesburg) for 24 h at 30°C. The cells were pelleted by centrifugation (12,500 *g* for 10 min), DNA was extracted using the Quick-gDNA Mini-Prep kit (Zymo Research, Irvine, USA) and the DNA concentration was determined using the Qubit dsDNA Broad Range Assay and a Qubit 2.0 fluorometer (Life Technologies, Johannesburg). PCR was performed using the DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Johannesburg) with specific primers and thermocycling conditions for each of the genes as described in Table 11. PCR products were sequenced using BigDye Terminator v3.1 cycle sequencing on an ABI 3500XL sequencer in forward and reverse direction (InquabaBiotec, Johannesburg). The sequences were edited with Chromas 2.6 and BioEdit sequence alignment editor software and consensus sequences were subjected to BLAST nucleotide search analysis to identify the antimicrobial resistance genes.

Table 11 Primers used for screening of broad-spectrum β-lactamase, ESBL and AmpC genetic determinants (Dallenne *et al.*, 2010) as well as integron prevalence (de Paula *et al.*, 2018) in selected Enterobacteriaceae isolated from water, fresh produce and contact surfaces

Target genes	Primer sequences	Thermocycling conditions	Expected amplicon size (bp)
bla$_{TEM}$	TEM-F: 5'-CATTTCCGTGTCGCCCTTATTC-3' TEM-R: 5'-CGTTCATCCATAGTTGCCTGAC-3'	94°C, 10min; 30 cycles of 94°C, 40s, 58°C, 40s, 72°C 1min; 72°C 7min	800
bla$_{SHV}$	SHV-F: 5'-AGCCGCTTGAGCAAATTAAAC-3' SHV-R: 5'-ATCCCGCAGATAAATCACCAC-3'		713
bla$_{OXA\text{-}1\ like}$	OXA-F: 5'-GGCACCAGATTCAACTTCAAG-3' OXA-R: 5'-GACCCCAAGTTTCCTGTAAGTG-3'		564
bla$_{CTX\text{-}M\ Group\ 8/25}$	CTX-M Gp8/25-F: 5'-AACRCRCAGACGCTCTAC-3' CTX-M Gp8/25-R: 5'-TCGAGCCGGAASGTGTYAT-3'	94°C, 10min; 30 cycles of 94°C, 40s, 60°C, 40s, 72°C 1min; 72°C 7min	326
bla$_{CTX\text{-}M\ Group\ 9}$	CTX-M Gp9-F: 5'-TCAAGCCTGCCGATCTGGT CTX-M Gp9-R: 5'-TGATTCTCGCCGCTGAAG-3'		688
bla$_{CTX\text{-}M\ Group\ 1}$	CTX-M Gp1-F: 5'-TTAGGAARTGTGCCGCTGYA-3' CTX-M Gp1-R: 5'-CGATATCGTTGGTGGTRCCAT-3'		561
bla$_{ACC}$	ACC-F: 5'-CACCTCCAGCGACTTGTTAC-3' ACC-R: 5'-GTTAGCCAGCATCACGATCC-3'	94°C, 10min; 30 cycles of 94°C, 40s, 60.5°C, 40s, 72°C 1min; 72°C 7min	346
bla$_{FOX}$	FOX-F: 5'-CTACAGTGCGGGTGGTTT-3' FOX-R: 5'-CTATTTGCGGCCAGGTGA-3'	94°C, 10min; 30 cycles of 94°C, 40s, 59.6°C, 40s, 72°C 1min; 72°C 7min	162
bla$_{MOX}$	MOX-F: 5'-GCAACAACGACAATCCATCCT-3' MOX-R: 5'-GGGATAGGCGTAACTCTCCCAA-3'		895
bla$_{DHA}$	DHA-F: 5'-TGATGGCACAGCAGGATATTC-3' DHA-R: 5'-GCTTTGACTCTTTCGGTATTCG-3'		997
bla$_{CIT}$	CIT-F: 5'-CGAAGAGGCAATGACCAGAC-3' CIT-R: 5'-ACGGACAGGGTTAGGATAGY-3'		538
bla$_{EBC}$	EBC-F: 5'-CGGTAAAGCCGATGTTGCG-3' EBC-R: 5'-AGCCTAACCCCTGATACA-3'		683
IntI1	Int1-F: 5'-GGT CAAGGATCTGGATTTCG-3' Int1-R: 5'-ACATGCGTGTAAATCATCGTC-3'	94°C, 12min; 30 cycles of 94°C, 30s, 60°C, 30s, 72°C 1min; 72°C 8min	436
IntI2	Int2-F: 5'-CACGGATATGCGACAAAAAGG-3' Int2-R: 5'-TGTAGCAAACGAGTGACGAAATG-3'		788
IntI3	Int3-F: 5'-AGTGGGTGGCGAATGAGTG-3' Int3-R: 5'-TGTTCTTGTATCGGCAGGTG-3'		600

3.5.6 DNA fingerprinting of Enterobacteriaceae isolates using ERIC-PCR analysis

Enterobacterial repetitive intergenic consensus ERIC-PCR analysis was conducted using ERIC-2 and ERIC-1R primers (Table 12) with Dream Taq Green PCR master mix (2X) (Thermo scientific, Johannesburg, South Africa). The PCR reaction was subjected to the following cycling conditions; an initial activation step at 95°C for 4 min, followed by 30 cycles of denaturing at 94°C for 30s, annealing at 40°C for 1 min and extension at 72°C for 8 min, with a final extension at 72°C for 15 min. Amplicons were visualised on a 2% agarose gel stained with Roti®-safe at 45V for 5h followed by molecular imager in conjunction with the Image LabTM software (BioRad). Banding patterns were captured, analysed and compared using BioNumerics 7.6 (Applied Maths, Saint-Marten-Latem). Percentage similarities of digitized bands were calculated using the Pearson's correlation coefficient and the unweighted pair group method with arithmetic mean, and complete linkage algorithms were used to derive a dendrogram (Du Plessis *et al.*, 2015).

3.5.7 Virulence gene profiling of *Escherichia coli* isolates

The presence of the enterohaemorrhagic virulence genes (*stx1, stx2* and *eae*) with an internal amplification control (*mdh*) was conducted using the primers outlined in Table 12 as previously described by Omar and Barnard (2010). A 25 µl PCR reaction consisted of 2x DreamTaq (Life Technologies). The PCR reaction was subjected to an initial activation step at 95°C for 15 min, followed by 35 cycles consisting of denaturing at 94°C for 45s, annealing at 55°C for 45s, extension at 68°C for 2 min and final extension at 72 C for 5 min (Omar and Barnard, 2010). Amplicons were visualised on a 2% agarose gel as previously described.

3.5.8 Assignment of *Escherichia coli* isolates to phylogenetic groups

Escherichia coli isolates were assigned to phylogroups by performing a quadruplex PCR assay based on the presence or absence of four genes (Clermont *et al.*, 2013). The genes targeted included the *chuA* gene (a heme transport gene), *yjaA* gene (function unknown, from the complete genome of *E. coli* K-12), TspE4.C2 gene (putative lipase esterase gene (and *arpA gene (unknown* function, from an *E. coli* strain associated with neonatal meningitis) genes as outlined in Table 12. A 25 µl

PCR reaction as previously described was used. The PCR reaction was subjected to an initial activation step at 95°C for 4 min, followed by 30 cycles consisting of denaturing at 94°C for 5s, annealing at 57°C (Group E) or 59°C (quadruplex and Group C) for 20s and final extension at 72°C for 5 min (Clermont *et al.*, 2013). When an isolate was classified into group A or group C or into group D or group E using the quadruplex primers described above, a second PCR was performed to confirm that the isolate belonged to group E by amplification of the 301-bp arpA gene using the ArpAgpE.f and ArpAgpE.r primers or to group C by amplification of the 219-bp trpA gene using the trpAgpC.1 and trpAgpC primers.

Table 12 Summary of genes targeted and primers used for antibiotic resistance gene detection, diarrheagenic *Escherichia coli* virulence gene detection, phylogenetic grouping of *E. coli* and ERIC-PCR analysis

Gene	Primer Sequence F / R	Size (bp)	Tm (˚C)	Ref.	Control
	Antibiotic Resistance Genes				
Aminoglycosides					
aac(6')-IB	5'-TTGCGATGCTCTATGAGTGGCTA3'/5'-CTCGAATGCCTGGCGTGTTT-3'	482	55	*	NO
strA-B	5'-TATCTGCGATTGGACCCTCTG-3'/5'-CATTGCTCATCATTTGATCGGCT-3'	538	60	†	NO
aadA1a	5'-GAGAACATACGCTTGCCTTGG-3'/5'-TCGGCGCGATTTTGCCGGTTAC-3'	198	48	†	
β Lactams (AmpC- β Lactamases) (as described in table 7)					
VEB	5'-CATTTCCCGATGCAAAGCGT-3'/5'-CGAAGTTTCTTTGGACTCTG-3'	648	60	‡	NO
IMP	5'-TTGACACTCCATTTACDG-3'/5'-GATYGAGAATTAAGCCACYCT-3'	139	55	‡	NO
VIM	5'-GATGGTGTTTGGTCGCATA-3'/5'-CGAATGCGCAGCACCAG-3'	390	55	‡	NO
Cefotaxime					
ampC	5'-GTGACCAGATATGGCCACA-3'/5'-TTACTGTAGCGCCTCGAGGA-3'	822	55,8	§	NCTC 13406
Fluoroquinolones					
qnrD	5'-CGAGATCAATTTACGGGGAATA-3'/5'-AACAAGCTGAAGCGCCTG-3'	465	50	¶	NO
qnrS	5'-GCAAGTTCATTGAACAGGGT-3'/5'-TCTAAACCGTCGAGTTCGGCG-3'	428	54	¶	NO
Penicillin					
blaZ	5'-ACTTCAACACCTGCTGCTTTC-3'/5'-TGACCACTTTTATCAGCAACC-3'	173	56	**	ATCC 43300
Phenicols					
cat I	5'-AGTTGCTCAATGTACCTATAACC-3'/5'-TTGTAATTCATTAAGCATTCTGCC-3'	547	50	††	NO
cat II	5'-ACACTTTGCCCTTTATCGTC-3'/5'-TGAAAGCCATCACATACTGC-3'	543	50	††	NO
cat III	5'-TTCGCCGTGAGCATTTTG-3'/5'-TCGGATGAGTATGGGCAAC-3'	286	50	††	NO
Quinolones					
gyrA	5'-TACACCGGTCAACATTGAGG-3'/5'-TTAATGATTGCCGCCGTCGG-3'	648	64	‡‡	NO
parC	5'-AAACCTGTTCAGCGCCGCATT-3'/5'-GTGGTGCCGTTAAGCAAA-3'	395	64	§§	
Sulfonamides					
sulI	5'-TTCGGCATTCTGAATCTCAC-3'/5'-ATGATCTAACCCTCGGTCTC-3'	822	50	††	NO
sul II	5'-CGGCATCGTCAACATAACC-3'/5'-GTGTGCGGATGAAGTCAG-3'	722		††	NO
Tetracycline					
tet(A)	5'-GCTACATCCTGCTTGCCTTC-3—'/5'-CATAGATCGCCGTGAAGAGG-3'	210	55	¶¶	NO
tet(B)	5'-TTGGTTAGGGGCAAGTTTTG-3'/5'-GTAATGGGCCAATAACACCG-3'	659	55	¶¶	NO
tet(C)	5'-CTTGAGAGCCTTCAACCCAG-3'/5'-ATGGTCGTCATCTACCTGCC-3'	418	55	¶¶	NO
tet(D)	5'-AAACCATTACGGCATTCTGC-3'/5'-GACCGGATACACCATCCATC-3'	787	55	¶¶	NO
tet(E)	5'-AAACCACATCCTCCATACGC-3'/5'-AAATAGGCCACAACCGTCAG-3'	278	55	¶¶	NO
	Diarrheagenic *Escherichia coli* virulence genes				
stx1	5'-ACACTGGATGATCTCAGTGG-3'/5'-CTGAATCCCCCTCCATTATG-3'	614	55	***	ATCC 35150
stx2	5'-CCATGACAACGGACAGCAGTT-3'/5'-CCTGTCAACTGAGCACTTTG-3'	779	55	***	ATCC 35150
eaeA	5'-CTGAACGGCGATTACGCGAA-3'/5'-GACGATACGATCCAG-3'	917	55	†††	ATCC 35150

Table 12 cont.

Gene	Primer Sequence F / R	Size (bp)	Tm (°C)	Ref.	Control
mdh	5'-GGTATGGATCGTTCCGACCT-3'/5'-GGCAGAATGGTAACACCAGAGT-3'	300	55	‡‡‡	ATCC 35150
LT	5'-GGCGACAGATTATACCGTGC-3'/5'-CGGTCTCTATATTCCCTGTT-3'	410	55	***	DSM 10973, DSM 27503
ST	5'-TTTCCCCTCTTTTAGTCAGTCAACTG-3'/5'-GGCAGGATTACAACAAAGTTCACA-3'	162	55	***	DSM 10973, DSM 27503
ial	5'-GGTATGATGATGATGATGGGC-3'/5'-GGAGGCCAACAATTATTTCC-3'	630	50	***	DSM 9028, DSM 9034
ipaH	5'-GTTCCTTGACCGCCTTTCCGATACCGTC-3'/5'-GCCGGTCAGCCACCCTCTGAGAGTAC-3'	600	60	†††	DSM 9028, DSM 9034
AA PR	5'-CTGGCGAAAGACTGTATCAT-3'/AATGTATAGAAATCCGCTGTT-3'	630	57	†††	DSM 27502
	Phylogeneric grouping of *Escherichia coli*				
chuA	5'-ATGGTACCGGACGAACCAAC-3'/5'-TGCCGCCAGTACCAAAGACA-3'	288	59	§§§	ATCC 25922
yjaA	5'-CAAACGTGAAGTGTCAGGAG-3'/5'-AATGCGTTCCTCAACCTGTG-3'	211	59	§§§	ATCC 25922
TspE4.C2	5'-CACTATTCGTAAGGTCATCC-3'/5'-AGTTTATCGCTGCGGGTCGC-3'	152	59	§§§	ATCC 25922
arpA	5'-AACGCTATTCGCCAGCTTGC-3'/5'-TCTCCCCATACCGTACGCTA-3'	400	59	§§§	ATCC 25922
Group C Phylotyping confirmation					
trpA	5'-AGTTTTATGCCCAGTGCGAG-3'/5'-TCTGCGCCGGTCACGCCC-3'	219	57	§§§	NO
Group E Phylotyping confirmation					
arpA	**5'-GATTCCATCTTGTCAAAATATGCC-3'/5'-GAAAAGAAAAAGAATTCCCAAGAG-3'**	301	59	§§§	ATCC 35150
	ERIC- PCR analysis				
ERIC-2	5'-AAG TAA GTG ACT GGG GTG AGC G-3'				
ERIC-1R	5'-ATG TAA GCT CCT GGG GAT TCA C-3'				

*: Park *et al.*, 2006; †: Sunde and Norström, 2005; ‡: Dallenne *et al.*, 2010; §: Böckelmann *et al.*, 2009; ¶: Li *et al.*, 2012; **: Martineau *et al.*, 2000; ††: Maynard *et al.*, 2004; ‡‡: Oram and Fisher, 1991; §§: Vila *et al.*, 1996; ¶¶: Ng *et al.*, 2001; ***: Omar and Barnard, 2010; †††: Aranda *et al.*, 2004; ‡‡‡: Tarr *et al*., 2002; and §§§: Clermont *et al*., 2013.

3.5.9 Virulence gene screening in *Salmonella* spp. isolates

The virulence genes targeted and primers used for screening the *Salmonella* isolates from water and fresh produce from small-scale farms and at the point-of-sale (formal and informal traders) were summarised in Table 13.

Table 13 Summary of genes targeted and primers used for virulence gene detection in *Salmonella* spp. isolates

Gene	Primer	Sequence (5' – 3')	Amplicon size	Annealing temperature	Ref.
hilA	hilA-F	5'-CGTGAAGGGATTATCGCAGT-3'	296 bp	56°C	1
	hilA-R	5'-GTCCGGGAATACATCTGAGC-3'			
invA	invA-F	5'-ACAGTGCTCGTTTACGACCTGAAT-3'	243 bp	56°C	2
	invA-R	5'-AGACGACTGGTACTGATCGATAAT-3'			
iroN	iroN F	5'-ACTGGCACGGCTCGCTGTCGCTCTAT-3'	1205 bp	66°C	3
	iroN R	5'-CGCTTTACCGCCGTTCTGCCACTGC-3'			
misL	misLF	5'-GTCGGCGAATGCCGCGAATA-3'	510 bp	55°C	4
	misLR	5'-GCGCTGTTAACGCTAATAGT-3'			
orfL	orfLF	5'-GGAGTATCGATAAAGATGTT-3'	345 bp	55°C	4
	orfLR	5'-GCGCGTAACGTCAGAATCAA-3'			
pefA	pefA-F	5'-TTGCACTGGGTGGTTCTGG-3'	485 bp	56°C	5
	pefA-R	5'-TGTAACCCACTGCGAAAG-3'			
pipD	pipD-F	5'-CGGCGATTCATGACTTTGAT-3'	400 bp	55°C	4
	pipD-R	5'-CGTTATCATTCGGATCGTAA-3'			
sefA	sefA-F	5'-GCAGCGGTTACTATTGCAGC-3'	321 bp	55°C	6
	sefA-R	5'-TGTGACAGGGACATTTAGCG-3'			
sifA	sifA F	5'-TTTGCCGAACGCGCCCCCACACG-3'	449 bp	55°C	3
	sifA R	5'-GTTGCCTTTTCTTGCGCTTTCCACCCATCT-3'			
sipA	sipA-F	5'-CCATTCGACTAACAGCAGCA-3'	449 bp	56°C	1
	sipA-R	5'-CGGTCGTACCGGCTTTATTA-3'			
sopB	sopB-F	5'-CCTCAAGACTCAAGATG-3'	1987 bp	56°C	7
	sopB-R	5'-TACGCAGGAGTAAATCGGTG-3'			
sopE	sopE-F	5'-CGAGTAAAGACCCCGCATAC-3'	362 bp	58°C	7
	sopE-R	5'-GAGTCGGCATAGCACACTCA-3'			
spvC	spvC-F	5'-ACTCCTTGCACAACCAAATGCGGA-3'	510 bp	56°C	2
	spvC-R	5'-TGTCTCTGCATTTCGCCACCATCA-3'			
spvR	spvR	5'-CCCCGGGAATTCGCTGCATAAGGTAGAAGG-3'	890 bp	57°C	4
	spvR	5'-CCCCGGGTACCATGGATTTCTTGATTAATAAA-3'			
ssrA	ssrA-F	5'-CTTACGATTACGCCATTTACGG-3'	706 bp	58°C	8
	ssrA-R	5'-ATTTGGTGGAGCTGGCGGGACT-3'			
stn	stnP1	5'-TTGTCTGCTATCACTGGCAACC-3'	617 bp	59°C	9
	stnM13	5'-ATTCGTAACCCGCTCTCGTCC-3'			

1: Fardsanei *et al*., 2018a; 2: Chiu and Ou, 1996; 3: Hughes, *et al*., 2008; 4: Gassama-Sow *et al*., 2006; 5: Heithoff *et al*., 2008; 6: Mirzaie *et al*., 2010; 7: Raffatellu *et al*., 2005; 8: Kutsukake *et al*., 2006; 9: Prager *et al*., 1995.

3.6 Description of formal and informal sampling sites and fresh vegetables collected for analysis in Cape Town metropole

3.6.1 Commercial fresh vegetable processing/packaging facility situated in Philippi, Western Cape, South Africa

Commercial fresh vegetable processing/packaging facility situated in Philippi, Western Cape, South Africa The selected packhouse has a Food Safety Audit certificate, and it supplies the processed produce to the fresh marks markets (Debbie Greeff, 2019, Food Safety Management representative, personal communication). The packhouse receives a range of fresh produce from various farms including broccoli, carrot, red cabbage, and lettuce from which coleslaw mixes are prepared. Fresh vegetables were chosen in collaboration with the owner, based on the fact that it is not subject to seasonal variability and contains more than one raw ingredient (shredded broccoli stems, cabbage and carrot). The pack store also supplies loose lettuce heads, wrapped lettuce heads as well as RTE lettuce to retailers. All fresh produce including those received from other farms go through several processing steps. The end products are supplied to the retailer 2 outlets; some are supplied to the informal markets in the packhouse vicinity. Valuable insights regarding risk reduction/increase during processing and distribution could be obtained by comparing the microbial quality of these three forms of lettuce at the various stages in the supply chain (since they will all come from a common source).

3.6.2 Informal vendors in the Cape Town Metropole

The site selection was done in with Cape Town Scientific Services (CTSS) which was essential for representative informal street vendor data collection in the Cape Town Metropolitan area. Five sites were selected by the CTSS based on information collected from the street vendors via surveys previously conducted by CTSS. According to the CTSS the fresh produce is sourced from various nearby farms or the Epping fruit and vegetable market (S.D. Ariefdien, 2017, Senior Environmental Health Practitioner, Klipfontein sub-District, South Africa, personal communication, 3 November). There were no formal food safety complaints linked to any of the five sites. All five sites have permits from the Economic Development Department and have been

trading for decades (S.D. Ariefdien, 2017, Senior Environmental Health Practitioner, Klipfontein sub-District, South Africa, personal communication, 3 November).
Location of the selected five informal vendor sites in the Cape Town metropolitan area (Table 14).

Table 14 Summary of informal vendor sites in Cape Town Metropole

Site	Area
A	Delft
B	Mitchells Plain
C	Gatesville
D	Rylands
E	Epping

3.7 Collection and processing of fresh vegetables from formal and informal supply chains in the Cape Town Metropole

3.7.1 A commercial fresh vegetable processing/packaging facility situated in Philippi, Western Cape, South Africa

Broccoli, cabbage, and carrots are used as ingredients for fresh cut coleslaw bags. RTE broccoli-coleslaw were collected both during production in the pack store, as well as before and after entering the retail distribution chain. To determine to the potential contamination points in the packhouse, broccoli stems were sampled from three points: before peeling, after peeling and washing in chlorinated municipal water, as well as after shredding (just prior to packaging). Cabbage was sampled from two processing points: after outer leaves removal before washing, and after shredding just prior to packaging. Carrot was also sampled from three points: before peeling and washing, after submerging in chlorinated municipal water, and after shredding just prior to packaging. All samples were collected in triplicate from each sampling point, and this was repeated five times, in different weeks. A total of 45 broccoli stem, 45 carrot, 18 red cabbage, and 75 lettuce samples were collected. 18 broccoli stem, 18 carrots, 18 red cabbage, 9 packhouse coleslaw bags, 9 retail coleslaw bags, and 54 lettuce samples were collected. It was decided to include washwater testing in the pack store only if the results from the first round of sampling indicate a concern.

Lettuce samples were sampled following the processing steps of each different lettuce "pack" - loose lettuce which is sold to individuals at the packhouse, pre-packaged which is supplied to retail markets, and also pillow-packs for the retail market. Lettuce sampling was done on three different occasions for each lettuce type, and each sample was collected in triplicate. All samples collected were packed in sealable plastic bags and put in a cooler box with ice cubes, and then transported to the Department of Food Science. Upon arrival, samples were stored at 4^{o}C until analysis which was done within 24h.

From each sampling point, sampling was done in triplicate. From the triplicate samples collected at each sampling point, each sample was cut in half, and then half of each of these samples was cut into smaller pieces on a sterile metal tray, with a sterile knife. A 100 g was then weighed from each of the three trays, and mixed on a separate sterile tray to form a 300 g composite sample. From the composite sample, 25 g samples were weighed in triplicate into sterile polyethylene stomacher bags. Thereafter, 225 mL of 0.1% buffered peptone water (BPW) (Merck, South Africa) was added to the bags containing 25 g samples, and stomached at 230 rpm for 2 min in a 220v Interscience Bag Mixer (SANS, 2004).

3.7.2 Informal vendors in the Cape Town Metropole

Two produce types (five samples each) were collected at each site. The one product was always lettuce and the other product was selected based on the availability at the site at that time. The other fresh produce products sampled included any of the following: cabbage, spinach, green beans, green peppers or tomatoes. In total ten samples were collected at each site. One site was sampled each week. After all five sites (Site A-E) were sampled once over a time period of five weeks, the same pattern was repeated to have a total of three repetitions for each site. Three-week time lapses were left between repetitions which resulted that a site was visited every eight weeks.

At each site, the samples were collected and placed in plastic bags. All samples were placed in a cool box and transported to the laboratory to be stored at 4˚C until analysed within 24hours. Upon arrival, the fresh produce samples were placed in individual plastic bags and given a unique randomised number. All samples were weighed out (25 g) in stomacher bags and stored at 4˚C

before analysis took place the day after the samples were collected. Before analysis, the 25 g sample was macerated in 225 mL sterilised buffered peptone water (Merck) for 2 minutes at 230 rpm in a 220V Interscience Bag Mixer.

3.8 Microbiological analysis of fresh vegetables from the formal and informal supply chains in the Cape Town Metropole

3.8.1 Enumeration of hygiene indicator bacteria and Enterobacteriaceae in water and fresh produce samples

The RAPID'E. coli 2 method that was used for the enumeration of coliforms and *E. coli* has AFNOR approval as a valid alternative to the NF ISO 4832 (standard for the enumeration of coliforms) and NF ISO 16649-2 (enumeration of β-Glucuronidase-positive *E. coli*) according to the ISO 16140 protocol for method validation. The RAPID'E. coli 2 method was used according to the manufacture's (Bio-Rad, South Africa) protocol. The 25 g sample was macerated with 225 mL of BPW and a dilution series (10-2 - 10-6) was prepared. The dilution series were plated out in duplicate (pour plate technique) using RAPID'E. coli 2 agar (Bio-Rad, South Africa) and incubated for 24 hours at 37˚C. After incubation, the plates that had a count between 30 and 300 were counted and reported. The chromogenic RAPID'E. coli 2 agar (Bio-Rad, South Africa), distinguish between coliforms and *E. coli* based on different colour reactions. The colonies displaying a blue colour were considered presumptive coliforms and the colonies displaying a pink-violet colour, presumptive *E. coli*.

The enumeration of Enterobacteriaceae was based on SANS 21528-2:2005. The same preparation process was followed as for the enumeration of *E. coli* and coliforms. The macerated fresh produce samples were plated out (pour plate technique) in duplicate on VRBG (Merck) agar followed by 24-hour incubation at 37˚C. The plates that have colony numbers between 30 and 300 were enumerated and reported (SANS, 2008).

Testing of the water for *E. coli* and coliforms was be conducted using membrane filtration (ISO 9308-1: Water Quality – Enumeration of *Escherichia coli* and coliform bacteria – Membrane filtration method for waters with low bacterial background flora.

3.8.2 Pathogen detection and isolation

The BAX® Q7 system (Hygiena) and the appropriate BAX® assay kits (Microsep) were used for the detection of *Salmonella* spp., STEC and *Listeria monocytogenes* according to the manufacturer's protocol. The appropriate BAX® system kit used for the detection of the pathogens is listed in Table 15. If the detection result was positive, the isolation process for each pathogen was followed. This PCR-based system is a rapid molecular pathogen detection system that handles both real-time and end-point assays. The BAX® system results are available 24 hours after sample incubation and makes use of internal controls with every assay to validate negative results. Positive control cultures were selected for the validation process and included *Salmonella typhimurium* ATCC 14028 as well as an *E. coli* (STEC) strain previously isolated from meat and *Listeria monocytogenes* isolated from a food processing environment (P. Gouws, 2017, Professor, Department of Food Science, Stellenbosch, South Africa, personal communication).

Table 15 The BAX® system compatible kits used for the detection of the appropriate pathogens

Pathogen being tested	**Appropriate BAX® system kit**
Shiga toxin-producing *E. coli*	Real Time *E. coli* STEC (screening *stx* & *eae*) kit
Salmonella spp.	*Salmonella* 2 assay kit
Listeria monocytogenes	*L. monocytogenes* 24E assay kit

***Listeria monocytogenes* isolation**

The RAPID'L. mono method used for the isolation of *Listeria monocytogenes* and other species has been certified by NF validation as an alternative method to the standard ISO 11290-1 (*Detection of Listeria monocytogens and other species of Listeria in all food products for human consumption and in environmental samples*) according to the ISO 16140 protocol for method validation. The RAPID'L. mono method is used according to the protocol of the manufactures (Bio-Rad, 2014). A pre-weighed 25 g sample was macerated in 225 mL Frazer broth (OXOID) enriched with half Frazer supplement (OXOID) and incubated for 24 hours at 30˚C. The sample

was streaked onto RAPID'L. mono agar (Bio-Rad) in duplicate and incubated for 24 hours at 35˚C. Any colonies displaying a black centre were considered as presumptive *Listeria monocytogenes.*

***Salmonella* spp. isolation**

The isolation of *Salmonella* spp. was done by following the protocol laid out in the Bacteriological Analytical Manual governed by the Food and Drug Administration (Andrews *et al.,* 2014). The pre-weighed 25 g sample was macerated in 225 mL BPW and incubated for 24 hours at 35˚C. After the incubation, 0.1 mL was transferred to 10 mL RV broth (OXOID) and incubated for a further 24 hours at 42˚C. This was then streaked out in duplicate onto XLD agar (Merck) and Hektoen agar (OXOID) and incubated for 24 hours at 35˚C. The small black colonies on XLD agar and dark green colonies on Hektoen agar are presumptive *Salmonella.*

***Escherichia coli (STEC)* isolation**

STEC isolation was done according to Kim *et al.* (2014) with some modifications. The 25 g samples homogenised in 225 mL BPW were incubated at 35℃ for 24 h. Following incubation, 1 mL cultured sample was transferred to 9 mL of *Escherichia coli* (EC) broth (Oxoid, South Africa) then incubated at 35oC for 24 h. After incubation, the EC broth with sample was streaked onto L-EMB agar (Oxoid, South Africa), and incubated at 35°C for 24 h. A single *E. coli* colony was transferred to 5 mL of TSB and incubated at 37°C overnight. After incubation, 800 µL was stored at -80℃ in 40% sterile glycerol. The pre-weighed 25 g sample was macerated in 225 mL BPW and incubated for 24 hours at 35℃. After the incubation, 1 mL of the sample was transferred to 9 mL EC broth (OXOID) and incubated for a further 24 hours at 35˚C. The incubated EC broth sample was streaked out in duplicate onto L-EMB agar (OXOID) and incubated for 24 hours at 35℃. The isolated colonies were tested again to confirm using the BAX® system.

3.8.3 Extended β-lactamase (ESBL)-producing Enterobacteriaceae detection and isolation

Detection of ESBL was done according Zurfluh *et al.* (2015) procedures with a few modifications to suit this study. Samples (25 g) homogenised in 225 BPW were incubated at 37℃ for 2 h. After incubation, 1 mL was transferred into 10 mL of Enterobacteriaceae enrichment (EE) broth (Merck,

South Africa), and then incubated at 37°C for 24h. A loop full was then streaked on ChromID Brilliance ESBL agar (bioMérieux, South Africa) and incubated at 37°C for 24 h. Growth on plates was considered as presumptive ESBL positive colonies. Colony colours were recorded according to the manufacturer's colour chart. The presumptive colonies were then sub-cultured into TSB (Merck, South Africa), and 800 µL was stored in 40% glycerol for further analysis.

3.8.4 Statistical analysis

The statistical analysis including both the calculation of the means, standard deviations and the construction of bar graphs were completed using Sigma Plot version 13 software. The variance estimation and precision Analysis Calculation (VEPAC) to determine the least significant differences was done by using Statistica 13.0 software. A 95% confidence interval is used to determine significant differences ($p < 0.05$).

3.9 References

ARANDA KRS, FAGUNDES-NETO U and SCALETSKY ICA (2004). Evaluation of Multiplex PCRs for Diagnosis of Infection with Diarrheagenic *Escherichia coli* and *Shigella* spp. Journal of Clinical Microbiology **42**, 5849-5853.

HETTY BLAAK H, VAN HOEK AHAM, VEENMAN C, DOCTERS VAN LEEUWEN AE, LYNCH G, VAN OVERBEEK WM and DE RODA HUSMAN AM (2014). Extended spectrum ß-lactamase- and constitutively AmpC-producing Enterobacteriaceae on fresh produce and in the agricultural environment. International Journal of Food Microbiology **168-169**, 8-16.

BÖCKELMANN U, DÖRRIES H, NEUS AYUSO-GABELLA M, DE MARÇAY MS, TANDOI V, LEVANTESI C, MASCIOPINTO C, VAN HOUTTE E, SZEWZYK U, WINTGENS T and GROHMANN E (2009). Quantitative PCR monitoring of antibiotic resistance genes and bacterial pathogens in three European artificial groundwater recharge systems. Applied and Environmental Microbiology **75**(1), 154-163.

CHIU CH and OU JT (1996). Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture- multiplex PCR combination assay. Journal of Clinical Microbiology **34**(10), 2619-2622.

CLERMONT O, CHRISTENSON JK, DENAMUR E and GORDON DM (2013). The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environmental Microbiology Reports **5,** 58-65

CLINICAL LABORATORY STANDARD INSTITUTE [CLSI] (2018). Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

DALLENNE C, DA COSTA A, DECRE D, FAVIER C and ARLET G (2010). Development set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy **65**, 490-495 Https://doi: 10.1093/jac/dkp498).

DU PLESSIS EM, DUVENAGE F and KORSTEN L (2015). Determining the potential link between irrigation water quality and the microbiological quality of onions by phenotypic and genotypic characterization of *Escherichia coli* isolates. Journal of Food Protection, **78**, 643-651.

DE PAULA ACL, MEDEIROS JD, DE AZEVEDO AC, CHAGAS J, DE ASSIS M, DA SILVA VL and DINIZ VG (2018). Antibiotic resistance genetic markers and integrons in white soft cheese: aspects of clinical resistome and potentiality of horizontal gene transfer. Genes **9**:106.

EUCAST (2013). EUCAST guidelines for detection of resistance mechanisms and specific resistance of clinical and/or epidemiological importance'. European Society of Clinical Microbiology and Infectious Diseases **1**, 1-43.

FARDSANEI F, SOLTAN DALLAL MM, DOURAGHI M, MEMARIANI H, BAKHSHI B, ZAHRAEI SALEHI T and NIKKHAHI F (2018). Antimicrobial resistance, virulence genes and genetic relatedness of *Salmonella enterica* serotype Enteritidis isolates recovered from human gastroenteritis in Tehran, Iran. Journal of Global Antimicrobial Resistance, **12**, 220-226.

GASSAMA-SOW A, WANEAA, CANU NA, UZZAU S, AIDARA-KANE A and RUBINO S (2006). Characterization of virulence factors in the newly described *Salmonella* enterica serotype Keurmassar emerging in Senegal (sub-Saharan Africa). Epidemiology and Infection **134**(4), 741-743.

HUGHES LA, SHOPLAND S, WIGLEY P, BRADON H, LEATHERBARROW AH, WILLIAMS NJ, BENNETT M, DE PINNA E, LAWSON B, CUNNINGHAM AA and CHANTREY J (2008). Characterisation of *Salmonella* enterica serotype Typhimurium isolates from wild birds in northern England from 2005-2006. BMC Veterinary Research **4**, 4.

HEITHOFF DM, SHIMP WR, LAU PW, BADIE G, ENIOUTINA EY, DAYNES RA, BYRNE BA, HOUSE JK and MAHAN MJ (2008). Human *Salmonella* clinical isolates distinct from those of animal origin. Applied and Environmental Microbiology **74**(6), 1757-1766.

KUTSUKAKE K, NAKASHIMA H, TOMINAGA A and ABO T (2006). Two DNA invertases contribute to flagellar phase variation in *Salmonella* enterica serovar typhimurium strain LT2. Journal of Bacteriology, **188**(3), 950-957.

LI J, WANG T, SHAO B, SHEN J, WANG S and WU Y (2012). Plasmid-Mediated quinolone resistance genes and antibiotic residues in wastewater and soil adjacent to swine feedlots: Potential transfer to agricultural lands. Environmental Health Perspectives **120**(8), 1144-1149.

MARTINEAU F, PICARD FJ, LANSAC N, MÉNARD C, ROY PH, OUELLETTE M and MICHEL G (2000). Correlation between the resistance genotype determined by multiplex PCR assays and the antibiotic susceptibility patterns of Staphylococcus aureus and Staphylococcus epidermidis correlation between the resistance genotype determined by multiplex PCR assay. Antimicrobial Agents Chemotherapy **44**(2):231-238.

MAYNARD C, BEKAL S, SANSCHAGRIN F, LEVESQUE RC, BROUSSEAU R, MASSON L, LARIVIÈRE S, HAREL J and LARIVIE S (2004). Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. International Journal of Clinical Microbiology **42**(12), 5444-5452.

MIRZAIE S, HASSANZADEH M and ASHRAFI I (2010). Identification and characterization of *Salmonella* isolates from captured house sparrows. Turkish Journal of Veterinary and Animal Sciences **34**(2), 181-186.

NG LK, MARTIN I, ALFA M and MULVEY M (2001). Multiplex PCR for the detection of tetracycline resistant genes. Molecular Cellular Probes **15**(4):209-215.

PRAGER R, FRUTH A and TSCHÖPE H (1995). *Salmonella* enterotoxin (*stn*) gene is prevalent among strains of *Salmonella enterica*, but not among *Salmonella bongori* and other Enterobacteriaceae. FEMS Immunol Medical Microbiology **12**(1), 47-50.

OMAR KB and BARNARD TG (2010). The occurrence of pathogenic *Escherichia coli* in South African wastewater treatment plants as detected by multiplex PCR. Water South Africa **36**, 172-176.

ORAM M and FISHER LM (1991). 4-Quinolone resistance mutations in the DNA gyrase of *Escherichia coli* clinical isolates identified by using the polymerase chain reaction. Antimicrobial Agents Chemotherapy **35**(2), 387-389.

PARK CH, ROBICSEK A, JACOBY GA, SAHM D and HOOPER DC (2006). Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemotherapy **50**(11), 3953-3955.

RAFFATELLU M, WILSON RP, CHESSA D, ANDREWS-POLYMENIS H, TRAN QT, LAWHON S, KHARE S, ADAMS LG and BÄUMLER AJ (2005). SipA, SopA, SopB, SopD, and SopE2 contribute to *Salmonella* enterica serotype typhimurium invasion of epithelial cells. Infection and Immunity **73**(1), 146-154.

SOUTH AFRICAN NATIONAL STANDARDS/)/ INTERNATIONAL STANDARDS ORGANIZATION (ISO) (2004). Microbiology of food and animal feeding stuffs — Horizontal methods for the detection Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae-Part 1: Detection and enumeration by MPN technique with pre-enrichment.

STANDING TA, Du PLESSIS E, DUVENAGE S and KORSTEN L (2013). Internalisation potential of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica* subsp. *enterica* serovar Typhimurium and *Staphylococcus aureus* in lettuce seedlings and mature plants. Journal of Water and Health **11**, 210-223.

SUNDE M and NORSTRÖM M (2005. The genetic background for streptomycin resistance in *Escherichia coli* influences the distribution of MICs. Journal of Antimicrobial Chemotherapy **56**(1):87-90.

TARR CL, LARGE TM, MOELLER CL, LACHER DW, TARR PI, ACHESON DW and WHITTAM TS (2002). Molecular characterization of a serotype O121: H19 clone, a distinct Shiga toxin-producing clone of pathogenic *Escherichia coli*. Infectious Immunity **70**(12), 6853-6859.

VILA J, RUIZ J, GOÑI P and JIMENEZ DE ANTA MT (1996). Detection of mutations in parC in quinolone-resistant clinical isolates of *Escherichia coli.* Antimicrobial Agents Chemothera **40**(2),491-493.

CHAPTER 4

RESULTS, DISCUSSION AND CONCLUSIONS

4.1 Questionnaires South African consumers' food safety knowledge, attitudes and practices, fresh vegetables consumed in Tshwane and Cape Town Metropoles

4.1.1 Questionnaire A: South African consumers' food safety knowledge, attitudes and practices, fresh vegetables consumed in Tshwane and Cape Town Metropoles

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A total of 159 consumers responded about their food safety knowledge, attitudes and practices of consumption of fresh vegetables. This included 83 (52%) residing in the Western Cape and 76 (48%) from the Tshwane Metropole in Gauteng Province. In total, 95 (60%) of the consumers responded as male and 64 (40%) as female. The respondent age groups ranged from younger than 25 years to older than 65 years with the majority of the respondents aged between 56-65 years old (Table 16).

Ninety-one percent of consumers knew that *Salmonella* is a harmful bacteria that causes food poisoning, 93% knew that bacteria multiplies rapidly at room temperature and 93% knew that cutting boards and cooking utensils should be washed thoroughly with hot soapy water (Table 17).

Table 16 Demographic information of consumers

Characteristic	%
Residents residing in Metropoles (n=159)	
Pretoria – Gauteng Province	52%
Cape Town – Western Cape Province	48%
What is your gender? (n=159)	
Male	60%
Female	40%
What is your age at your last birthday? (n=159)	
<25	1%
26-35	9%
36-45	15%
46-55	25%
56-65	29%
>65	20%

Table 16 cont.

Characteristic	%
What is your highest level of Education? (n=159)	50%
Graduate (Degree or Diploma)	18%
Complete secondary schooling (passed grade 12/standard 10)	1%
Undergraduate (currently busy with after school graduate studies)	15%
Honours Graduate	12%
Masters graduate	1%
Unclassified	2%
Doctors graduate	1%

Table 17 General food safety knowledge (In your opinion, how knowledgeable are you about food safety?) of consumers

Questions	True %	False %	Uncertain %
Foodborne illnesses are diseases that are passed on to people by harmful bacteria that are present in contaminated food.	94%	2%	4%
Cross contamination is the passing of harmful substances or bacteria to food from food or from dirty equipment, utensils or hands.	97%	2%	1%
The temperature danger zone at which harmful bacteria will flourish is 0-15˚C.	16%	59%	25%
Cross contamination is the main cause of food poisoning.	64%	20%	16%
Harmful bacteria multiply quickly at room temperature.	93%	3%	4%
Cutting boards and cooking utensils should be washed thoroughly with hot soapy water.	98%	2%	0%
Salmonella is a harmful bacteria which causes food poisoning.	91%	2%	7%
Nausea and vomiting are common symptoms when you have food poisoning.	97%	2%	1%

Fifty-eight percent of respondents were neutral about how other people perceived their food safety knowledge, while 53% reported that they were knowledgeable about the causes of food poisoning. As far as storage of food to prevent spoilage of food and cleaning of work surfaces to prevent bacterial growth are concerned, 61% and 67% respectively of the respondents reported that they know what the correct practices are (relevant information highlighted in Table 18).

On average 94% of respondents felt that personal hygiene of people that work with food can prevent foodborne illness and that ensuring food safety lies with the food handlers. Ninety-seven percent were willing to learn about food safety and to change their food handling practices (Table 19).

Table 18 Food safety knowledge of the consumer in comparison to other people (In your opinion, how knowledgeable are you about food safety?)

Questions	Strongly Disagree	Disagree	Neutral	Agree	Strongly Agree
Compared to other people, I know more about food safety.	2%	8%	58%	29%	4%
People who know me consider me to be a food safety expert.	3%	26%	58%	11%	1%
Compared to other people, I know more about how to assess the safety of food.	1%	16%	50%	30%	2%
I am knowledgeable about the causes of food poisoning.	1%	11%	31%	53%	4%
I am knowledgeable about how to store perishable foods (spoil easily) correctly to prevent food poisoning.	0%	7%	24%	61%	8%
I am knowledgeable about how to clean work surfaces correctly to prevent bacterial growth.	1%	1%	10%	67%	21%

Table 19 Food safety thoughts of consumers (How do you feel about the following?)

Questions	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
Good personal hygiene of those that work with food can prevent foodborne illness.	0%	1%	5%	45%	49%
It is the responsibility of all food handlers to ensure that food is safe to eat.	0%	0%	4%	30%	65%
I am willing to change my food handling behaviours when I know they are incorrect.	0%	0%	3%	38%	59%
I am willing to learn more about food safety.	0%	0%	3%	42%	55%
It is more important to have tasty food than safe food.	57%	34%	6%	0%	3%
I select a place to buy food from based on its reputation for cleanliness.	0%	1%	10%	47%	42%
Washing my hands before touching food reduces the risk of spreading harmful bacteria.	0%	0%	3%	33%	64%
I can reduce the risk of foodborne illness by thoroughly washing areas where foods are prepared beforehand.	1%	0%	3%	33%	64%
Food products purchased from a trustworthy retailer are always safe.	13%	39%	32%	13%	3%
Branded food products are always safe.	16%	58%	22%	4%	0%
Ready-made foods purchased from retailers are prepared in a clean environment.	6%	27%	57%	9%	1%
Ready-made foods purchased from retailers cannot make me sick.	34%	55%	9%	1%	%
The ‘’best before date’’ is an indication of whether food is safe to eat or not.	4%	23%	13%	50%	9%
The quality of food products in general keeps improving in South Africa.	5%	28%	42%	23%	3%
In recent years, my confidence in fresh produce food safety has increased.	4%	30%	40%	22%	3%
I am concerned about the safety of food products in general.	1%	8%	23%	53%	15%
Food products in general have never been as safe as they are nowadays.	9%	31%	41%	17%	2%

Fifty percent of respondents indicated that they were fairly confident about the safety of food originating from retailers Table 20. Second highest confidence was in food from restaurants (43%), followed by food markets (35%), farm stalls (28%), fast food outlets (22%), imported food (22%) and least confidence in street food (3%). Fifty-five percent of consumers reported that they had no confidence in street food (Table 20).

Between 50% and 70% respondents were concerned about potential food contaminants including rodent dropping, pesticides, mercury, lead, bacteria, human excretions and dirty dishcloths (Table 21).

Table 20 Consumer confidence in suppliers (How confident are you about the safety of food originating from the following sources?)

Questions	Highly confident	Fairly confident	Uncertain	Not very confident	Not confident at all
Fast food outlets	2%	22%	23%	14%	14%
Restaurants	3%	43%	25%	25%	5%
Farm stalls	2%	28%	40%	23%	7%
Imported	1%	22%	43%	25%	9%
Retailers	1%	50%	35%	25%	4%
Food markets	3%	35%	36%	9%	3%
Street food vendors	1%	3%	14%	23%	55%

Table 21 Food safety concerns of consumers (How concerned are you about food contamination with the following?)

Questions	Highly concerned	Fairly concerned	Uncertain	Not very concerned	Not concerned at all
Insect and rodent droppings	56%	34%	5%	5%	0%
Pesticides	53%	35%	7%	4%	1%
Mercury	51%	28%	11%	10%	0%
Lead	52%	28%	10%	10%	0%
Irradiation	38%	30%	9%	14%	9%
Bacteria	65%	34%	1%	0%	0%
Human excretion	71%	21%	6%	2%	0%
Dirty dishcloths	59%	36%	3%	1%	0%

Respondents were most confident in the safety of fresh produce purchased from Woolworths (87%), followed by Pick n Pay (72%), Spar (69%), Checkers (70%), Food Lovers Market (58%) and least confident in Shoprite (44%) (Table 22).

As far as the safety of fresh whole and washed prepackaged vegetables are concerned 67% of respondents indicated that they were fairly confident and 15% were highly confident. As far as Greek salad, coleslaw and roasted vegetable salads are concerned approximately 37% respondents indicated that they were uncertain about the safety of the products are concerned, while between 32% and 40% were fairly confident in the product safety (Table 23).

Table 22 Food safety confidence of consumers (How confident are you about the safety of fresh produce in general purchased from the following retailers?)

Questions	Highly confident	Fairly confident	Uncertain	Not very confident	Not confident at all
Checkers	8%	62%	17%	13%	1%
Food Lovers Market	8%	50%	26%	12%	4%
Pick n Pay	9%	63%	18%	9%	1%
Shoprite	4%	40%	31%	18%	7%
Spar	11%	58%	21%	8%	2%
Woolworths	35%	52%	9%	3%	1%

Table 23 Food safety confidence of consumers (How confident are you about the safety of the following food items?)

Questions	Not confident at all	Not very confident	Uncertain	Fairly confident	Highly confident
Fresh whole vegetables	0%	7%	11%	67%	15%
Washed prepackaged vegetables	0%	7%	11%	67%	15%
Ready-prepared mixed salads, i.e.					
Greek salad	4%	19%	37%	36%	4%
Coleslaw	6%	23%	38%	32%	1%
Roasted vegetable salad	3%	15%	37%	40%	5%
Beetroot salad	11%	48%	3%		5%

At home consumers (76%) immediately store perishable food from retailers in the fridge after purchasing. The percentage of consumers that wash fresh produce, before eating or cooking them and irrespective if the skin is eaten or not ranged between 50-60%. Hands and cooking utensils, cutting boards are washed regularly (62% to 68% respondents) (Table 24).

The retailer of choice for safe food purposes was Woolworths (42%), followed by Pick n Pay at 25%, Spar, Checkers, Food Lovers Market and Shoprite (Table 25).

Seventy-nine percent of respondents indicated that the manufacturers/ producers and food safety inspectors should definitely be responsible for making sure that food is safe. This was followed by the respondents themselves at 74% that should accept responsibility for keeping food safe, retailers (73%), Department of Health (69%), government (51%) and farmers (42%) (Table 26).

Table 24 Food handling practices of consumers (How often do you perform the following food handling practices?

Questions	Always	Frequently	Sometimes	Rarely	Never
AT HOME:					
Store perishable foods bought from the retailer immediately in the refrigerator.	76%	16%	1%	6%	0%
Use perishable foods after the expiration date.	3%	6%	36%	29%	26%
Use non-perishable foods after the expiration date.	4%	15%	31%	31%	18%
Keep fruits and vegetables separate from raw meat in the refrigerator.	75%	18%	4%	3%	1%
Check the temperature of refrigerators/freezers regularly to prevent food from going off.	53%	26%	6%		4%
Wash fresh fruits and vegetables even if the skin is not eaten.	50%	26%	8%	14%	3%
Wash fresh fruits and vegetables before cooking them.	57%	25%	4%	11%	3%
Wash fresh fruits and vegetables before eating them.	59%	26%	3%	12%	0%
Wash hands with soap and water after handling raw foods.	64%	26%	2%	8%	0%
Clean food preparation areas in the kitchen with hot soapy water.	62%	25%	1%	12%	0%
Wash cutting boards/utensils before preparing raw foods.	68%	16%	4%	11%	1%
Use the same work surface for raw and cooked meat.	62%	25%	1%	12%	0%
Consult the label for storage conditions of food items.	68%	16%	4%	11%	1%
Store raw food items separate from cooked food items.	2%	9%	19%	17%	52%

Questions	Always	Frequently	Sometimes	Rarely	Never
AT THE RETAILER:	1	2	3	4	5
Examine food packaging to check for damages before purchasing.	55%	34%	7%		1%
Consult the expiry date of food products to decide if it is safe to purchase.	58%	30%	9%		2%
Purchase only branded food products thinking they are the safest.	5%	20%	41%		13%
Leave perishable foods in the car for more than an hour after purchasing it at the retailer.	2%	3%	10%		49%
Choose a clean trolley/basket for shopping.	44%	38%	10%		1%
Place fresh vegetables you won't peel or cook before eating directly into the trolley/basket.	44%	38%	10%		1%
Re-use the same plastic shopping bags for food purchases.	7%	23%	21%		31%
Use insulated cooler bags to carry perishable foods home in from the store.	11%	12%	25%		30%
Shop for non-perishable foods first and leave the perishable foods for last.	23%	24%	25%		13%

Table 25: Retailer preference of consumers (Who is your retailer of choice for safe food purchases?)

Retailer	Consumer preference (%)
Woolworths	42%
Pick n Pay	25%
Checkers	12%
Spar	14%
Food Lovers Market	4%
Shoprite	2%

Table 26 Food safety assurance responsibility (In your opinion, who is responsible for making sure that food is safe to eat?)

	Definitely	Definitely not	To some extent	To a lesser extent	Uncertain
Government	51%	4%	35%	6%	4%
Farmers	42%	0%	47%	7%	4%
Manufacturers/producers	79%	0%	21%	0%	0%
Retailers	73%	0%	23%	3%	1%
Department of Health	69%	3%	22%	3%	4%
Food Safety inspectors	79%	1%	15%	3%	2%
Myself	74%	1%	23%	2%	1%

Questionnaire results showed that between 86% and 99% of the respondents eat all the vegetables listed, while morogo/ African leafy and kale were not as popular, consumed and 24% and 32% respondents respectively. As expected lettuce and cucumbers were consumed raw by 97% and 95% of respondents indicated in the survey. Butternut, pumpkin, sweet potato and potatoes were popular vegetables consumed by between 86% and 99% of respondents, mostly cooked. However, butternut (5% respondents) and sweet potatoes (6% respondents) were reported to be consumed both raw and cooked (Table 27).

Table 27 Fresh produce preference of consumers (which vegetables and fruit from the list below do you typically buy; do you eat it raw, cooked or both?

Vegetable consumed	Eat	Don't eat	Cooked	Raw & cooked	Raw
Cabbage	89%	11%	28%	56%	5%
Carrot	99%	1%	10%	86%	3%
Green beans	93%	6%	65%	28%	1%
Spinach	91%	9%	70%	19%	1%
Morogo/African leafy vegetables	24%	76%	14%	3%	7%
Lettuce	97%	3%	3%	3%	91%
Kale	32%	68%	16%	7%	9%
Cucumber	95%	5%	1%	4%	90%
Vegetable consumed	**Eat**	**Don't eat**	**Cooked**	**Raw & cooked**	**Raw**
Sweet corn	92%	8%	72%	14%	5%
Peas	92%	8%	63%	25%	5%
Onions	99%	1%	27%	69%	3%
Butternut	93%	6%	82%	5%	6%
Beetroot	89%	11%	67%	17%	4%
Pumpkin	86%	14%	84%	1%	0%
Sweet potato	95%	5%	89%	6%	0%
Potatoes	99%	1%	97%	1%	0%
Cauliflower	94%	6%	70%	25%	0%
Broccoli	92%	8%	70%	21%	0%
Green pepper	91%	9%	14%	65%	11%
Tomatoes	99%	1%	2%	84%	13%

4.1.2 Questionnaire B: Food safety knowledge, attitude and practices among consumers in the informal settlements of Gauteng Province, South Africa

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Demographic information of consumers in the informal settlements

Proportionally there were more female (67.04%) than male (32.06%) participants in the study. More than 93.82% of the participants were South African, while approximately 40% reported were permanent residents of Gauteng Province. The majority of people who were from outside Gauteng Province were from Limpopo Province. Only 6% of the population were from foreign countries including Zimbabwe, Lesotho, Malawi and Kenya. The age range was from 18 to 63 years. The largest age groups were 20-29 and 30-39 years of age, (Table 28). More than 82% (n=203) of the participants had education to and above grade 12, with 38% (n=94) reported to have some level of tertiary studies (Table 28). More than 60% of the participants were responsible for buying or cooking of fresh produce. Approximately 65% (n=85) of the people earn between R0.00 and R4990.00, with 55.29% of families reporting have 4 to 14 members.

Food safety knowledge of consumers in the informal settlements

The majority of the participants (65% and above) in the current study were aware of foodborne pathogen (*Salmonella* spp.), and the type of symptoms. A large proportion (79%) acknowledged that cross contamination is the main cause of foodborne illnesses and that separation of raw and cooked food is essential. Over 77% of the participants knew that fresh produce can be contaminated by bacteria and that microorganisms can multiply rapidly at room temperature (Table 29). Although over 70% of the people knew that leftover cooked food kept at room temperature for more than six hours could cause food poisoning, More than 82% of the participants reported that utensils should be washed toughly before they are used or in between preparing meat and fresh produce. However, approximately one third indicated that washing hands before handling food is not essential.

Table 28 Demographic information of consumers

Characteristic	Response	
	N	%
What is your gender? (n=262)		
Male	84	32.06
Female	178	67.94
What is your age at your last birthday? (n=218)		
18-19	21	9.63
20-29	85	38.99
30-39	66	30.28
40-49	34	15.60
50-69	12	5.50
What is your highest level of Education? (n=248)		
Grade 7	6	2.42
Lower than matric	37	14.92
Grade 12	109	43.95
Degree/diploma	64	25.81
Post-graduate qualification	32	12.90
Who mainly purchases fresh produce at your house? (n=243)		
Myself	149	61.32
Someone else	94	38.68
Who mainly cooks food at your house? (n=249)		
Myself	159	63.86
Someone else	90	36.14
What is the monthly household income? (n=132)		
R0-990	18	13.64
R1000-4990	67	50.76
R5000-9900	18	13.64
R10000-50000	29	21.97
How many people reside in your home and are dependent on the income? (n=252)		
1-3	117	46.43
4-6	112	44.44
7-9	20	7.94
10-14	3	1.19

Table 28 cont.

Characteristic	Response	
	N	%
What is your country of origin, If South Africa please specify your native province (n=259)?		
Gauteng	106	40.93
Limpopo	84	32.43
Eastern Cape	23	8.88
Free State and Kwa Zulu Natal	8	3.01
Northern Cape and North West	6	2.32
Mpumalanga and South Africa	16	6.18
Foreign countries	16	6.18

Table 29 Food safety knowledge of consumers (What do you know about food safety?)

Questions	True	False	Uncertain
Foodborne illnesses are diseases that are passed on to people by harmful bacteria/germs that are present in contaminated food.	80.53	2.29	17.18
Cross contamination is the passing of harmful substances or bacteria/germs from food to food or from dirty equipment, utensils or hands.	86.59	2.30	11.11
Cross contamination is the main cause of food poisoning.	79.01	8.40	12.60
Fresh produce can be contaminated with bacteria/germs that cause disease.	77.48	12.60	9.92
Harmful bacteria/germs multiply quickly at room temperature.	78.93	12.26	8.81
It is not important to wash hands before handling food.	33.97	64.50	1.53
Insects such as cockroaches and flies might transmit foodborne pathogens.	76.72	11.07	12.21
Cutting boards and cooking utensils should be washed thoroughly with hot water.	88.55	4.96	6.49
The same cutting board can be used for raw meat and fresh produce provided it is clean.	82.44	9.54	8.02
Raw food and cooked food need to be stored separately	89.69	3.82	6.49
Eating covered leftover cooked food, kept at room temperature for more than 6 hours, is at high risk to cause food poisoning.	70.61	13.36	16.03
Fresh produce can be stored at 20°C for more than 24 hours.	74.81	8.02	17.18
Cooked food can be kept at room temperature for more than two hours.	68.70	17.18	14.12
Salmonella is a harmful bacteria/germ that can cause foodborne illnesses.	70.23	03.05	26.72
All *Escherichia coli* are harmful bacteria that can cause foodborne illnesses.	66.41	06.11	27.48
Vomiting, diarrhea are symptoms of food poisoning.	87.79	04.96	7.25

Food safety attitude of consumers in the informal settlements

The majority of the participants had a good general attitude towards food safety, with approximately 80% showing concern about the safety of their fresh produce. More than 90% believe that food handlers have a significant role in ensuring the safety of food, and that good hygiene is important in order to prevent the spread of foodborne pathogens. Similarly, 96% of the consumers believed that washing hands and preparing fresh produce in a clean area can reduce the risk of spread of foodborne pathogens or illnesses (Table 30). Majority of the consumers (71%) wrongly believed that fresh produce kept outside the fridge overnight is unsafe for consumption.

Approximately 70% of the consumers reported that their confidence in fresh produce have increased in recent years. Eighty seven percent reported that the quality of the product is a priority as compared to the price. More than 68% of the participants reported that fresh produce from street vendors were of good quality. However, when consumers were asked about the safety of the fresh produce purchased from formal retailers as compared to informal greengrocers the responses were different. Approximately 50% of the consumers believed that fresh produce from formal retailers were safer. About 21% of the consumers believed that fresh produce from greengrocers were safer with 28% of the people not sure of which supplier is better. Consumers (60%) trust that food products are safer now than in the past with 69% reporting that their confidence in fresh produce has increased (Table 30).

Table 30 Food safety attitude of consumers ('What are thoughts about food safety')

Questions	Disagree %	Neutral %	Agree %
Good personal hygiene of those that work with food can prevent foodborne illness.	2.73	7.42	89.84
It is the responsibility of all food handlers to ensure that food is safe to eat.	2.33	6.23	91.44
Quality is more important than price.	4.69	8.20	87.11
Washing my hands before touching food reduces the risk of spreading harmful bacteria/germs.	1.56	1.95	96.50
Preparing food in a clean area can reduce the risk foodborne illness.	3.11	5.06	91.83
Food sold at the green grocers are of good quality.	5.86	25.39	68.75
I'm concerned about the safety of fresh produce in general.	1.95	15.56	82.49
I think it is unsafe to leave fresh produce outside the fridge overnight.	12.11	16.40	71.48
Do you think fresh produce sold at retailers are safer than at green grocers or markets?	20.62	28.40	50.97
In recent years, my confidence in fresh produce food safety has increased.	10.94	20.31	68.75
Food products in general have never been as safe as they are nowadays.	13.57	25.97	60.47

Food safety practices of consumers

Food safety practices of all the participants were similar, irrespective of age, gender and educational level. Ninety-six percent of the consumers reported that they wash hands before handling fresh produce. However, there was a large variation of how and why people wash hands, with only 48% reporting to wash hands with soap in order to remove bacteria (Table 31).

Only 3.83% people responded that they do nothing before they handle fresh produce. It was observed that most people (78%) who have higher education prefer to wash their fresh produce. In contrast, 48% of the people who have education lower than grade 12 that indicated that they do not wash the fruit if they do not eat the peel (Table 31).

Of the 258 participants only 64% (n=162) responded that they use the same cutting board for fresh produce and raw meat. From 162 people, only 20% reported to use soap to wash the cutting board before they prepare fresh produce or ready-to-eat foods. Meanwhile, approximately 62% reported that they just rinse the cutting board with water before cutting fresh produce after preparing raw meat (Table 31).

The results on the choice of vendor showed that consumers considered quality, hygiene and affordability over access to the markets and fresh produce. Furthermore, consumers in the study reported that they buy most of their fresh produce from formal retailers (62.35%). With the most popular retailers being Pick n Pay (23.92%) and Shoprite (20%). However, the proportion of consumers who purchase fresh produce from the street vendors (37.65%) are higher than those reported for these retailers individually. In addition, 39% and 42% (n=258) of the respondents use public transport or walk to get to the market where they purchase the fresh produce (Table 30). Sixty-four percent of the people who buy fresh produce from street vendors walk, while those who buy from formal retailers either use public transport or private cars (Table 30). Consumers are not concerned about the availability of fresh produce. Overall, in the study, consumers choose the vendor for fresh produce based on quality and affordability (Table 30). In addition to buying fresh produce, consumers reported to store fresh produce in a refrigerator (81.40%) loose without packaging (47.56%). Sixty percent of the consumers reported to buy fresh produce that is enough for a week or a month. Similarly, 67.94% reported that they store keep fresh produce at home/ for one to six days or even two weeks. Sixty-two% of the people who reported to keep food for one to six days also stated that they buy only enough fresh produce for a week.

Over 96% (n=254 out of 260) of the consumers in the current study had access to a toilet. Eighty two percent consumers in the current study responded that they use soap and running water to wash hands after using the toilet.

Respondents (25%) reported that they have problems with overflowing sewage near their homes for at least once a week to a few times a year. Shockingly, 14% of the consumers reported that they have an issue with overflowing sewage daily. Approximately 50% of the people had problems with waste disposal next to their home with waste prevalent on weekly basis (Table 30).

Table 31 Food Safety practices of consumers (What do you do regarding Food Safety?)

Practices	%
What do you typically do before handling fresh produce? (n= 261)	
I don't do anything before handling fresh produce	3.83
I wash my hands with water to remove dirt	25.67
I wash my hands with water and soap to remove dirt	22.22
I wash my hands with water and soap to prevent the spread of bacteria	48.28
Do you always wash fruits and vegetables before preparation even if the skin is not eaten? (n=261)	
No	19.54
No, since I don't eat the skin	6.13
Yes, I rinse with water to remove dirt	33.33
Yes, I wash with water to remove bacteria on the surface	41.00
Where do you buy fresh produce? (n= 255)	
Pick n Pay	23.92
Spar	11.76
Shoprite	20.00
Checkers	6.67
Fruit and vegetable stall (Street vendor)	37.65
How do you decide on where to buy fresh produce? (n= 258)	
Hygiene (How clean the place and is the seller)	24.03
Affordability of the fresh produce	30.23
Quality of the fresh produce	35.27
Accessibility of the market (distance)	7.36
Accessibility of the fresh produce	3.10
How do you transport fresh produce to your home? (n= 160)	
Private car	1.87
Taxi	30.00
Walking	68.13

Table 31 cont.

Practices	%
Where do you store your fresh produce? (n= 258)	
In a refrigerator	81.40
AT room temperature in an open vegetable rack	18.60
At room temperature in a closed cupboard	3.49
How do you store fresh produce? (n= 246)	
Loose without packaging	47.56
In the package from the store	25.2
In a separate container	27.24
How long do you keep the fresh produce before use? (n= 262)	
1-6 days	67.94
1-2 weeks	17.94
Month and more	5.34
Until the fresh produce spoils	8.78
How much fresh produce do you purchase at any given time? (n= 250)	
Enough for one meal	11.20
Enough for one day	13.60
Enough for one week	60.00
Enough for one month	15.20
Do you use the same work surface for raw and cooked food? (yes, n= 162)	
Yes, do nothing between working with raw and cooked food	3.1
Yes, I wipe it before I prepare the raw fresh produce	15.43
Yes, I wash it with water before I prepare raw fresh produce	61.72
I wash it with water and dishwashing liquid before I prepare raw fresh produce	19.75
Do you have a toilet in your home? (n=260)	
Yes	96.54
No	3.46
When you leave the toilet what do you do? (n= 249)	
Nothing	0.40
I wash my hands with running water and soap	84.74
I wash my hands with water and soap from a water storage container	14.86
Have you ever had a problem with rubbish next to your home (n= 255, yes = 130)	
Yes, Daily	20.77
Yes, Weekly	46.15
Yes, Monthly	9.23
Yes, A few times a year	15.38
Yes, Once a year	4.62
Do you have problems of sewage overflowing into your home (n=254, yes= 65)	
Yes, Daily	12.31
Yes, Weekly	13.85
Yes, Monthly	26.15
Yes, A few times a year	27.69
Yes, Once a year	12.31

Discussion and Conclusion

Evaluating the KAP of the consumers in these areas is of great importance as they regularly consume fresh produce from informal street vendors. The consumers and street vendors in these areas are challenged with poor infrastructure that can affect the quality and safety of the fresh produce. From the 31 outbreaks reported by the National Institute for Communicable Diseases in 2017 in South Africa, 11 resulted from contaminated food in the home or local community (NICD, 2017). It is of very important that consumers are aware of foodborne illnesses that can result from cross-contaminated food with pathogenic microorganisms. This study showed that most people know about the importance of fresh produce safety and most show a good general attitude towards food safety practices. They realised the importance of washing hands and clean environments to prevent cross contamination. However, less than half indicated that they use soap to wash hands. Most people also reported that the safety of food is the responsibility of the food handler. Another concern is that most people use the same cutting board for raw meat and fresh produce and a large proportion just use water to rinse in between the two different products.

These results show that interventions on the safety of fresh produce is needed. This could however be solved by food safety education programs that will provide information at regular intervals to ensure that the knowledge people have is turned into positive attitudes and result in general good practices. The government need to ensure that sewage treatments and waste removal in the informal settlements are done on a regular basis. The recent Covid-19 outbreak will have a significant impact on all of these practices.

4.1.3 Questionnaire C: "South African street trading green grocers' food safety knowledge, attitudes and practices, fruit and vegetables sold" (Appendix A)

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Demographic information of consumers in the informal settlements

Gender distribution: The total 183 (n=183) street vendors, with 62.0% (n=113) males and 38.0% females (n=70) were interviewed. These results were quite surprising as street vending has been

widely reported in the literature as a female-dominated sector. Street vending is often reported as a sector that enables women to juggle between family life and the generation of an income that provides for their family's needs. This might be a result of gender equality initiatives put in place not only in South Africa, but in Africa as a whole which has afforded women equal chances to compete with their counterparts for other employment opportunities besides street vending and domestic work, as was the case in the past. It was therefore fascinating to realise that this study indicated that in Tshwane, it was mostly men who identified street vending as not only a lucrative business opportunity, but also often as their main source of income. While we celebrate gender equality and mainstreaming, these results do raise some concern as a study done in Kenya reports that women as street vendors tend to be much more careful when it comes to ensuring food safety as compared to men (e.g. 68% of the women were more concerned about food safety while the majority of men were not)

Age distribution: Most of the participants (39%) were between the age of 26 and 35, followed by 36-45 years (29%), 18-25 years (20%), 46-55 (8%) years and the minority being 56 and older (4%). These results suggest a mean of 34.5 years, which indicates that in Tshwane, the younger population (18-35 years) is mostly involved in street vending as compared to older generations. These results are in line with reports made by StatsSA in 2019 that unemployment in South Africa is most prevalent between the ages of 15 and 34, hence leading the youth to temporal income generation strategies, with street vending being one of those opportunities (StatsSA, 2019).

Country of origin: It has been reported that the increase in street vending is due to migration and urbanisation both in developed and developing countries (Sariffuddin *et al.*, 2017). The participants were therefore requested to indicate their countries of origin in an open-ended question. The findings suggest that the respondents originated from 13 different countries with less than half of the sample (47%) originating from South Africa (see Table 32 below).

Table 32 Participants' country of origin

Country of Origin	Percentages %
South Africa	47%
Zimbabwe	18%
Mozambique	24%
Congo	0.5%
Malawi	4.4%
Swaziland	0.5%
Lesotho	0.5%
India	0.5%
Bangladesh	1.1%
Tanzania	2%
Ivory coast	0.5%
Kenya	0.5%
Nigeria	0.5%

The intensity of urbanisation is thus quite well supported with reference to the results above. This was furthermore highlighted by the results collected through conversation, which revealed that of the 47% that are South African citizens, only a few of the respondents were natively from Tshwane. Further investigation revealed that most moved to Tshwane from other provinces in the hope of better chances and employment. These results support those found in other studies such as that of Skinner (2008), which note that urbanisation has contributed profusely to street vending in Africa and globally.

Level of education: The results from the level of education question indicated that 46.4% of the sample were matriculants (successfully graduated Grade 12), 25% had high school education but did not reach Matric/Grade 12, 17% had Grade 7 certificates, finished their primary school education, and only 7% of the sample had gone to college or university.

These results support the results of a study done Nkrumah-Abrese and Schachteck (2017) where they profiled street vendors in Tshwane. They reported that 67% of their participants had matriculated, while 30% had a tertiary qualification, with only 3% who had no form of education. Although the percentages in the current study are slightly lower than theirs, this shows that most Tshwane street vendors have basic education, unlike the reports from other countries. It is therefore assumed that a lack of education is perhaps not the primary factor contributing to street vending, but rather a lack of lucrative employment opportunities. Results

from this study showed that people should be trained in food safety practices irrespective of their educational background.

Street vending location

Street vending in Tshwane is concentrated in the Central Business District (CBD) of Pretoria, accounting for 40% of the participants, followed by Marabastad (16%), Bosman (15.8%) and Sunnyside (14%). These results clearly show the effects of urbanisation in the country. When people move from rural settlements, they usually move to the centre of a chosen town in the hopes of better employment opportunities. *Since urbanisation is high in South Africa (65%) compared to other countries such as China (54%), India (32%) and Nigeria (47%) (Plecher, 2019), the majority of people who have moved to urban areas to get work find themselves unemployed, thus reverting to street vending.*

Street vending as a profitable income

To find out how much profit is made from street vending monthly, the participants were asked to indicate the amount they made from three profit ranges (less than R1000, between R1000-R3000, R3000-R5000, R5000-R10,000 or above R10,000). As indicated in Figure 4.4, 29% of the sample made less than R 1000 in profits monthly from street vending, less than half (48%) of the respondents managed to make between R1000-R3000 per month on average, 15% between R3000 and R5000, 4% between R5000-R10,000 and 4% above R10,000.

Type of street vending (stationary or mobile)

Street vending is often defined in terms of its mobility (Bhowmik, 2005). In order to determine the category in which the vendors fell under, the respondents were asked if they had a fixed or mobile stall. Owning a stall indicates sustainability and compliance with the municipal bylaws (Masonganye, 2010). Street vendors that have a designated area where they work daily are less likely to have unwanted issues like those experienced by mobile street vendors, e.g. eviction or having their goods confiscated. The findings revealed that 80% of the participants met the criteria of being stationary vendors compared to the rest (20%) who were therefore defined as mobile.

Fresh produce sold by street vendors: Over the years, fruits and vegetables have been implicated in several foodborne illness outbreaks globally (Callejón *et al*., 2015b; Herman *et al*., 2015). This has come as a result of improper production and post-harvest handling and preparation practices (Beharielal *et al*., 2018; Duvenage and Korsten, 2017). It is also true that different types of fruits and vegetables have been implicated in these outbreaks. Thus, in order to not only investigate food safety knowledge, attitudes, and practices, but also the types of fruits and vegetables sold in street vending, as an area of concern, the respondents were asked to list the types of fresh produce they predominately had in their stalls daily. Seasonality, as a factor in the fresh produce market, raised some concern as it influenced the products available at the time of data collection and, ultimately, the results. Nonetheless, the participants were provided with a detailed list of possible fresh produce options irrespective of their seasonality. They were then asked to tick what they had available in their stalls at the time of data collection (between the 26th of November and 7th of December 2018).

Popularity of vegetables amongst street vendors

The most popular fresh vegetables sold included cabbages (n=82), carrots (n=81), onions (n=77), potatoes (n=57) and green beans (n=55) respectively (Figure 9).

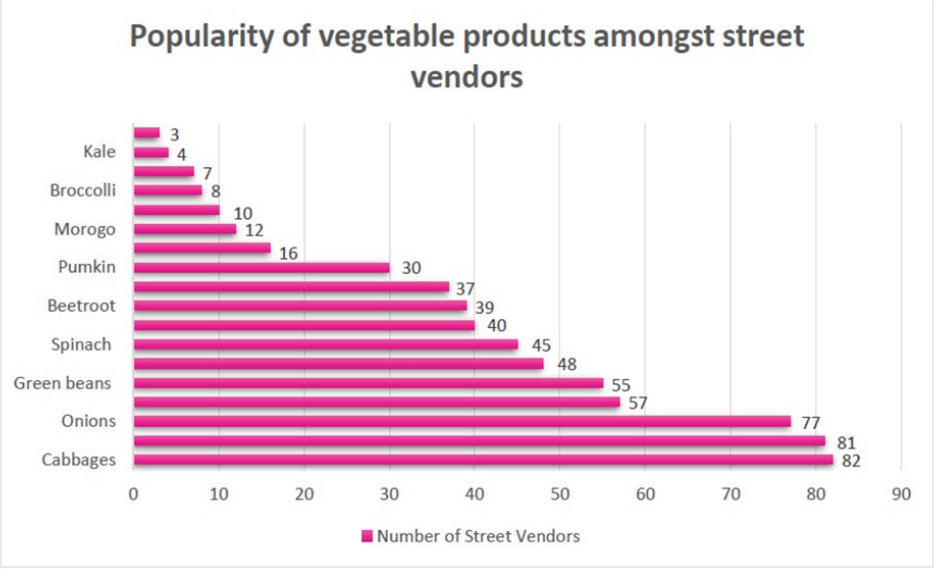

Figure 9: Number of street vendors selling each type of food.

4.2 Microbiological analysis of fresh vegetables from formal and informal supply chains in the Tshwane Metropole

4.2.1 Microbiological quality, safety and characterisation of potential human pathogenic bacteria isolated from fresh vegetables at the point-of-sale (formal and informal traders)

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Accepted publication: "High prevalence of multidrug resistant *Escherichia coli* isolated from fresh vegetables sold by selected formal and informal traders in the most densely populated Province of South Africa" in Journal of Food Science, 26 October 2020, DOI:10.1111/1750-3841.15534.

Specific aim: This study aimed to determine the microbiological safety, presence of potential human pathogenic bacteria in vegetables at sold at formal retailers, informal street- and mobile trolley vendors, and from farmers' markets in Gauteng Province, SA.

Experimental procedures

Details of the point-of-sale vegetable samples collected , hygiene indicator bacteria enumeration, foodborne pathogen and (ESBL) and AmpC-producing Enterobacteriaceae occurrence, identification and antimicrobial resistance characterisation were described in Chapter 3, sections 3.2.1, 3.3.1 and 3.4.

Results

Microbiological analysis of fresh vegetable samples. Vegetable samples (n=545) including spinach, tomato, lettuce, cucumber and green beans were purchased from retailers, street traders, trolley vendors and farmers' markets. The coliforms counts ranged from 0.59-8.10 log CFU/g on spinach, 3.58-7.82 log CFU/g on lettuce, 0-8.21 log CFU/g on tomatoes, 0-6.48 log CFU/g on cucumber and 0.70-6.77 log CFU/g on green bean samples from the different products across all vendor types (Table 33). The Enterobacteriaceae counts were similar, ranging between 0.00-8.16 log CFU/g on spinach, 0.00-8.10 log CFU/g on tomatoes, 4.18-8.26 log CFU/g on lettuce, 0.00-6.45 log CFU/g on cucumbers and 0.00-7.71 log CFU/g on green beans. Coliform and

Enterobacteriaceae counts were mostly not significantly different between formal and informal markets, with exceptions noted on occasion (Table 33). Although the majority of *E. coli* counts on fresh produce were acceptable, some samples were of poor microbiological quality with counts as high as 5.88 log CFU/g for spinach, 5.10 log CFU/g for tomatoes, 3.3 log CFU/g for lettuce, 3.78 log CFU/g and 4.78 log CFU/g for cucumbers and green beans.

Detection of potential foodborne pathogens. None of the vegetable samples analysed tested positive for *Salmonella* spp. or *L. monocytogenes.* Following enrichment 14.86% of the 545 vegetable samples analysed from all the formal and informal vendors harboured *E. coli.* This included 25.3% farmers' market samples, 6% street traders' samples, 3% trolley vendor samples, and 10% samples from retailers, predominantly from spinach and lettuce samples.

Table 33 Total coliform, *Escherichia coli* and Enterobacteriaceae loads present in spinach, lettuce, cucumber and green bean samples purchased from retailers, street trading greengrocers, trolley vendors, and vendors at farmers' markets.

Product	No of samples (% harbouring coliforms)	**Total coliforms (log CFU/g)** Range	Mean[a]	No of samples (% harbouring *E. coli*)	***E. coli* (log CFU/g)** Range	Mean[a]	No of samples (% harbouring Enterobacteriaceae)	**Enterobacteriaceae (log CFU/g)** Range	Mean[a]
Spinach									
Retailers	50 (100)	2.90-7.17	5.61^{AB}	50 (20)	0.00-3.42	0.84^{AB}	50 (100)	2.78-8.16	5.79^{ABC}
Street traders	50 (100)	0.70-7.60	5.54^{AB}	50 (12)	0.00-2.08	0.25^{BC}	50 (98)	0.00-6.99	5.42^{ABCD}
Trolley vendors	50 (100)	0.59-7.04	5.05^{BCD}	50 (28)	0.00-1.29	0.72^{ABC}	50 (90)	0.00-7.27	6.63^{DE}
Farmers' market vendors	50 (100)	3.76-8.10	6^{A}	50 (44)	0.00-5.88	1.22^{A}	50 (100)	4.03-7.88	5.92^{AB}
Total for spinach	**200**								
Tomato									
Retailers	50 (100)	0.48-8.04	4.58^{CDE}	50 (94)	0.00-0.89	0.12^{C}	50 (100)	2.40-8.10	5.34^{ABCD}
Street traders	50 (100)	2.00-8.21	4.96^{BCDE}	50 (100)	0.00-2.30	0.05^{C}	50 (98)	0.00-7.82	4.76^{CDE}
Trolley vendors	50 (100)	0.00-6.36	4.42^{DE}	50 (98)	0.00-3.60	0.16^{BC}	50 (92)	0.00-7.94	4.51^{DE}
Farmers' market vendors	50 (100)	3.15-7.89	5.43^{ABC}	50 (20)	0.00-5.10	0.54^{ABC}	50 (100)	1.49-7.75	5.02^{BCDE}
Total for tomato	**200**								
Lettuce									
Farmers' market vendors	50 (100)	3.58-7.82	6.08^{A}	50 (26)	0.00-3.31	0.65^{ABC}	50 (100)	4.18-8.26	6.22^{A}
Total for lettuce	**50**								
Cucumber									
Farmers' market vendors	45 (96)	0.00-6.48	4.06^{E}	45 (20)	0.00-3.78	0.43^{BC}	45 (96)	0.00-6.45	4^{E}
Total for cucumber	**45**								
Green beans									
Farmers' market vendors	50 (100)	0.70- 6.77	4.97^{BCDE}	50 (28)	0.00-4.78	0.68^{ABC}	50 (98)	0.00-6.71	5.22^{ABCD}
Total for green beans	**50**								

Phenotypic antimicrobial resistance profiling of *Escherichia coli* isolates. A total of 67 isolates were selected which included one representative *E. coli* isolate per product type found from each supplier and tested further for antimicrobial resistance or susceptibility against seven antibiotic classes. Of the 67 selected *E. coli* isolates, 40.3% were multidrug resistant (resistance to ≥3 antibiotic classes). The highest resistance was against neomycin (73.13%) which belongs to the aminoglycoside class of antibiotics, followed by penicillins (ampicillin, 38.81%; amoxicillin, 41.79% and augmentin, < 10%), sulfonamides (cotrimoxazole, 22.39%), tetracycline (19.4%) and chloramphenicol (11.94%) (Figure 10). Less than 10% of the isolates were resistant to gentamycin (aminoglycoside), while 34.3% of the isolates were resistant to fourth-generation cephalosporin antibiotics (cefepime) and < 10% resistant to impenem (carbapenemase). The most frequent resistance patterns in the different antibiotic classes for the isolates included resistance to antibiotics in the penicillins-cephalosporins-aminoglycosides combination (13 MDR isolates), followed by the penicillins-aminoglycosides-sulfonamides-tetracyclines- Chloramphenicol combination (5 isolates) and the penicillins-cephalosporins-aminoglycosides-sulfonamides (3 isolates) combination (Table 34).

Figure 10: Phenotypic antimicrobial resistance profiles of *Escherichia coli* isolated from different fresh produce types sold at different vendors in Gauteng, South Africa.

Table 34 Summary of the number of antimicrobials, most frequent resistance patterns, number, and type of antibiotic classes to which generic *Escherichia coli* isolates from different fresh produce samples were resistant

No of antimicrobials to which isolates were resistant	No of isolates (n=67)	No of isolates with specific pattern	Most frequent pattern[a]	No of antibiotic classes to which isolates were resistant	Antibiotic class(es)
0	8				
1	21	17	NE10C	1	Aminoglycosides
		2	T30C	1	Tetracyclines
		1	AUG30C	1	Penicillins
		1	CPM30C	1	Cephalosporins
2	8	2	AUG30C - NE10C	2	Penicillins, Aminoglycosides
		1	AP10C - NE10C	2	Penicillins, Aminoglycosides
		1	TS25C - T30C	2	Sulfonamides, Tetracyclines
		1	A10C - NE10C	2	Penicillins, Aminoglycosides
		1	A10C - CPM30C	2	Penicillins, Cephalosporins
		1	CPM30C - NE10C	2	Cephalosporins, Aminoglycosides
		1	GM10C - NE10C	1	Aminoglycosides
3	5	3	A10C - CPM30C - NE10C	3	Penicillins, Cephalosporins, Aminoglycosides
		1	AP10C - A10C - CPM30C	2	Penicillins, Cephalosporins
		1	AP10C - A10C - NE10C	2	Penicillins, Aminoglycosides
4	12	7	AP10C - A10C - CPM30C - NE10C	3	Penicillins, Cephalosporins, Aminoglycosides
		1	FOX30C - CPM30C - TS25C - NE10C	3	Cephalosporins, Sulfonamides, Aminoglycosides
		1	AP10C - AUG30C - A10C - NE10C	2	Penicillins, Aminoglycosides
		1	AP10C - CPM30C - TS25C - NE10C	4	Penicillins, Cephalosporins, Sulfonamides, Aminoglycosides
		1	AP10C - A10C - CPM30C - TS25C	3	Penicillins, Cephalosporins, Sulfonamides
		1	TS25C - T30C - NE10C - C30C	4	Sulfonamides, Tetracyclines, Aminoglycosides, Chloramphenicol
5	6	1	AP10C - A10C - T30C - NE10C - C30C	4	Penicillins, Tetracyclines, Aminoglycosides, Chloramphenicol
		1	AP10C - A10C - CPM30C - TS25C - NE10C	4	Penicillins, Cephalosporins, Sulfonamides, Aminoglycosides
		1	AP10C - AUG30C - A10C - CPM30C - NE10C	3	Penicillins, Cephalosporins, Aminoglycosides
		1	AP10C - A10C - CPM30C - TS25C - IMI10C	4	Penicillins, Cephalosporins, Sulfonamides, Carbapenems
		1	AP10C - A10C - CPM30C - T30C - NE10C	4	Penicillins, Cephalosporins, Tetracyclines, Aminiglycosides
		1	AP10C - TS25C - T30C - NE10C - C30C	5	Penicillins, Sulfonamides, Tetracyclines, Aminoglycosides, Chloramphenicol
6	6	4	AP10C - A10C - TS25C - T30C - NE10C - C30C	5	Penicillins, Sulfonamides, Tetracyclines, Aminoglycosides, Chloramphenicol
		1	AP10C - A10C - CPM30C - TS25C - T30C - NE10C	5	Penicillins, Cephalosporins, Sulfonamides, Tetracyclines, Aminoglycosides
		1	AP10C - AUG30C - A10C - CPM30C - TS25C - NE10C	4	Penicillins, Cephalosporins, Sulfonamides, Aminoglycosides
7	1	1	AP10C - AUG30C - A10C - TS25C - T30C - NE10C - C30C	5	Penicillins, Sulfonamides, Tetracyclines, Aminoglycosides, Chloramphenicol

Molecular characterisation of diarrheagenic *Escherichia coli.* None of the 67 selected *E. coli* isolates tested harboured the diarrheagenic virulence genes tested for (lt, st, bfpA, eagg, eaeA, stx1, stx2, ipaH).

Discussion

This is the first report of the presence of MDR ESBL/AmpC-producing Enterobacteriaceae in raw vegetables sold at selected formal and informal retailers in Gauteng Province, South Africa. Leafy greens have previously been prioritised as the highest level of concern in terms of fresh produce safety from a global perspective (WHO, 2008). The guidelines with regard to acceptable hygiene indicator bacteria counts on ready-to-eat (RTE) produce differ across the world (FSAI, 2016; FSANZ, 2001; Health Protection Agency, 2009). The South African Department of Health microbiological guidelines for fresh fruits and vegetables, currently under revision, required coliform counts of <200 CFU/g (2.3 log CFU/g) and no *E. coli* or *Salmonella* in a 25 g sample. Other countries do not include coliform counts in the guidelines for interpretation of results of microbiological testing of RTE foods. Coliforms include amongst other Citrobacter, Klebsiella, Enterobacter and *E. coli*, that could potentially pose a threat to human health (Baylis *et. al.*, 2011). Coliform and Enterobacteriaceae counts of vegetables are often > 4 log CFU/g.

Overall, 2-8% of the tomato samples from the different vendors had unsatisfactory *E. coli* counts (*E. coli* ≥ 1000 CFU/g), according to the commission regulation on microbiological criteria for RTE pre-cut fruit and vegetables (EFSA, 2007a). Spinach samples from all different vendors had unsatisfactory *E. coli* counts ranging between 12% from farmers' market vendors to 6%, 4%, and 2% from trolley vendors, retailers and street traders respectively. Similarly, 6%, 4%, and 2% lettuce, green beans, and cucumber samples respectively, had unsatisfactory *E. coli* counts. When evaluated against international guidelines as specified in the UK (20 to 100 CFU/g), Australia (3 to 100 CFU/g), and Canada (100 most probable number per g), 13.03% (n=71) of the samples from the current study would not have been compliant (FSANZ, 2001; Health Canada, 2002; Health Protection Agency, 2009). This included 19.72% samples from the formal and 80.28% samples from the informal markets, respectively. Furthermore, *E. coli* levels from spinach and tomatoes from the retailers, street traders, trolley vendors and farmers' markets were not significantly different. All the vegetable samples tested negative for *Salmonella spp.* and

L. monocytogenes. The high percentage (50%) of the SA population that depend on informal trade, highlights the need to improve fresh produce safety in all the different markets (Petersen and Charman, 2018). In SA, 21.76% and 95.60% of the population purchasing from the informal sector consume raw and/or cooked spinach and tomatoes, respectively. The questionnaire survey results from the population purchasing from the formal sector, showed that 94%, 29% and 94% of the respondents eat lettuce, beans and cucumber raw, respectively (WRC, 2018; Baloyi, 2020). The coliform and Enterobacteriaceae counts on different vegetables from formal and informal markets reiterated the natural bacterial prevalence on the produce, regardless of food safety regulations being implemented or not in these contrasting points of sale with highly differing personal hygiene and sanitation standards and cold refrigeration capacity (Al-Kharousi *et al.*, 2016; Grace *et al*., 2019).

The prevalence of 40.3% multidrug-resistant *E. coli* in the fresh produce samples highlights the need for improved food safety practices in the supply chains and identification of fresh produce contamination sources with antimicrobial resistant bacteria. With a rise in antimicrobial resistance in both commensal and pathogenic bacteria in different environments, subsequent treatment options to infections become limited (Freitag *et al*., 2018).

Conclusion

E. coli levels from spinach and tomatoes from retailers, street traders, trolley vendors, and farmers' markets were not significantly different. No *Salmonella* spp. nor *L. monocytogenes* were present in any of the vegetables sampled and analysed in this study. Moreover, the prevalence of multidrug resistant commensal *E. coli* does indicate the need for improved food safety practices in the supply chains. The high antimicrobial resistance levels observed in commensal *E. coli* isolated from fresh produce at the point of sale further highlights the need to include detection and characterisation of Enterobacteriaceae (commensal and potential pathogenic bacteria) with expanded spectrum antimicrobial resistance. This can be achieved through improved surveillance of fresh produce production systems from farm to retail to identify potential sources of microbial contamination which contribute to the presence and dissemination of antimicrobial resistant microorganisms and their genetic determinants.

4.2.2 Occurrence, identification, and antimicrobial resistance profiles of extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae from fresh vegetables retailed in Gauteng Province, South Africa

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Specific aim: This study aimed to detect, identify, and characterise extended-spectrum β-lactamase (ESBL) and AmpC-producing Enterobacteriaceae isolates from point-of-sale vegetables, since these microorganisms represent increased risks related to environmental integrity, food safety and human health.

Experimental procedures

Details of (ESBL) and AmpC-producing Enterobacteriaceae detection, isolation, identification and antimicrobial resistance characterisation (phenotypic and genotypic) were described in Chapter 3, section 3.4.3, 3.4.4 and 3.4.5.

Identification of presumptive extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae isolates. Using MALDI-TOF analysis, 122/432 (28.24%) of the presumptive extended-spectrum/AmpC β-lactamase-producing isolates obtained from the fresh vegetable samples were confirmed as Enterobacteriaceae belonging to ten genera. The isolates were identified as Enterobacter spp. (28.68%), including *E. cloacae*, *E. absuriae*, *E. cowanii and E. ludwigii; Serratia* (18.85%), predominantly *S. fonticola*, *Escherichia coli* (18.03%); *Klebsiella* spp. (14.75%) including *K. pneumoniae* and *K. oxytoca*; *Rahnella aquatilis* (9.01%), *Proteus* spp. (4.91%) including *P. penneri* and *P. mirabilis*; Citrobacter spp. (2.46%), including *C. farmeri* and *C. freundii*; *Kluyvera ascorbata* (1.64%), *Achromobacter xylosixidans* (1.64%) and *Raoultella ornithinolytica* (0.82%). Presumptive ESBL/AmpC-producing Enterobacteriaceae were isolated from all the vegetable types tested.

Phenotypic antimicrobial resistance profiling. Of the 77 presumptive ESBL-producing Enterobacteriaceae (n=77) showed resistance with 96.1% being MDR (resistant to 3 antimicrobial classes) (Figure 11). Resistance to the aminoglycoside and chloramphenicol classes was dominant, observed in 94.8% and 85.7% of the isolates, respectively. ESBL production was shown in 61 (79.2%) of the 77 isolates, and AmpC production in 41.6% of the isolates (Figure 11).

Genotypic antimicrobial resistance profiling. Genes encoding β-lactamases were detected in 58/77 (75.3%) isolates obtained from all vegetable types, mainly in *E. coli* (n = 20), *Enterobacter* spp. (n = 12), and *Serratia spp*. (n = 11) isolates. This included 48%) broad-spectrum, 51% ESBL, and 25.9% AmpC genetic determinants (Figure 11). The most frequently detected β-lactamase genes were $bla_{\text{CTX-M}}$ (n = 36.3%), followed by bla_{SHV} (28.6%), bla_{TEM} (27.3%), and bla_{OXA} (6.5%). ESBLs encoded by $bla_{\text{CTX-M}}$ included CTX-M-14 (n = 15), CTX-M-15 (n = 6), CTX-M-27 (n = 4), and CTX-M-55 (n = 3); bla_{TEM} genes encoded TEM-3 (n = 3), while bla_{SHV} genes encoded SHV-18 (n = 6), SHV-28 (n = 1), and SHV-154 (n = 1). All the bla_{OXA}, 85.7% (n = 18) of the bla_{TEM}, and 63.6% (n = 14) of the bla_{SHV} sequences encoded broad-spectrum β-lactamases OXA-1, TEM-1, TEM-215, SHV-1, SHV-11, or SHV-26, respectively. Three isolates harboured more than one ESBL; one *E. coli* isolate carried the $bla_{\text{TEM-3}}$, $bla_{\text{SHV-18}}$, and $bla_{\text{CTX-M- 14}}$ genes, and two isolates (*E. coli* and *E. cowanii*) carried the $bla_{\text{TEM-3}}$ gene in association with $bla_{\text{CTX-M-14}}$ and $bla_{\text{SHV-18}}$ genes, respectively. In 12 isolates (*E. coli* [n = 3]; *Enterobacte*r spp. [n = 3]; *Serratia* spp. [n = 3]; *R. aquatilis* [n = 2]; and *P. mirabilis* [n = 1]), ESBL genes in association with broad-spectrum b-lactamases were detected (Figure 11).

AmpC resistance genes were detected in 18/58 (31%) isolates harboring β-lactamase genetic determinants (Figure 11). In 17 isolates, only one pAmpC genetic determinant was detected; $bla_{\text{MIR-20}}$ (n = 4), $bla_{\text{MIR-16}}$ (n = 3), $bla_{\text{ACT-58}}$ (n = 2), and one isolate each carried $bla_{\text{CMY-2}}$, $bla_{\text{MIR-14}}$, $bla_{\text{ACT-29}}$, $bla_{\text{ACT-10}}$, $bla_{\text{ACT-2}}$, bla_{EC}, $bla_{\text{CMY-161}}$, $bla_{\text{CMY-87}}$ respectively. Five isolates including *Enterobacter* spp. (n = 2), *E. coli* (n = 1), *R. aquatilis* (n = 1), and *S. fonticola* (n = 1) also harboured ESBL genetic determinants. One *P. penneri* isolate carried three AmpC genes

(*bla*$_{ACT10}$, *bla*$_{DHA-18}$, and *bla*$_{CMY-49}$). The EBC family of the AmpC genetic determinants was the most dominant type.

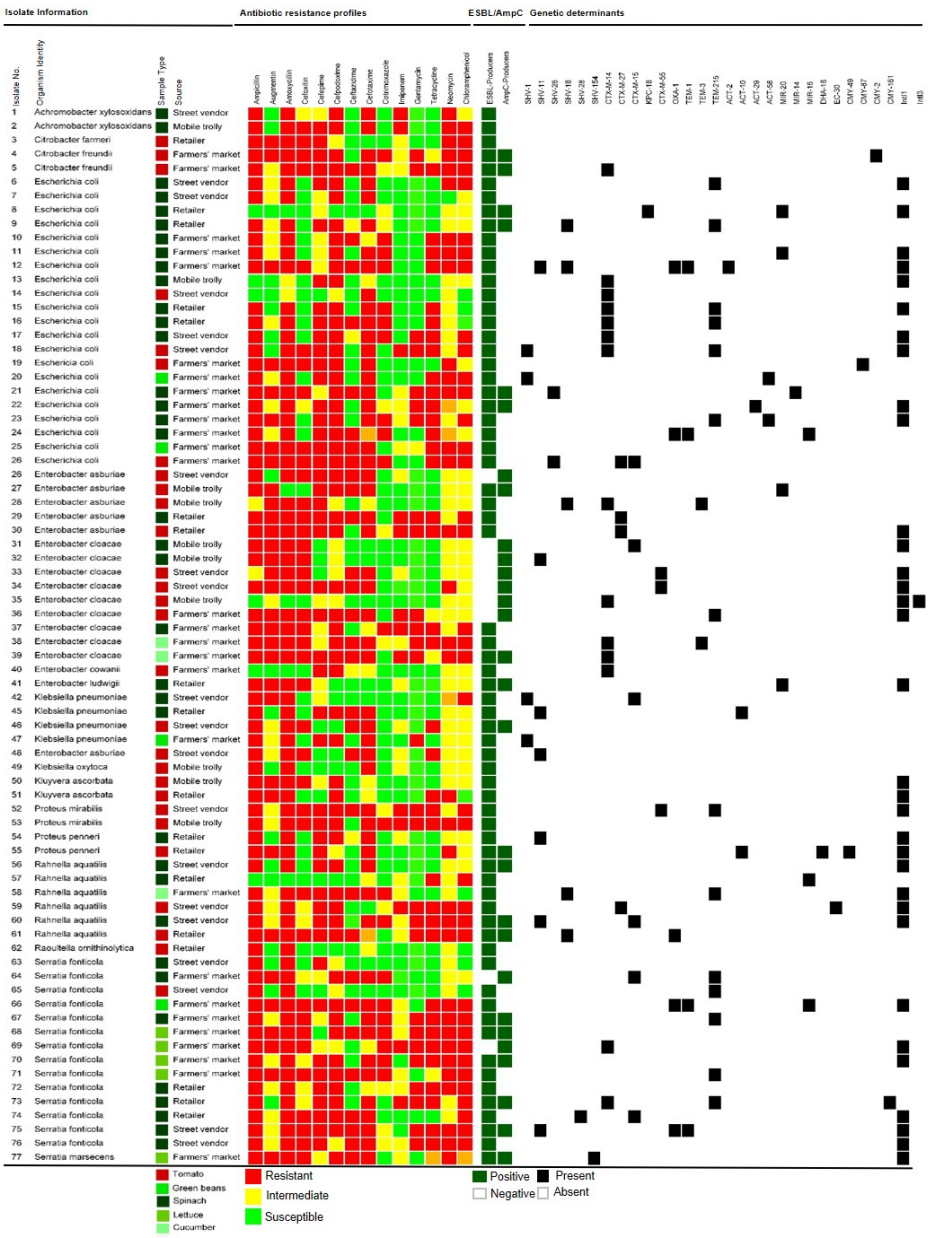

Figure 11 Summary of the species isolated from different fresh vegetables, indicating the phenotypic resistance profiles and the extended-spectrum β-lactamase/AmpC genetic determinants detected. AP10C, ampicillin; AUG30C, amoxicillin-clavulanic acid; A10C, amoxicillin; FOX30C, cefoxitin; CPM30C, cefepime; CPD10C, cefpodoxime; CPD10C/ CLAV1C, cefpodoxime-clavulanic acid; CAZ30C, ceftazidime; CAZ/CLAV10C, ceftazidime-clavulanic acid; CTX30C, cefo- taxime; CTX/CLAV10C, cefotaxime-clavulanic acid; TS25C, trimethoprim-sulfamethoxazole; IMI10C, imipenem; T30C, tetra-cycline; NE10C, neomycin; C10C, chloramphenicol.

Discussion

Multidrug resistant ESBL/AmpC-producing Enterobacteriaceae were detected for the first time in raw vegetables retailed at selected sites in Gauteng Province, SA. Antibiotic-resistant opportunistic pathogens on fresh produce are a serious health concern that contributes toward the burden of AR in different environments, leading to increased risk of infection if colonisation in humans occurs (Al-Kharousi *et al*., 2016). Enterobacteriaceae regarded as emerging bacterial threats include *E. coli*, *K. pneumoniae*, and *Enterobacter* spp. showing resistance to β- lactams and aminoglycosides (Fair and Tor, 2014). Presumptive ESBL producers, predominantly *E. coli*, *K. pneumoniae*, *E. cloacae*, and *E. asburiae*, were detected in 17.4% of our vegetable samples analysed. This is lower than the 25.4% reported by Zurfluh *et al.* (2015) for imported vegetables into Switzerland from the Dominican Republic, India, Thailand, and Vietnam, but higher than the 6% reported by Reuland *et al*. (2014) on retail vegetables in the Netherlands. Similar to Blaak *et al.* (2014), environmental ESBL-producing Enterobacteriaceae isolated from vegetables included *S. fonticola* and *R. aquatilis.*

MDR phenotypes (resistance to a least 3 antimicrobial classes) were observed in 96.1% of our analyzed isolates. The most prevalent non-β-lactam resistance profiles showed resistance against aminoglycoside (94.8%), chloramphenicol (85.7%), and tetracycline (53.2%). This is higher than reports from similar studies that showed resistance to aminoglycosides (46.7-66.7%), chloramphenicol (33.3%) (Zurfluh *et al*., 2015; Ben Said *et al*., 2016), and tetracycline (46.7%) (Ben Said *et al*., 2016) in ESBL-producing Enterobacteriaceae.

Genes expressing broad-spectrum β-lactamases, ESBLs, and/or AmpC β-lactamases were detected in 69.9% of our MDR isolates. Co-expression of ESBL and AmpC genes in environmental (van Hoek *et al*., 2015; Ye *et al*., 2017) and clinical (Tau *et al*., 2012; Kharat *et al*., 2017) Entero-bacteriaceae isolates has also been reported. Globally the blaCTX-M-type ESBL genes are predominant in Enterobacteriaceae, which was similar in our study, the majority detected in *E. coli* isolates. The main genetic determinant was $bla_{\text{CTX-M-14}}$, predominantly detected in *E. coli* and *C. freundii* isolates, which corresponds to results obtained for vegetable samples in Tunisia (Ben Said *et al*., 2016). Isolates harboring $bla_{\text{CTX-M-15}}$ included *E. coli*, *E. cloacae*, *K. pneumoniae*, *R. aqualtilis*, and *S. fonticola* and were second most prevalent in our study. This is in agreement

with reports that *bla*$_{\text{CTX-M-14}}$ and *bla*$_{\text{CTX-M-15}}$ are predominantly detected worldwide (Ehlers *et al*., 2009; Zurfluh *et al*., 2015).

The pAmpC resistance genes were detected predominantly included the EBC-type pAmpC β- lactamases (identified as *bla*$_{\text{ACT}}$/*bla*$_{\text{MIR}}$). This contrasts with two previous studies where blaCIT, blaDHA, or blaACC pAmpC β-lactamases were mostly detected in Enterobacteriaceae isolated from fresh produce and water samples (Njage and Buys, 2014; Ye *et al*., 2017). *bla*$_{\text{ACT/MIR}}$ genes have been reported to be the dominant AmpC genetic determinants in Enterobacter spp., causing intra-abdominal infections (Khari *et al*., 2016), and were detected in seven of the Enterobacter spp. isolates in our study. The fact that fresh produce can serve as a reservoir of MDR ESBL/AmpC-producing Enterobacteriaceae, including their genetic determinants, constitutes a potential health risk to the consumer as resistance to antimicrobials frequently used to treat human infections was shown.

Conclusion

This is the first exploratory study in SA to investigate the presence and characteristics of multidrug resistant ESBL/AmpC-producing Enterobacteriaceae isolated from raw vegetables produced sold by retailers (formal and informal). Results obtained during this study will contribute to the development of commodity-specific supply chain risk management systems. A global holistic One Health approach is required to address the increase in the levels of antimicrobial resistance not only in humans and animals, but also in the environment. More importantly, contributions from governmental departments as well as from the scientific community need to be integrated.

4.2.3 Microbiological analysis of spinach (baby spinach, spinach, bunches, RTE pillow packs) from three commercial spinach supply chains in Gauteng Province supplying retailers in Tshwane Metropole

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A draft manuscript "Microbiological quality and antimicrobial resistance profiles of *Escherichia coli* isolated throughout spinach supply chains in Gauteng Province, South Africa" has been prepared.

Experimental procedures

Details of the commercial spinach production sites, sample collection, processing, hygiene indicator bacteria enumeration, foodborne pathogen isolation, identification and characterisation were described in Chapter 3, section 3.2.2, 3.3 and 3.4.

Sampling Points: Commercial Spinach Supply Chains

(Refer to Chapter 3, section 3.2.2.)

On farm		Commercial processing facility	Retailer
Spinach supply chain scenario 1 (Farm A)			
River Holding dam Irrigation pivot point	**Open field:** Spinach Soil	**Packhouse/processing facility:** Spinach at receival, after cut Packhouse highcare: Spinach after wash; produce after pack Crates at receival; floors, cutting surfaces; cutting machine; outside wash water bath; spinach bunches; RTE spinach pillow packs; wash water	Spinach bunches RTE spinach pillow packs supplied directly to retailer 1
Spinach supply chain scenario 2 (Farm B)			
Two holding dams Overhead irrigation	**Tunnels:** Baby spinach Soil	**Packhouse/processing facility:** Baby spinach leaves transported in crates Unwashed baby spinach packed in punnets*	**Commercial processing facility (Retailer 1)** Unwashed baby spinach packed punnets at receival Crates at receival Core wash water Processing facility high care: Spinach after wash Spinach after pack	Unwashed spinach punnets Washed spinach pillow packs
Spinach supply chain scenario 3 (Farm C)			
Holding dam Overhead irrigation	**Tunnels** Baby spinach Soil	**Packhouse/processing facility:** Crates in refrigerator (receival for washed products) Unwashed baby spinach packed punnets*	**Commercial processing facility (Retailer1)** Unwashed baby spinach packed punnets at receival Crates at receival Core wash water Processing facility high care: Spinach after wash Spinach after pack	Unwashed spinach punnets Washed spinach pillow packs

In total 288 samples were collected, which included soil at harvest (n=6 composite samples); water samples at the source, irrigation point and during processing (n=72); spinach samples at harvest, during processing and at retail (n=192); and contact surface swab samples throughout production and processing of the fresh produce (n=18).

Results

Microbiological quality analysis. The Enterobacteriaceae, coliform and *E. coli* counts of the irrigation water, washwater, spinach and swab samples from the farm, through processing and at the retailer were determined for Farm A (Table 35, 36 and 37), Farm B (Table 38 and 39) and Farm C (Table 40 and 41). Enterobacteriaceae counts in river water from Farm A ranged from 2.84-3.20 log CFU/ml, while the holding dam water and irrigation pivot point water counts ranged from 1.61-3.78 log CFU/ml and 0.00-3.83 log CFU/ml, respectively. The trip by source interaction of Enterobacteriaceae counts from water sources on Farm A were not significantly different (p=0.0936) (Table 35). However, the Enterobacteriaceae levels were significantly different based on the source of the water (p=0.0083) with river water significantly higher than the dam reservoir and irrigation water in trip one. Enterobacteriaceae counts on spinach samples from Farm A were not significantly different (trip x source – p=0.1627, trip – p=0.3639, source – p=1.1646) (Table 36). The mean Enterobacteriaceae counts on spinach from Farm A ranged from 0.00-6.52 log CFU/g.

The coliform levels of river, holding dam and irrigation pivot point water samples from Farm A ranged from 3.38-4.76 log MPN/100 ml, 3.19-3.38 log MPN/100 ml and 3.11-4.76 log MPN/100 ml, respectively. Samples collected from river water during trip one exhibited higher coliform counts than the holding dam and irrigation pivot point water samples during the same trip (p=0.0077) (Table 35). The coliform levels on spinach from Farm A ranged from 3.90-6.50 log CFU/g. Interestingly coliform levels on spinach at harvest, at dispatch, at receival and bunches at the retailer were all significantly lower when river water was directly used for overhead irrigation (Trip 2) (p=0.0003) (Table 36). The coliform levels on spinach after wash and spinach after pack from trip one was significantly lower than during trip 2 (p = 0.0003).

Escherichia coli levels in river water ranged from 2.20-2.64 log MPN/100 ml, in the holding dam water from 1.43-1.50 log MPN/100 ml and in the water from the irrigation pivot from 1.50-2.56 log MPN/100 ml. Similar to the coliform levels, during trip one the river water *E. coli* levels was significantly higher than that of the holding dam and irrigation pivot point water samples during the same trip (p = 0.0257) (Table 35). Similarly to the coliform levels, *E. coli* in the irrigation pivot point water were not significantly different to the river water (p=0.0257), as river water was used for to directly irrigate (Table 35). The mean *E. coli* levels on spinach from Farm A ranged from 0.00-4.03 log CFU/g. The *E. coli* (trip x source) count interactions from spinach were significantly different (p = 0.0012) (Table 36). *Escherichia coli* levels during Trip 2 on spinach at receival were significantly higher than spinach after pack, spinach after cut and spinach at harvest during Trip 2, with all other samples having significantly lower *E. coli* levels (p=0.0012). *Escherichia coli* levels on spinach during Trip 1 did not differ significantly and were the lowest (p=0.0012) (Table 36).

The coliform levels from swab samples throughout processing on Farm A ranged from 2.60-6.32 log CFU/cm^2, with a significant difference between the trip x source interactions (p=0.0021) (Table 37). In contrast to the coliform levels from the contact surface swab samples, Enterobacteriaceae levels ranged from 2.70-6.13 log CFU/cm^2, with no significant difference in the trip x source interactions (p=0.1333) (Table 37). The *E. coli* levels on the contact surfaces ranged from 0.00-2.74 log CFU/cm^2. Similar to the Enterobacteriaceae counts, the trip x source interactions of *E. coli* from contact surfaces were not significantly different (p= .3325), however, the *E. coli* counts on per trip were significantly different (p=0.0034) with Trip 2 having higher levels than Trip 1 (Table 37).

Table 35 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from water samples throughout production on Farm A

		Production scenario 1					
		Farm A		Farm A		Farm A	
Source	**Trip**	**Enterobacteriaceae (log CFU/ml)**		**Coliforms (log MPN/100 ml)**		***Escherichia coli* (log MPN/100 ml)**	
		Mean ± SE[a]	**t-Test[b]**	**Mean ± SE[a]**	**t-Test[b]**	**Mean ± SE[a]**	**t-Test[b]**
River	1	2,88 ± 0,04	AB	3,92 ± 0,29	B	2,29 ± 0,09	A
	2	3,11 ± 0,05	A	4,63 ± 0,07	A	2,52 ± 0,08	A
Dam (Reservoir)	1	2,72 ± 0,63	AB	3,32 ± 0,06	C	1,47 ± 0,02	B
	2	-	-	-	-	-	-
Irrigation pivot point	1	2,52 ± 1,26	B	3,17 ± 0,03	C	1,55 ± 0,02	B
	2	1,95 ± 0,98	C	4,59 ± 0,08	A	2,49 ± 0,06	A
Packhouse dam	1	0,00 ± 0,00	-	0,00 ± 0,00	-	0,00 ± 0,00	-
	2	0,00 ± 0,00	-	0,00 ± 0,00	-	0,00 ± 0,00	-
Bunch wash basin	1	0,00 ± 0,00	D	0,30 ± 0,18	E	0,00 ± 0,00	D
	2	0,35 ± 0,35	D	1,78 ± 0,12	D	0,58 ± 0,31	C
Washwater	1	0,00 ± 0,00	D	0,00 ± 0,00	E	0,00 ± 0,00	D
	2	0,00 ± 0,00	D	0,10 ± 0,10	E	0,00 ± 0,00	D
p-value (source)			0,0083		<0,0001		<0,0001
p-value (trip)			0,9843		<0,0001		0,0012
p-value (trip x source)			0,0936		0,0077		0,0257

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different (p < 0,05).

Table 36 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from spinach samples throughout production on Farm A

Source	Trip	Production scenario 1					
		Farm A		Farm A		Farm A	
		Enterobacteriaceae (log CFU/g)		Coliforms (log CFU/g)		*Escherichia coli* (log CFU/g)	
		Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]
Spinach at Harvest	1	5,88 ± 0,10	A	5,73 ± 0,10	AB	0,00 ± 0,00	C
	2	5,00 ± 0,18	A	4,54 ± 0,16	E	0,78 ± 0,48	BC
Spinach bunches at dispatch (packhouse)	1	5,80 ± 0,00	A	5,89 ± 0,25	A	0,00 ± 0,00	C
	2	3,61 ± 1,84	A	4,94 ± 0,44	CDE	0,00 ± 0,00	C
Spinach at receival (packhouse)	1	4,06 ± 2,04	A	5,46 ± 0,61	ABC	0,00 ± 0,00	C
	2	4,49 ± 0,11	A	4,67 ± 0,42	DE	3,22 ± 0,76	A
Spinach after cut	1	6,09 ± 0,05	A	5,55 ± 0,04	ABC	0,00 ± 0,00	C
	2	5,77 ± 0,02	A	5,57 ± 0,05	ABC	1,34 ± 0,69	B
Spinach after wash	1	5,18 ± 0,13	A	4,54 ± 0,56	E	0,00 ± 0,00	C
	2	5,33 ± 0,26	A	5,40 ± 0,07	ABCD	0,00 ± 0,00	C
Spinach after pack	1	5,04 ± 0,22	A	4,98 ± 0,09	BCDE	0,00 ± 0,00	C
	2	5,90 ± 0,08	A	6,00 ± 0,12	A	1,49 ± 0,75	B
Spinach at Retailer	1	5,38 ± 0,18	A	5,39 ± 0,11	ABCD	0,00 ± 0,00	C
	2	6,17 ± 0,10	A	6,16 ± 0,11	A	0,34 ± 0,34	C
Spinach bunches at retailer	1	5,66 ± 0,16	A	5,70 ± 0,10	ABC	0,00 ± 0,00	C
	2	4,89 ± 0,18	A	4,55 ± 0,22	E	0,00 ± 0,00	C
p-value (source)			0,1646		0,0215		0,0012
p-value (trip)			0,3639		0,1412		<0,0001
p-value (trip x source)			0,1627		0,0003		0,0012

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

Table 37 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from contact surface samples throughout production on Farm A

		Production scenario 1					
		Farm A		Farm A		Farm A	
Source	Trip	Enterobacteriaceae (log CFU/cm²)		Coliforms (log CFU/cm²)		*Escherichia coli* (log CFU/cm²)	
		Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]
Crates	1	5,14 ± 0,10	AB	4,79 ± 0,18	AB	0,00 ± 0,00	B
	2	4,02 ± 0,19	AB	3,30 ± 0,35	D	1,21 ± 0,61	AB
Floors	1	4,53 ± 0,48	AB	4,42 ± 0,56	BC	0,00 ± 0,00	B
	2	4,99 ± 0,57	AB	5,57 ± 0,39	A	2,09 ± 0,41	A
Cutting surfaces	1	5,27 ± 0,20	A	5,36 ± 0,26	A	0,00 ± 0,00	B
	2	3,56 ± 0,44	B	3,96 ± 0,28	CD	0,94 ± 0,40	AB
p-value (source)			0,4228		0,1838		0,3326
p-value (trip)			0,0853		0,0222		0,0034
p-value (trip x source)			0,1333		0,0021		0,3326

[a]SE: Standard error
[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).
[c]Within each column, means (based on the trip interactions) followed by the same letters are not significantly different ($p < 0,05$).

The Enterobacteriaceae counts of the borehole water from Farm B were 0.00 log CFU/ml, while the counts of the reservoir dam and irrigation pivot point water samples ranged between 0.78-2.46 log CFU/ml and 0.00-2.49 log CFU/ml, respectively. The Enterobacteriaceae levels showed a significant increase in the borehole source water to the dam reservoir and irrigation pivot point water (p=0.0365) (Table 38). Additionally, the trip independently demonstrated significant differences with Trip 2 having higher Enterobacteriaceae counts than Trip 1 (p=0.0058). The Enterobacteriaceae counts on spinach from Farm B ranged between 0,00-7,05 log CFU/g (Table 39), with a significant difference (p=0,0006) in the trip x source interactions.

The coliform counts of the borehole water were 0.00 log MPN/100 ml, while the coliform counts from the reservoir dam and irrigation pivot point water samples ranged between 2.65-3.84 log MPN/100 ml, and 2.35-3.64 log MPN/100 ml, respectively (Table 38). The coliform counts were significantly different (trip x source interactions p=0,0074). Coliform counts on spinach from Farm B ranged between 0.00-6.65 log CFU/g (Table 39), with significant differences observed (trip x source interactions p=0.0002). Additionally, the coliform counts on the spinach samples from the different points throughout processing had a significant difference (p = 0.0037) with significantly higher coliform counts on spinach at retailer samples than that of the washed spinach samples at the processing facility.

Escherichia coli counts in irrigation water from Farm B were 0.00 log MPN/100 ml in the borehole source water, while the reservoir dam and irrigation pivot point *E. coli* counts ranged between

0.61-4.56 log MPN/100 ml, and 0.00-0.72 log MPN/100 ml, respectively (Table 38). Similar to the Enterobacteriaceae and coliform counts, the *E. coli* counts from water samples were significantly different ($p<0.0001$) (Table 38). During the second sampling trip, the reservoir dam water of Farm B had unacceptable *E. coli* levels according to the South African Department of Water Affairs guidelines for agricultural water used for irrigation of raw vegetables and crops, i.e. <1000 *E. coli* CFU/100 ml (Department of Water Affairs and Forestry [DWAF], 1996). However, the *E. coli* levels measured during the same trip at the irrigation pivot point in the field was significantly lower with levels that were of acceptable quality according to the guidelines (Table 38). *Escherichia coli* counts of the spinach samples from harvest up to the retailer ranged between 0.00-2.00 log CFU/g (Table 5), and were not found to be significantly different ($p = 0.7069$) (Table 39).

Table 38 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from water samples throughout production on Farm B

		Production scenario 2					
Source	**Trip**	**Farm B**		**Farm B**		**Farm B**	
		Enterobacteriaceae (log CFU/ml)		**Coliforms (log MPN/100 ml)**		***Escherichia coli* (log MPN/100 ml)**	
		Mean ± SE[a]	**t-Test[b]**	**Mean ± SE[a]**	**t-Test[b]**	**Mean ± SE[a]**	**t-Test[b]**
Dam (Source)	1	0,00 ± 0,00	C	0,00 ± 0,00	D	0,00 ± 0,00	D
	2	0,00 ± 0,00	C	0,00 ± 0,00	D	0,00 ± 0,00	D
Dam (Reservoir)	1	1,23 ± 0,27	B	2,71 ± 0,03	BC	0,84 ± 0,12	B
	2	2,46 ± 0,00	A	3,77 ± 0,05	A	4,40 ± 0,09	A
Irrigation pivot point	1	1,09 ± 0,56	B	2,45 ± 0,09	C	0,50 ± 0,12	C
	2	2,36 ± 0,05	A	3,09 ± 0,28	B	0,00 ± 0,00	D
Washwater	1	0,00 ± 0,00	C	0,00 ± 0,00	D	0,00 ± 0,00	D
	2	0,00 ± 0,00	C	0,00 ± 0,00	D	0,00 ± 0,00	D
p-value (source)			<0,0001		<0,0001		<0,0001
p-value (trip)			0,0058		0,0015		<0,0001
p-value (trip x source)			0,0365		0,0074		<0,0001

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

Table 39 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from spinach samples throughout production on Farm B

		Production scenario 2					
Source	**Trip**	**Farm B Enterobacteriaceae (log CFU/g)**		**Farm B Coliforms (log CFU/g)**		**Farm B *Escherichia coli* (log CFU/g)**	
		Mean ± SE[a]	**t-Test[b]**	**Mean ± SE[a]**	**t-Test[b]**	**Mean ± SE[a]**	**t-Test[b]**
Spinach at Harvest	1	4,10 ± 0,26	CDE	3,33 ± 0,87	G	0,40 ± 0,40	A
	2	5,34 ± 0,08	AB	5,16 ± 0,12	BCD	0,00 ± 0,00	A
Spinach at Dispatch (crates)	1	4,46 ± 0,79	BCDE	4,03 ± 0,91	DEFG	0,00 ± 0,00	A
	2	4,74 ± 0,24	BC	4,73 ± 0,14	CDE	0,00 ± 0,00	A
Spinach punnets at dispatch (packhouse)	1	4,56 ± 2,28	BCD	6,57 ± 0,07	A	0,00 ± 0,00	A
	2	3,50 ± 1,36	DEF	4,84 ± 0,07	BCDE	0,00 ± 0,00	A
Spinach at Receival (processing facility)	1	4,90 ± 0,25	BC	3,92 ± 0,16	EFG	0,00 ± 0,00	A
	2	5,56 ± 0,15	AB	5,20 ± 0,24	BCD	0,00 ± 0,00	A
Spinach punnets at receival (processing facility)	1	-	-	-	-	-	-
	2	5,35 ± 0,15	AB	5,05 ± 0,28	BCDE	0,00 ± 0,00	A
Spinach after wash	1	3,33 ± 0,21	EF	3,49 ± 0,31	FG	0,00 ± 0,00	A
	2	5,42 ± 0,61	AB	4,35 ± 0,04	CDEFG	0,00 ± 0,00	A
Spinach after pack	1	2,47 ± 1,24	F	3,39 ± 0,27	G	0,00 ± 0,00	A
	2	4,82 ± 0,13	BC	4,84 ± 0,32	BCDE	0,00 ± 0,00	A
Spinach at Retailer	1	5,37 ± 0,08	AB	5,43 ± 0,14	ABC	0,00 ± 0,00	A
	2	4,49 ± 0,28	BCDE	4,43 ± 0,24	CDEFG	0,00 ± 0,00	A
Spinach punnets at retailer	1	6,10 ± 0,14	A	5,99 ± 0,14	AB	0,00 ± 0,00	A
	2	5,14 ± 0,33	ABC	4,65 ± 0,28	CDEF	0,00 ± 0,00	A
p-value (source)			0,4192		0,0037		0,7439
p-value (trip)			0,1034		0,3915		0,3488
p-value (trip x source)			0,0006		0,0002		0,7069

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

The Enterobacteriaceae counts from Farm C ranged between 2.41-3.23 log CFU/ml and 0.00-1.71 log CFU/100 ml in the borehole source and irrigation water samples, respectively (Table 40). Enterobacteriaceae counts per trip were significantly lower ($p<0.0001$) in the irrigation pivot point water compared to the initial borehole source water (Table 40). The Enterobacteriaceae levels on spinach from Farm C ranged from 0.00-7.07 log CFU/g (Table 41). There were significant differences ($p<0.0001$) in the Enterobacteriaceae enumerated from the spinach samples on Farm C (Table 41). From the different sources of spinach samples throughout processing, the Enterobacteriaceae counts differed significantly ($p=0.0042$) between washed and unwashed spinach at retail. However, when compared to the counts on the spinach at harvest, neither the counts on the washed nor unwashed retail spinach samples differed significantly implying the washing of the baby spinach leaves seems to have no beneficial effect (Table 41). Additionally, the trip interactions showed a significant difference with trip two having significantly higher Enterobacteriaceae counts on spinach ($p<0.0001$).

Coliform counts in the irrigation water from Farm C ranged between 4.44-5.44 log MPN/100 ml and 0.93-2.44 log MPN/100 ml in the borehole source and irrigation pivot point water samples, respectively (Table 40). Although the trip x source water coliform count interactions on Farm C were not significantly different (p=0,0804), the coliform counts from samples from the sources had a significant difference (p<0.0001) with counts from the irrigation pivot point water significantly lower than that of the source water in the dam (Table 40). Additionally, coliform count interactions between the two trips were significantly different (p=0.0166) (Table 40), with Trip 1 demonstrating higher coliform counts. The coliform counts on spinach from Farm C ranged between 1.04-7.01 log CFU/g (Table 41). The coliform counts on spinach samples differed significantly (p<0.0001) (Table 41).

On Farm C, *E. coli* was enumerated in low levels during trip one from the source dam water (borehole) only, with counts ranging between 0,00-0,61 log MPN/100 ml. The *E. coli* from the water samples were significantly different (p=0.0014) (Table 40), with water from the dam source being significantly higher during Trip 1. *Escherichia coli* counts on spinach from Farm C ranged between 0.00-3.70 log CFU/g (Table 41), with no significant difference (p = 0,6166) on *E. coli* levels on spinach from harvest up to retail (Table 41).

In the second production scenario, swab samples were only taken from the cutting surfaces of the packhouse on Farm C and ranged from 0.00-4.93 log CFU/cm^2. A significant difference (p=0,045) was observed for the coliform levels between the two trips, with Trip 1 having higher coliform counts (Table 42). No *E. coli* was enumerated from the contact surfaces. Similar to the coliform levels, the Enterobacteriaceae levels from the cutting surface swab samples differed significantly (p=0,0333) between the two trips (Table 42).

The composite soil samples of the three farms had similar mean counts with Enterobacteriaceae that ranged from 3.29-5.22 log CFU/g, coliform counts that ranged from 3.05-5.19 log CFU/g, and with no *E. coli* enumerated from soil on any of the farms.

Table 40 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from water samples throughout production on Farm C

Source	Trip	Production scenario 2					
		Farm C		Farm C		Farm C	
		Enterobacteriaceae (log CFU/ml)		Coliforms (log MPN/100 ml)		*Escherichia coli* (log MPN/100 ml)	
		Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]
Dam (Source)	1	3,21 ± 0,01	A	5,24 ± 0,10	A	0,51 ± 0,10	A
	2	2,45 ± 0,02	B	4,46 ± 0,01	B	0,00 ± 0,00	B
Irrigation pivot point	1	1,41 ± 0,15	C	2,28 ± 0,09	C	0,00 ± 0,00	B
	2	0,00 ± 0,00	D	1,44 ± 0,44	D	0,00 ± 0,00	B
Washwater	1	0,00 ± 0,00	D	0,10 ± 0,10	E	0,00 ± 0,00	B
	2	0,00 ± 0,00	D	0,20 ± 0,20	E	0,00 ± 0,00	B
p-value (source)			<0,0001		<0,0001		0,0014
p-value (trip)			<0,0001		0,0166		0,0027
p-value (trip x source)			<0,0001		0,0804		0,0014

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

Table 41 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from spinach samples throughout production on Farm C

Source	Trip	Farm C		Farm C		Farm C	
		Enterobacteriaceae (log CFU/g)		Coliforms (log CFU/g)		*Escherichia coli* (log CFU/g)	
		Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]	Mean ± SE[a]	t-Test[b]
Spinach at Harvest	1	3,92 ± 0,11	G	3,93 ± 0,10	D	0,00 ± 0,00	A
	2	6,03 ± 0,55	ABC	6,36 ± 0,35	AB	0,74 ± 0,74	A
Spinach punnets at dispatch (packhouse)	1	6,17 ± 0,30	AB	6,11 ± 0,22	ABC	0,00 ± 0,00	A
	2	5,66 ± 0,03	BCDE	5,66 ± 0,12	BC	0,00 ± 0,00	A
Spinach at receival (packhouse)	1	3,32 ± 1,67	G	3,37 ± 1,19	D	0,57 ± 0,57	A
	2	3,33 ± 1,68	G	4,01 ± 1,48	D	0,57 ± 0,57	A
Spinach at receival (processing facility)	1	4,66 ± 0,28	F	4,06 ± 0,06	D	0,00 ± 0,00	A
	2	5,30 ± 0,05	DEF	6,03 ± 0,37	ABC	0,00 ± 0,00	A
Spinach punnets at receival (processing facility)	1	6,52 ± 0,11	A	4,06 ± 0,06	E	0,00 ± 0,00	A
	2	5,32 ± 0,07	CDEF	4,01 ± 1,48	D	0,00 ± 0,00	A
Spinach after wash	1	3,35 ± 0,06	G	4,06 ± 0,61	D	0,00 ± 0,00	A
	2	4,95 ± 0,15	EF	5,87 ± 0,08	ABC	0,00 ± 0,00	A
Spinach after pack	1	3,84 ± 0,06	G	3,86 ± 0,09	D	0,00 ± 0,00	A
	2	5,48 ± 0,23	BCDE	5,80 ± 0,14	ABC	0,00 ± 0,00	A
Spinach at Retailer	1	3,72 ± 0,07	G	3,84 ± 0,05	D	0,00 ± 0,00	A
	2	5,27 ± 0,15	DEF	5,35 ± 0,17	C	0,00 ± 0,00	A
Spinach punnets at retailer	1	6,57 ± 0,09	A	6,64 ± 0,10	A	0,00 ± 0,00	A
	2	5,90 ± 0,19	ABCD	5,73 ± 0,10	ABC	0,80 ± 0,49	A
p-value (source)			0,0042		0,0006		0,6275
p-value (trip)			<0,0001		<0,0001		0,1109
p-value (trip x source)			<0,0001		<0,0001		0,6166

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

Table 42 Enterobacteriaceae, coliforms and *Escherichia coli* enumerated from contact surface samples throughout production on Farm C

Source	Trip	Farm C		Farm C		Farm C	
		Enterobacteriaceae (log CFU/cm^2)		Coliforms (log CFU/cm^2)		*Escherichia coli* (log CFU/cm^2)	
		Mean ± SE[a]	t-Test[c]	Mean ± SE[a]	t-Test[c]	Mean ± SE[a]	t-Test[c]
Cutting surfaces	1	2,85 ± 0,41	A	0,91 ± 0,91	A	0,00 ± 0,00	A
	2	5,71 ± 0,29	B	4,93 ± 0,06	B	0,00 ± 0,00	A
p-value (source)			-		-		-
p-value (trip)			0,0333		0,045		-
p-value (trip x source)			-		-		-

[a]SE: Standard error

[b]Within each column, means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

[c]Within each column, means (based on the trip interactions) followed by the same letters are not significantly different ($p < 0,05$).

Detection of foodborne pathogens. Overall, 65/288 samples (22.57%) were found to be contaminated with *E. coli* after enrichment. From the two spinach production scenarios, a total of 80 *E. coli* isolates were recovered. This included 35 isolates from the first production scenario from water (n=13), fresh produce (n=14), soil (n=1) and contact surfaces (n=7), whilst the 45 *E. coli* isolates recovered from the second production scenario were from water (n=29) and fresh produce (n=16). Only one *E. coli* isolate, from the holding dam water in the first production scenario, was positive for the stx2 virulence gene, whilst none of the other *E. coli* isolates were positive for any of the diarrheagenic virulence genes tested for. From the first production scenario, *Salmonella* spp. isolates (n=11) were recovered from two river samples, one holding dam and one irrigation water sample, respectively. No *Listeria* spp. were isolated from any of the samples tested.

Phenotypic antimicrobial resistance profiling of *Escherichia coli* isolates. Of the 80 *E. coli* isolates recovered, 95.00% showed resistance against at least one antimicrobial agent, this included resistance to aminoglycosides (73.42%), cephalosporins (50.62%), penicillins (44.30%), tetracycline (37.98%), sulfonamides (21.52%), chloramphenicol (15.19%) and carbapenems (5.06%). Overall, a greater percentage of resistance phenotypes were from water *E. coli* isolates (52.50%), followed by isolates from spinach (37.50%) and contact surfaces (10.00%). Multidrug resistance was observed in 35/80 (43.75%) of the isolates; 26.30% from production scenario one and 17.50% from the second production scenario, where borehole water was used for irrigation (Table 43). The multidrug resistant *E. coli* isolates predominantly showed, in the β-lactam group, resistance to penicillins (66.3%), followed by 4th generation cephalosporins (61.3%) and carbapenems (11.3%). Multidrug resistant

phenotypes predominantly included resistance profiles of β-lactams combined with aminoglycosides, followed by β-lactams combined with tetracyclines, sulfonomides, and chloramphenicol, respectively (Table 43).

ERIC-PCR and antimicrobial resistance clustering analysis of *Escherichia coli* isolates. At a 70% similarity cut-off, cluster analysis of ERIC-PCR DNA fingerprints generated 7 distinct *E. coli* profiles for the 35 isolates from the first production scenario (Figure 12 A-G). The largest cluster (Cluster A) included *E. coli* isolates (n=24) from water, soil, spinach from farm to retail, as well as contact surfaces through processing. Several water and contact surface samples, as well as spinach at different points throughout production and irrigation water samples clustered together in cluster A with ≥94.0% similarity values. Cluster B included isolates from spinach at different points in the packhouse and irrigation water with similarity values of 78.0%. Similarly, cluster C included an *E. coli* isolate from spinach after cut that was 72.0% similar to a river water isolate. Cluster D was composed of two *E. coli* isolates from spinach (at harvest and at retail) at similarity values >90.0%, whilst in cluster F, two *E. coli* isolates from the river and holding dam water clustered together at 75.0% similarity. Cluster G consisted of a single *E. coli* isolate from the floor swab samples.

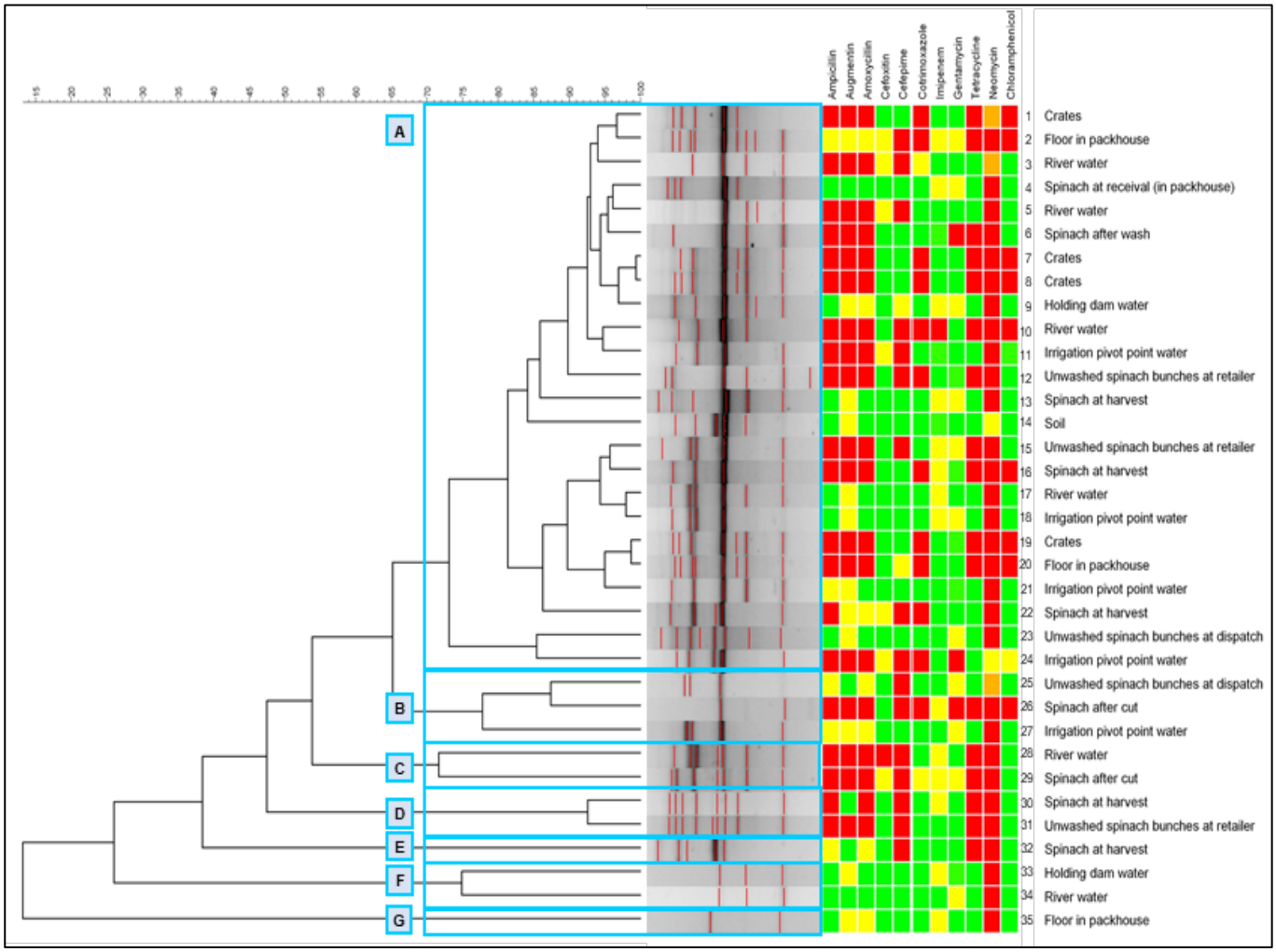

Figure 12: Dendrogram showing the genetic relatedness of *Escherichia coli* isolates from irrigation water sources (river, holding dam, and irrigation pivot point), soil, spinach (at harvest, throughout processing and at retail) and contact surfaces throughout spinach production.

Table 43 Summary of number of antibiotics, antibiotic resistance patterns and antibiotic classes to which ESBL/AmpC-producing Enterobacteriaceae isolates from irrigation water, soil, spinach and lettuce samples were resistant

No of antimicrobials to which isolates were resistant	No of isolates (n=79)	No of isolates per production scenario		No of isolates with specific pattern	Most frequent patterna	No of antibiotic classes to which isolates were resistant	Antibiotic class(es)
		Production scenario 1	Production scenario 2				
0	4	1	3	4			
		11	6	17	NE10C	1	Aminoglycosides
1	22	1	3	4	CPM30C	1	Cephalosporins
			1	1	A10C	1	Penicillins
			2	2	GM10C - NE10C	1	Aminoglycosides
			3	3	T30C - NE10C	2	Tetracyclines, Aminoglycosides
			1	1	NE10C - C30C	2	Aminoglycosides, Chloramphenicol
2	10		1	1	FOX30C - NE10C	2	Cephalosporins, Aminoglycosides
			1	1	CPM30C - T30C	2	Cephalosporins, Tetracyclines
			1	1	A10C - CPM30C	2	Penicillins, Cephalosporins
			1	1	TS25C - T30C	2	Sulfonomides, Tetracyclines
			1	1	FOX30C - GM10C - NE10C	2	Cephalosporins, Aminoglycosides
			1	1	CPM30C - GM10C - NE10C	2	Cephalosporins, Aminoglycosides
3	5		1	1	GM10C - T30C - NE10C	2	Aminoglycosides, Tetracyclines
			1	1	AP10C - A10C - CPM30C	2	Penicillins, Cephalosporins
		1		1	CPM30C - T30C - NE10C	3	Cephalosporins, Tetracyclines, Aminoglycosides
			2	2	FOX30C - CPM30C - GM10C - NE10C	2	Cephalosporins, Aminoglycosides
		1		1	AP10C - AUG30C - A10C - CPM30C	2	Penicillins, Cephalosporins
			1	1	AP10C - A10C - GM10C - C30C	3	Penicillins, Aminoglycosides, Chloramphenicol
4	8		1	1	AUG30C - A10C - CPM30C - NE10C	3	Penicillins, Cephalosporins, Aminoglycosides
			1	1	AP10C - A10C - FOX30C - CPM30C	2	Penicillins, Cephalosporins
			1	1	AP10C - A10C - CPM30C - TS25C	3	Penicillins, Cephalosporins, Sulfonomides
		1		1	AP10C - CPM30C - TS25C - NE10C	4	Penicillins, Cephalosporins, Sulfonomides, Aminoglycosides
			1	1	AP10C - AUG30C - A10C - FOX30C - CPM30C	2	Penicillins, Cephalosporins
		2		2	AP10C - AUG30C - A10C - CPM30C - NE10C	3	Penicillins, Cephalosporins, Aminoglycosides
			1	1	AP10C - A10C - CPM30C - GM10C - NE10C	3	Penicillins, Cephalosporins, Aminoglycosides
			1	1	FOX30C - CPM30C - IMI10C - GM10C - NE10C	3	Cephalosporins, Carbapenems, Aminoglycosides
			1	1	AP10C - A10C - FOX30C - CPM30C - T30C	3	Penicillins, Cephalosporins, Tetracyclines
5	11	1		1	AP10C - A10C - CPM30C - T30C - NE10C	4	Penicillins, Cephalosporins, Tetracyclines, Aminoglycosides
			1	1	AP10C - A10C - CPM30C - T30C - C30C	4	Penicillins, Cephalosporins, Tetracyclines, Chloramphenicol
			1	1	AP10C - A10C - FOX30C - T30C - NE10C	4	Penicillins, Cephalosporins, Tetracyclines, Aminoglycosides
			1	1	CPM30C - IMI10C - GM10C - T30C - NE10C	4	Cephalosporins, Carbapenems, Aminoglycosides, Tetracyclines
		1		1	CPM30C - TS25C - T30C - NE10C - C30C	5	Cephalosporins, Sulfonomides, Tetracyclines, Aminoglycosides, Chloramphenicol

Table 43 cont.

No of antimicrobials to which isolates were resistant	No of isolates (n=79)	No of isolates per production scenario		No of isolates with specific pattern	Most frequent patterna	No of antibiotic classes to which isolates were resistant	Antibiotic class(es)
		Production scenario 1	Production scenario 2				
6	7	1		1	AP10C - AUG30C - A10C - GM10C - T30C - NE10C	3	Penicillins, Aminoglycosides, Tetracyclines
		3		3	AP10C - AUG30C - A10C - CPM30C - T30C - NE10C	4	Penicillins, Cephalosporins, Tetracyclines, Aminoglycosides
		1		1	AP10C - AUG30C - A10C - TS25C - T30C - C30C	4	Penicillins, Sulfonamides, Tetracyclines, Chloramphenicol
		1		1	AP10C - AUG30C - A10C - CPM30C - TS25C - GM10C	4	Penicillins, Cephalosporins, Sulfonomides, Aminoglycosides
			1	1	AP10C - A10C - TS25C - IMI10C - T30C - NE10C	5	Penicillins, Sulfonamides, Carbapenems, Tetracyclines, Aminoglycosides
7	9	1		1	AP10C - AUG30C - A10C - FOX30C - CPM30C - T30C - NE10C	4	Penicillins, Cephalosporins, Tetracyclines, Aminoglycosides
		5		5	AP10C - AUG30C - A10C - TS25C - T30C - NE10C - C30C	5	Penicillins, Sulfonamides, Tetracyclines, Aminoglycosides, Chloramphenicol
		1		1	AP10C - AUG30C - A10C - CPM30C - TS25C - T30C - NE10C	5	Penicillins, Cephalosporins, Sulfonamides, Tetracyclines, Aminoglycosides
			1	1	AP10C - A10C - CPM30C - TS25C - GM10C - T30C - NE10C	5	Penicillins, Cephalosporins, Sulfonamides, Aminoglycosides, Tetracyclines
			1	1	AP10C - AUG30C - A10C - CPM30C - TS25C - T30C - C30C	5	Penicillins, Cephalosporins, Sulfonamides, Tetracyclines, Chloramphenicol
8	1		1	1	AP10C - AUG30C - A10C - FOX30C - CPM30C - TS25C - GM10C - NE10C	4	Penicillins, Cephalosporins, Sulfonamides, Aminoglycosides
9	2	1		1	AP10C - AUG30C - A10C - CPM30C - TS25C - GM10C - T30C - NE10C - C30C	6	Penicillins, Cephalosporins, Sulfonamides, Aminoglycosides, Tetracyclines, Chloramphenicol
		1		1	AP10C - AUG30C - A10C - CPM30C - TS25C - IMI10C - T30C - NE10C - C30C	7	Penicillins, Cephalosporins, Sulfonamides, Carbapenems, Tetracyclines, Aminoglycosides, Chloramphenicol

The *E. coli* ERIC-PCR DNA fingerprints in the second production scenario generated 12 distinct clusters. This included seven clusters in the supply chain from the first supplier, Farm B (Figure 13 A-G) and five clusters in the supply chain from the second supplier, Farm C (Figure 13 H-L). Cluster E was composed of three *E. coli* isolates from the irrigation pivot point and spinach at retailer, with 86.0% similarity values. In cluster F, several *E. coli* isolates from the water reservoir, spinach at receival in the packhouse as well as washed and unwashed retail spinach clustered together at similarity values ranging from 73.0-99.0%. In cluster I, 3 *E. coli* isolates from the washed and unwashed spinach product lines at the retailer clustered together with 92.0% similarity. Clusters K consisted of 9 *E. coli* isolates, including 3 spinach at receival isolates and 1 holding dam isolate with 94.0% similarity. Furthermore, *E. coli* isolates from spinach at harvest, holding dam (source water) and the unwashed spinach at retailer had 98.0% similarity. The 5 isolates in cluster L included 3 *E. coli* isolates from spinach at harvest, and holding dam (source) water with 90.0% similarity.

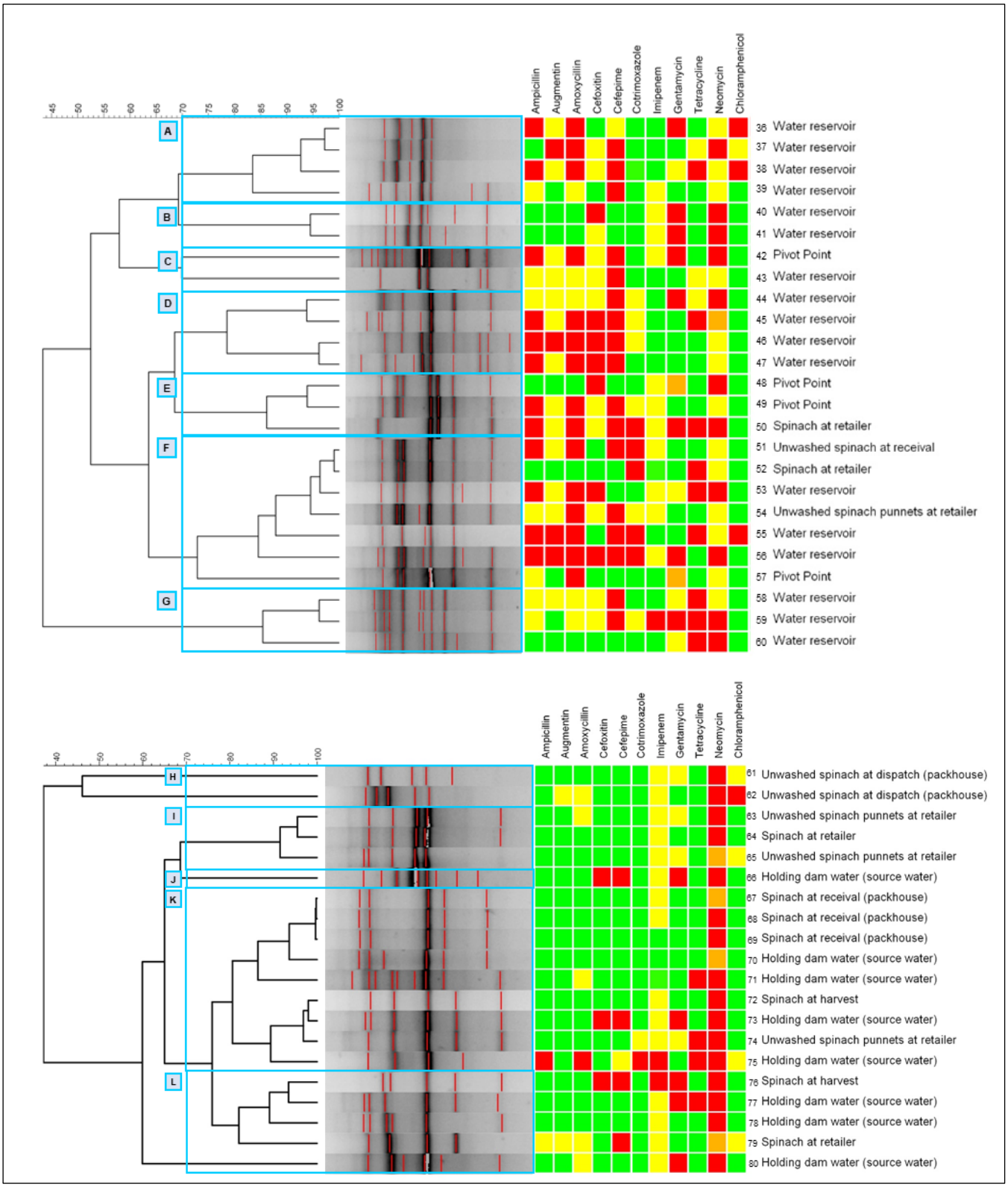

Figure 13: Dendrogram showing the genetic relatedness of *Escherichia coli* isolates from irrigation water sources (borehole water sources) and spinach (at harvest, throughout processing and at retail) from two farms supplying spinach to a central processing facility.

Discussion

To the authors knowledge, this is the first study in South Africa where complete spinach production systems with different irrigation water sources from the farm, throughout processing and up to retail, were investigated for the presence of foodborne pathogens. The coliform counts on spinach in the present study were similar to other South African studies including coliform counts obtained from baby spinach from a farm and processing facility supplying retailers (Jongman *et al*., 2016) and an exploratory study of spinach obtained from formal and informal markets (Du Plessis *et al*., 2017).

Internationally, no consensus exist regarding the microbiological standards that apply to ready-to-eat minimally processed vegetables (Health Protection Agency, 2009; [Food Safety Authority of Ireland (FSAI), 2016]; Fresh Produce Safety Centre Australia & New Zealand [FPSC A-NZ], 2019). Furthermore, regulations or standards specifying the microbial critical limits for irrigation water, water for washing whole fresh produce or water used during fresh produce processing also differ, or lack entirely [Department of Water Affairs and Forestry (DWAF), 1996]; FPSC A-NZ, 2019).

The presence of *E. coli* on spinach (or leafy green vegetables) has been studied worldwide (Buyukunal *et al*., 2015; Cardamone *et al*., 2015; Korir *et al*., 2016). The guidelines for *E. coli* count limits for fresh vegetables however differ for each country. Where river water had been directly applied via overhead irrigation in the current study, *E. coli* was enumerated from the spinach samples, contact surfaces and from the river, irrigation, and washwater throughout the supply chain. These results correspond to previous studies indicating that enteric bacteria can be transferred onto irrigated produce via irrigation with polluted water (Du Plessis *et al*., 2015, Ijabadeniyi *et al*., 2012). Similar to previous South African studies (Gemmell and Schmidt, 2012; Du Plessis *et al*., 2015; Jongman and Korsten, 2016), the river water *E. coli* levels from the first production scenario in the current study exceeded acceptable levels according to the South African Department of Water Affairs guidelines of <1 to 1000/100 ml for irrigation water of vegetables to be eaten raw (DWAF, 1996). In the second production scenario where borehole water was used for irrigation, the *E. coli* levels in the source water from the first supplier farm (Farm B) was found to meet the current irrigation water standard (DWAF, 1996; Du Plessis *et al*., 2017), however, *E. coli* levels in the holding dam water did not meet this requirement, reiterating that different pre-harvest production methods may affect the microbiological quality of produce. Similarly, *E. coli* levels from the second supplier farm in production scenario two

from the current study was also acceptable according to the South African Department of Water Affairs guidelines of <1 to 1000/100 ml for irrigation water of vegetables to be eaten raw. After enrichment, generic *E. coli* was isolated from 40.30% and 14.60% of water and spinach samples respectively, which is lower than the 84.80% and 38.30% generic *E. coli* prevalence in irrigation water and lettuce samples in Brazil (Decol *et al.*, 2017). The results of this study further reiterates the importance of irrigation water as contamination source of leafy green vegetables in accordance to previous studies also showing the potential link between the microbiological quality of irrigation water and contamination of fresh vegetables (Du Plessis *et al*., 2015; Jongman and Korsten, 2016a). Similar to Du Plessis *et al.* (2015) and Decol *et al*. (2017), more irrigation water samples in the current study were found to be contaminated with *E. coli* than fresh produce samples.

Similar to Vital *et al*. (2018), more antimicrobial resistant *E. coli* isolates were detected from irrigation water (52.5%) than from spinach (37.5%) isolates. Resistance to antibiotics that are traditionally first-line drug treatment options for gastrointestinal infections (tetracycline, ampicillin and cotrimoxazole) (Alanazi *et al*., 2018; Kim *et al*., 2019) were observed in *E. coli* isolates from both irrigation water and spinach in the current study. Multidrug resistant *E. coli* isolates were more prevalent in irrigation water isolates compared to spinach and contact surface isolates, similar to results reported by Vital *et al*. (2018).

The ERIC-PCR profiles from the current study showed high similarity values (> 90.0%) for irrigation water *E. coli* isolates and spinach *E. coli* isolates at different points of production, processing or retail of each of the respective supply chains which is similar to results reported by Du Plessis *et al.* (2015) have highlighted the link between irrigation water quality and microbiological quality of onions. Cluster analysis in each spinach supply chain (regardless of the water source and overall microbiological quality of the irrigation water) showed *E. coli* isolates from irrigation water clustering together with *E. coli* from spinach at retail at similarity of at least 85.0%. This indicates that contamination that occur on the farm can influence the safety of the final product at retail, regardless of processing steps followed through production.

Conclusion

The results from this study provide valuable background information regarding the prevalence of antimicrobial resistant *E. col*i throughout spinach production from the farm, during processing and at retail. The necessity of using clean and safe irrigation water was highlighted

with the need for standardised microbiological safety parameters for irrigation water of ready-to-eat fresh vegetables, as a link between *E. coli* from irrigation water and spinach at different points of the respective production systems were shown.

4.2.4 Occurrence, phenotypic and molecular characterisation of extended-spectrum- and AmpC- β-lactamase-producing Enterobacteriaceae isolated from selected commercial spinach supply chains in South Africa

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Specific aim: This study aimed to determine the presence of ESBL/AmpC-producing Enterobacteriaceae in typical commercial spinach production systems from the farm to retail, and to characterise the isolated strains by (i) phenotypic antimicrobial resistance profiles, (ii) identification of ESBL/AmpC genetic determinants, and (iii) detection of Class 1, 2 and 3 integrons.

Experimental procedures

Details of (ESBL) and AmpC-producing Enterobacteriaceae detection, isolation, identification and antimicrobial resistance characterisation (phenotypic and genotypic) were described in Chapter 3, section 3.4.3, 3.4.4 and 3.4.5.

Results

Isolation and identification of presumptive ESBL/AmpC-producing Enterobacteriaceae isolates. Presumptive ESBL/AmpC-producing Enterobacteriaceae (n=59) from the selective chromogenic media belonged to six genera including *Escherichia*, *Klebsiella*, *Serratia*, *Rahnella*, *Salmonella*, and *Enterobacter*, with MALDI-TOF analysis. This included isolates from the water (n=20), fresh produce (n=35) and contact surface samples (n=4), while no presumptive ESBL/AmpC-producing Enterobacteriaceae isolates were recovered from the soil samples.

Prevalence of ESBL/AmpC-producing Enterobacteriaceae and antimicrobial susceptibility testing. In total, screening using DDST, 48/59 (81.36%) isolates tested positive

for ESBL production (Figure 14). All cefoxitin resistant isolates (20/59) were additionally screened with the AmpC detection set of which 11/20 (55%) tested positive (Figure 15). From the 48 ESBL/AmpC-producing isolates, 16 isolates were from water and 32 from produce samples. Irrigation water isolates (n=15) included *E. coli* (14.58%) and *Serratia fonticola* (6.25%) from both scenarios, while *K. pneumoniae* (6.25%) and *Salmonella* spp. (4.17%) were isolated only from scenario 1 where river water was used for irrigation. Isolates from the spinach at harvest and throughout processing (n=13) included predominantly *S. fonticola* (16.67%), followed by *K. pneumoniae* (4.17%), *Rahnella aquatilis* (4.17%) and *E. coli* (2.08%). From the retailer spinach isolates (n=19), ESBL/AmpC-producing *S. fonticola* (16.67%), K. *pneumoniae* (8.33%), *R. aquatilis* (6.25%), *E. coli* (4.17%) and *Enterobacter asburiae* (2.08%) were recovered. One *R. aquatilis* isolate was also recovered from the washwater used during processing in scenario 1 (Figure14).

Multidrug resistance was observed in 98% of the confirmed ESBL/AmpC-producing isolates, including 16 and 31 isolates from water and fresh produce, respectively (Figure 14). Resistance to the aminoglycoside (89.58%) and chloramphenicol (79.17%) classes were dominant. In the β-lactam group, further analysis showed resistance against amoxicillin (31.25% in water and 66.67% in produce), followed by ampicillin (29.17% in water and 66.67% in produce), augmentin (29.17% in water and 52.08% in produce), and cefoxitin (14.58% in water and 27.08% in produce). The resistance rate to carbapenems (imipenem) were 8.33% and 4.17% in water and produce, respectively, with 10.42% and 41.67% of the water and produce isolates that showed intermediate resistance to imipenem. Resistance to other antibiotics included cotrimoxazole (22.92% in water and 29.17% in produce) and tetracycline (22.92% in water and 27.08% in produce).

Genotypic antibiotic resistance profiling. Genes encoding β-lactamases were detected in 29/48 (60.42%) isolates obtained from water and produce samples, mainly in *S. fonticola* (n=13), followed by *E. coli* (n=7) and *K. pneumoniae* (n=5). The most frequently detected β-lactamase genes were $bla_{\text{CTX-M}}$ (n=25), followed by bla_{TEM} (n=18), bla_{SHV} (n=17) and bla_{OXA} (n=12). Extended-spectrum β-lactamase variants encoded by blaCTX-M Group 1 included CTX-M-3, CTX-M-12, and CTX-M-15 amongst others, whilst blaCTX-M Group 9 encoded for CTX-M-14. The bla_{TEM} sequences encoded the broad-spectrum β-lactamase TEM-1 and TEM-234. The bla_{SHV} sequences encoded SHV-187, SHV-203 or SHV-61. All the blaOXA sequences encoded broad-spectrum β-lactamases OXA-1. Only the CIT family (identified as

bla$_{CMY}$ variants) of AmpC genetic determinants was detected in six *S. fonticola* isolates from scenario 2 (Figure 14).

Detection of integrons. The integrase 1 gene (IntI1) was detected in 23/48 (47.92%) of the isolates, predominantly in *S. fonticola* (n=11), followed by *K. pneumoniae* (n=6), *R. aquatilis* (n=2), *E. coli* (n=3), and one *E. asburiae* isolate. The IntI3 gene associated with class 3 integrons were detected in 35/48 (72.92%) of the isolates, including *S. fonticola* (n=16), six *E. coli*, six *K. pneumoniae*, five *R. aquatilis*, and one *E. asburiae* and *Salmonella* spp. isolate, respectively. Both the class 1 and class 3 integrase genes were detected in 29 isolates, which included *S. fonticola* (n=9), *K. pneumoniae* (n=5), *E. coli* (n=3), *R. aquatilis* (n=2) and *E. asburiae* (n=1). Class 2 integrons were not detected in any of the isolates (Figure 14).

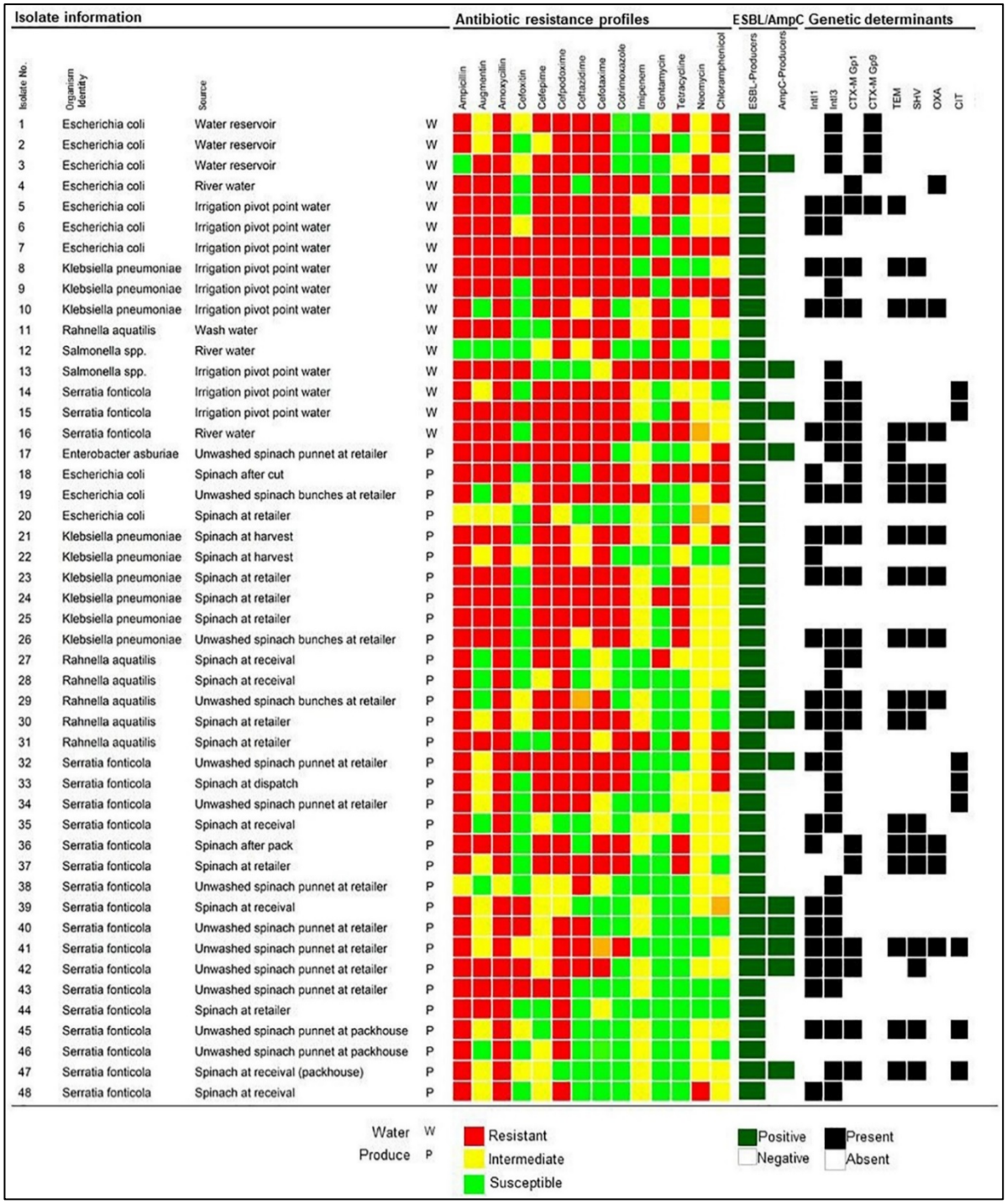


Figure 14: Extended-Spectrum- and AmpC-β-Lactamase-producing Enterobacteriaceae isolated from water, spinach and contact surface sources, indicating the phenotypic antibiotic resistance profiles and the detection of ESBL and/or AmpC, and integron genetic determinants. The colour code of the antimicrobial resistance profiles indicate the resistant, intermediate resistant or susceptible phenotypes to specific antibiotics from seven different classes. ESBL/AmpC production is indicated as positive or negative *detection* of genetic determinants indicated as present or absent.

Discussion

This study documents the prevalence of ESBL/AmpC-producing Enterobacteriaceae in spinach production, from the agricultural environment, during processing, and subsequent retailed products in South Africa. Overall, six ESBL/AmpC-producing Enterobacteriaceae genera, including environmental bacteria (*S. fonticola* and *R. aquatilis*), and potential human pathogens (*E. coli*, *K. pneumoniae*, *Salmonella* spp. and *E. asburiae*) were detected from 42 of the 288 samples. From the first production scenario, ESBL-producing potential pathogenic Enterobacteriaceae were mainly isolated, whereas the predominance of ESBL-producing *S. fonticola* from the second production scenario correspond to environmental ESBL-producing Enterobacteriaceae previously reported (Blaak *et al.*, 2014).

Irrigation water is a known source of antimicrobial resistant bacterial contamination in fresh produce production (Vital *et al.*, 2018). In both spinach production scenarios, the prevalence of ESBL/AmpC-producing Enterobacteriaceae (n=48) was higher in samples from produce (29.17% and 37.5%, respectively) than river (20.83%) and borehole (10.42%) water. Similarly, Njage and Buys (2014) reported highest prevalence of ESBL-producing *E. coli* isolates in fresh produce (lettuce) at harvest (90%), followed by different irrigation water (canal, 73% and river, 6%) samples in South Africa. In contrast, 100% irrigation water samples and only 14.7% of the harvested lettuce samples were found to be positive for ESBL/AmpC-producing environmental Enterobacteriaceae in the Netherlands (Blaak *et al.*, 2014). The 20.83% (10/48) occurrence of ESBL/AmpC-producing isolates from river irrigation water was higher than the 13.2% reported in a similar study from river water in China (Ye *et al*., 2017). Potential pathogenic ESBL-producing *K. pneumoniae*, E. coli and *Salmonella* spp. found in our river water samples were similar to the ESBL-producing potential pathogenic *E. coli*, *Citrobacter freundii* and *K. pneumoniae* reported by Ye *et al.* (2017). In contrast to Zekar *et al.* (2017) ESBL/AmpC-producing isolates (*E. coli* and *S. fonticola*) occurred in 10.4% borehole irrigation water samples from the second production scenario. The occurrence of ESBL/AmpC-producing Enterobacteriaceae on all our spinach samples increased from 6.25% at harvest, to 34.38% after processing, up to 59.36% in retail spinach samples in both production scenarios. Furthermore, an increase in species diversity from harvested, to processed-, and subsequent retail spinach were also observed. The identified species on retailer spinach samples included ESBL/AmpC-producing *K. pneumoniae*, *S. fonticola*, *R. aquatilis, E. coli* and *E. asburiae*, similar to other studies (Ye *et al.*, 2017; Zekar *et al*., 2017; Richter *et al.*, 2019). Interestingly, no ESBL/AmpC-producing Enterobacteriaceae isolates were detected

in soil samples from any of the farms analysed in the current study, which contrasts to Ben Said *et al*. (2015) and Blaak *et al*. (2014).

In this study, 98% of the ESBL/AmpC-producing isolates were multidrug resistant, while 93.3% MDR have been reported for ESBL-producing isolates from a similar study in Tunisia (Ben Said *et al*., 2015). Moreover, 100% of the river irrigation water isolates from this study showed MDR phenotypes, which is significantly higher than the 42.3% MDR previously reported in ESBL-producing Enterobacteriaceae isolates from river water sampled in China (Ye *et al.*, 2017). Overall, 63.16% (12/19) of the isolates from retailed spinach showed a MDR phenotype, which is lower than the 83.78% MDR previously reported on retail spinach in South Africa (Richter *et al*., 2019). In addition, resistance to as many as four additional non-β-lactam antibiotic classes were observed in the MDR ESBL-producing potential pathogenic isolates from river water and spinach samples. This included *K. pneumoniae* isolates with resistance to cotrimoxazole, a clinically relevant antibiotic, similar to clinical isolates in a recent South African study (Vasaikar *et al*., 2017). The occurrence (36%) of MDR ESBL-producing *K. pneumoniae* throughout the first production scenario was high, compared to similar studies where 0% (the Netherlands) and 15% (China) occurrence have been reported (Blaak *et al.*, 2014; Ye *et al*., 2017). This highlights the potential role that the agricultural environment may have as a reservoir of MDR opportunistic pathogens in fresh produce production.

However, the importance of not only assessing the agricultural environment as a possible source of antimicrobial contamination in fresh produce, but also the processing and distribution steps were discussed in a recent review (Hölzel *et al*., 2018). Accordingly, all ESBL-producing isolates from spinach (n=18) in the second production scenario of this study were isolated from produce during processing and retail (distribution), of which 94.4% showed a MDR phenotype.

Molecular characterisation of the MDR ESBL/AmpC-producing Enterobacteriaceae isolates from both spinach production scenarios revealed the dominance of $bla_{\text{CTX-M}}$, followed by bla_{SHV} and bla_{TEM}. Worldwide SHV, TEM and CTX-M β-lactamases are the major ESBLs detected in clinical and agricultural settings, including fresh produce (Njage and Buys, 2014, Zhang *et al*., 2015, Ye *et al*., 2017). The most common variants reported in literature to date include $\text{bla}_{\text{CTX-M-14}}$ (CTX-M Group 9) and $\text{bla}_{\text{CTX-M-15}}$ (CTX-M Group 1). In our study, CTX-M group 9 ($\text{bla}_{\text{CTX-M-14}}$) was found in *E. coli* isolates from river irrigation water as well as the holding dam borehole water. This corresponds to *E. coli* isolates from river water reported by

Njage and Buys (2014). Previous studies have reported $bla_{CTX\text{-}M\text{-}14}$ and $bla_{CTX\text{-}M\text{-}15}$ as the most broadly dispersed in clinical isolates, whilst in environmental isolates, CTX-M Group 1 variants ($bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}3}$ among other), have been reported (Borgogna *et al*., 2016; Cantón *et al*., 2012). Additionally, CTX-M Group 1 variants ($bla_{CTX\text{-}M\text{-}15}$, $bla_{CTX\text{-}M\text{-}3}$ and $bla_{CTX\text{-}M\text{-}12}$) found in the different Enterobacteriaceae isolates from vegetables corresponded to other studies (Ye *et al*., 2017, Richter *et al*., 2019).

Apart from the ESBL genes, pAmpC resistance genes were detected in six *S. fonticola* isolates from the second production scenario, but only included the CIT type (identified as bla_{CMY} variants). This is in contrast to the point of sale fresh produce where the EBC type was predominantly detected from different Enterobacteriaceae species (Richter *et al*., 2019), but corresponds to a study by Njage and Buys (2014), who predominantly detected the CIT type pAmpC β-lactamases in *E. coli* isolated from lettuce and irrigation water samples in the North West Province, SA.

A high percentage of the ESBL/AmpC-producing isolates in the current study further harboured integrons, which is consistent with previous reports (Ben Said *et al*., 2015; Ye *et al.*, 2017). Class 1 integrons were detected in 47.96% of the MDR ESBL/AmpC-producing isolates from both scenarios, corresponding to results reported (Ma *et al.*, 2017; Ye *et al.*, 2017). Similar to results reported by Freitag *et al.* (2018), no class 2 integrons were detected in the current study. This contrasts to previous studies where class 2 integrons were predominantly detected, followed by class 1 integrons from raw salad vegetables retailed in Canada (Bezanson *et al.*, 2008).

Conclusion

This is the first study to show the presence of ESBL/AmpC-producing Enterobacteriaceae in the agricultural environment, throughout processing, and the retailer spinach samples. Where river water was used for irrigation, higher contamination levels were seen, including an increase in ESBL/AmpC-producing Enterobacteriaceae genera isolated, as well as the phenotypic multidrug resistance profiles. Furthermore, the abundance and diversity of ESBL/AmpC-producing Enterobacteriaceae were the highest for retailer spinach samples for both commercial supply chains. The importance of using water of acceptable microbiological quality for irrigating fresh produce to be eaten raw is clear from the results of this study. The fact that Enterobacteriaceae with expanded spectrum antimicrobial resistance and their genetic

determinants occurred and persisted throughout the fresh produce supply chains evaluated, highlighted the importance of further surveillance of antimicrobial resistance in different environmental settings. In addition, this study adds to the global knowledge base regarding the prevalence and characteristics of ESBL/AmpC-producing Enterobacteriaceae in fresh vegetables and the agricultural environment required for future risk analysis.

4.2.5 Commercial lettuce and spinach (whole, minimally processed, packaged) from supply chains in Gauteng Province and North West Province supplying retailers in the Tshwane Metropole

Authors: Muneiwa Ratshilingano, Erika du Plessis, Stacey Duvenage and Lise Korsten

A draft publication has been prepared and will be submitted to The Journal of Food Protection: Multidrug resistance and molecular characteristics of generic and extended-spectrum β-lactamase-producing *Escherichia coli* isolated from selected commercially produced lettuce and spinach supply chains.

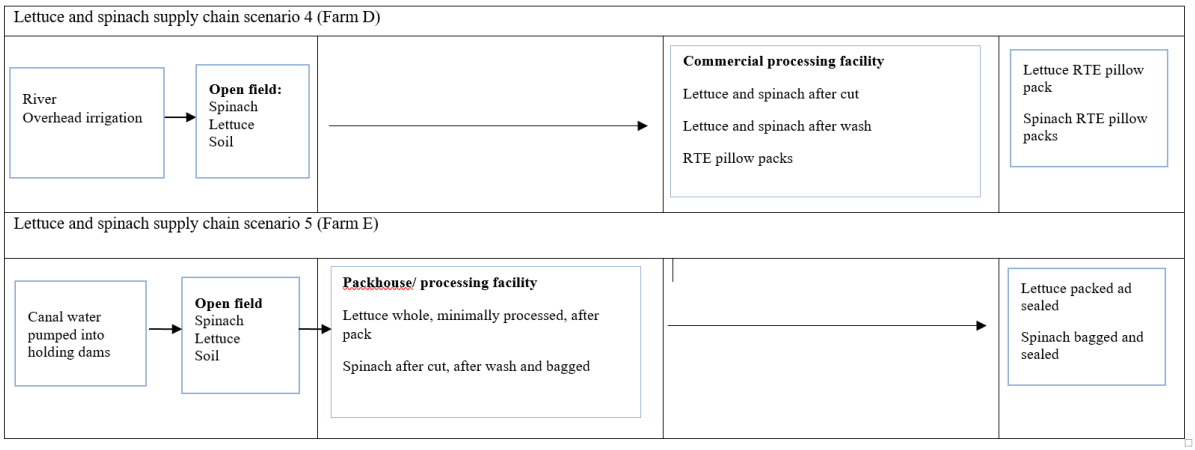
Specific aim: This study aimed to evaluate the microbiological quality of commercially produced lettuce and spinach as well as the associated production environment by enumerating total coliform, *E. coli* and Enterobacteriaceae and determining the prevalence of potential human pathogenic bacteria (including generic *E. coli*, *Salmonella* spp., *L. monocytogenes*) from irrigation water, soil and leafy greens from the farm.

Experimental procedures

Details of the commercial spinach and production sites selected, sample collection, processing, hygiene indicator bacteria enumeration, foodborne pathogen isolation, identification and characterisation were described in Chapter 3, section 3.2.2, 3.3 and 3.4.

Sampling Sites and Points: Lettuce and spinach supply chains

(Refer to Chapter 3, section 3.2.2.)



A total of 239 samples were collected comprising of lettuce (n=68), spinach (n=68), water samples (n=63) and soil samples (n=40) from two commercial farms (in the field followed through to the packhouses and associated major retail outlets) in Gauteng Province and North West Province, South Africa.

Results

Enumeration of total coliforms, *Escherichia coli* and Enterobacteriaceae on Farm A. The coliform, *E. coli* and Enterobacteriaceae counts of irrigation water, washwater, lettuce and spinach from commercial farms and through processing from Farm A and B were summarised in Table 44, Table 45 and Table 46. The total coliform levels on Farm A water samples ranged from 3.43-4.29 log MPN/100 ml in river water, 2.47-4.04 log MPN/100 ml in dam and 3.45-4.01 log MPN/100 ml in irrigation pivot water. No coliforms were recorded in the washwater from either trips (Table 44). The coliform counts of the river water used for irrigation of lettuce on Farm A sampled during trip two was significantly higher than during trip one (p=0.0006) (Table 44). Similarly, coliform counts in the storage dam used on Farm A for lettuce irrigation were significantly higher during trip two when compared to trip one (p=0.0006) (Table 44). Coliform counts in the irrigation pivot water during trip two were not significantly different to dam water during trip two (p=0.0006). Washwater coliform levels were consistently significantly lower when compared to other water sources on Farm A (p=0.0006) (Table 44). Coliform levels in river, dam and irrigation pivot water on Farm A for spinach irrigation did not differ significantly (p=0.1336) (Table 44). Although the source by

trip interaction was not significant, taking both trips into account coliform levels in the river were higher than the dam, the irrigation pivot water and the washwater ($p<0.0001$).

The coliform levels on lettuce from Farm A (production and through processing) ranged from 2.26-4.61 log CFU/g, counts were not significantly different through different lettuce sampling points ($p=0.1487$) (Table 44). Although the source by trip interaction was not significant, lettuce collected from trip one was significantly lower than trip two ($p=0.0123$). Additionally, the source of the sampling displayed significant difference, with lettuce at receival, lettuce at harvest and lettuce at retail significantly higher than lettuce after wash and lettuce after pack ($p<0.0001$). The coliform levels on spinach from Farm A (production and through processing) ranged from 2.89-4.64 log CFU/g, with no significant differences observed ($p=0.4133$) (Table 44). However, there was a significant difference in coliform counts on spinach dependant on the source of sampling, with spinach at retail and spinach at harvest significantly higher than spinach at receival, spinach after pack and spinach after wash ($p<0.0001$).

Escherichia coli levels in river water ranged between 0.00-3.53 log MPN/100 ml on Farm A; with *E. coli* levels in storage dam and irrigation pivot ranging between 0.00-2.93 log MPN/1000 ml and 0.00-2.79 log MPN/100 ml, respectively (Table 45). No *E. coli* was recorded in washwater from both trips of lettuce and spinach production. *Escherichia coli* counts in water sources used during lettuce production did not differ significantly ($p=0.0892$) (Table 45). Although the source by trip interaction was not significant, taking both trips into consideration *E. coli* counts from the river were significantly higher than irrigation pivot, dam and washwater counts ($p = 0.0283$). Similarly, water sources used during spinach production did not demonstrate significantly different *E. coli* levels ($p=0.1162$) (Table 45). However, there was a significant difference when taking the source of sampling of both trips into account; with *E. coli* counts from the river significantly higher than dam, irrigation pivot and washwater ($p = 0.0301$).

The mean *E. coli* levels on lettuce from Farm A (production and through processing) ranged from 0.00-2.70 log CFU/g (Table 45). A significant difference ($p <0.0001$) in *E. coli* levels was observed with lettuce at harvest during trip 1 having higher *E. coli* counts when compared to lettuce at harvest during trip 2 and other sampling points ($p <.0001$) (Table 45). Additionally, trip one had higher *E. coli* counts when compared to trip two ($p=0.0002$). The mean *E. coli* levels on spinach from Farm A ranged from 0.00-3.39 log CFU/g. A significant difference was

observed in *E. coli* levels for the spinach at harvest, in the packhouse, at retailer samples for both trips on Farm A (p = 0,0011) (Table 45). Additionally, washed spinach on Farm A during trip one showed significantly higher *E. coli* levels when compared to spinach at harvest in trip one, although trip two showed a significantly lower *E. coli* levels on unwashed spinach (at harvest and at receival) compared to washed spinach (p = 0,0011) (Table 45).

The Enterobacteriaceae levels in river water ranged from 3.06-3.64 log CFU/ml on Farm A, whilst the storage dam and irrigation pivot Enterobacteriaceae levels ranged from 2.50-3.16 log CFU/ml and 2.26-2.91 log CFU/ml, respectively (Table 46). Enterobacteriaceae levels river water from Farm A used for lettuce irrigation was significantly higher than the dam and irrigation pivot water (p=0.0270) (Table 46). Enterobacteriaceae levels in the washwater were consistently lower than other sampling points (lettuce p=0.0270, spinach p=0.0099) (Table 46). Moreover, Enterobacteriaceae levels from river water during trip one was significantly higher than river water during trip two as well as dam and irrigation pivot water (p=0.0099) (Table 46).

The mean Enterobacteriaceae counts on lettuce from Farm A ranged between 0.00-4.59 log CFU/g and did not differ significantly (p=0.1957) (Table 46). The mean Enterobacteriaceae counts on spinach from Farm A ranged from 2.14-4.52 log CFU/g. The Enterobacteriaceae enumerated from spinach samples differed significantly with the spinach at retail for trip one and spinach at harvest during trip two harbouring the highest Enterobacteriaceae levels (p=0.0125) (Table 46). Interestingly, Enterobacteriaceae levels were not significantly different at harvest, at receival, after wash and after pack, but then significantly increased at retail (p=0.0125) (Table 46).

Enumeration of total coliforms, *Escherichia coli* and Enterobacteriaceae on Farm B. The coliform, *E. coli* and Enterobacteriaceae counts of irrigation water, washwater, lettuce and spinach from commercial farms and through processing from Farm A and B were summarised in Table 44, Table 45 and Table 46. The total coliform levels in irrigation water samples from Farm B ranged from 2.72-4.27 log MPN/100 ml in storage dam and 3.16-4.90 log MPN/100 ml in irrigation pivot and 0.00-1.69 log MPN/100 ml in washwater (Table 44). The coliform levels in dam water used for irrigation of lettuce sampled during trip one and trip two on Farm B was significantly different to irrigation pivot ($p<0.05$). Dam water used for irrigation of spinach on Farm A was not significantly different in coliform levels when compared to

irrigation pivot (p=0.1529). The coliform counts of the dam water used for irrigation of spinach on Farm B sampled during trip two was significantly higher than during trip one (p = 0.0077) (Table 44). Coliform counts in the irrigation pivot water during trip two were not significantly different to dam water during trip two (p = 0.0006). Although the source by trip interaction was not significant, taking both trips into account coliform levels in irrigation pivot was higher than the dam and the washwater (p = 0.2365).

The coliform levels on lettuce from Farm B (production and through processing) ranged from 3.28-4,66 log CFU/g, with no significant difference observed (p=0.2069) (Table 44). Although the source by trip interaction was not significant, coliform levels on lettuce collected from trip one was significantly lower than trip two (p = 0.0036). Additionally, the source of the sampling displayed no significant difference, with lettuce at harvest, lettuce at receival, lettuce at afterwash and lettuce afterpack significantly higher than lettuce at retail (p=0.0784). The coliform levels on spinach from Farm B ranged from 2.65-4.71 log CFU/g. A significant difference in coliform levels was observed during trip one, with fluctuating coliform levels and no significant difference on spinach during trip two (Table 44). Moreover, a significant difference in coliform levels was observed, with coliform levels on spinach samples significantly higher in trip two than trip one (p<0.0001).

On Farm B storage dam *E. coli* levels ranged between 0.00-3.30 log MPN/100 ml and 0.00-3.00 log MPN/100 ml in samples from the irrigation pivot (Table 45). The *E. coli* levels in the storage dam during trip one were significantly lower than that during trip two (p=0.0095). In contrast, *E. coli* levels did not differ significantly between storage dam and irrigation pivot water sampled during spinach production on Farm B, (p= 0.0556). Otherwise, no *E. coli* was enumerated in washwater from on-farm processing facility collected from Farm A and Farm B (Table 45).

The mean *E. coli* levels on lettuce from Farm B (production and through processing) ranged from 0.00-1.66 log CFU/g, with no significant difference observed (p=0.1505) (Table 45). The mean *E. coli* levels on spinach from Farm B ranged from 0.00-4.02 log CFU/g. *Escherichia coli* levels on spinach after wash, after pack and at retail during trip two were significantly higher than other sampling points. Additionally, trip two demonstrated significantly higher *E. coli* levels on spinach when compared to trip one (p<0.0001).

The Enterobacteriaceae levels in water from the storage dam ranged from 2.53-4.04 log CFU/ml and 2.90-4.06 log CFU/ml in the irrigation pivot on Farm B (Table 46). No Enterobacteriaceae levels were recorded in washwater (lettuce p=0.0272, spinach p=0.0077) (Table 46). The levels of Enterobacteriaceae in water sources used during lettuce production were significantly different, with dam water during and irrigation pivot water during trip one showing the highest Enterobacteriaceae counts, followed by dam water in trip two and then by irrigation pivot water during trip two (p=0.0272). Moreover, significant difference was also observed on the trips, with levels of Enterobacteriaceae in water sources significantly higher in trip one compared to trip two (p=0.0108). Similarly, Enterobacteriaceae counts in water sources during spinach production was significantly different, with dam water and irrigation pivot water during trip two being significantly higher than the same sources during trip one (p=0.0077). Moreover, it was interesting to note that Enterobacteriaceae levels in trip two were significantly higher compared to trip one (p=0.0007).

The mean Enterobacteriaceae counts on lettuce from Farm B ranged between 2.89-4.67 log CFU/g. The Enterobacteriaceae counts enumerated from lettuce samples were significantly different, fluctuating from at harvest to at retail (p=0.0229). As expected, the lettuce at retail Enterobacteriaceae counts were lower than at other points. Without taking the trip into account the source of the lettuce played a significant role in terms of the Enterobacteriaceae counts (p=0.0081), with lettuce at harvest, lettuce at receival, lettuce after wash and lettuce after pack being not significantly different. The mean Enterobacteriaceae counts on spinach from Farm B ranged from 1.78-4.53 log CFU/g. During trip two the spinach Enterobacteriaceae levels were significantly higher when compared to trip one (p = 0.0032), with (Table 46).

The mean coliform levels from all the soil samples from the commercial farms ranged between 2.83 to 3.87 log CFU/g on Farm A and 3.04 to 4.08 log CFU/g on Farm B. No *E. coli* was enumerated from any of the soil samples tested.

Table 44 Total coliforms counts from irrigation water, washwater, lettuce and spinach sampled from Farm A and Farm B

Source	Sample point	Trip	Farm A			Farm B		
			Range	Mean ± SE[a,b]	P value	Range	Mean ± SE[a,b]	P value
Lettuce Production								
Irrigation water	River	1	3,43-3,66	3,57 ± 3,57^{B}	0.0006	-	-	0.0001
		2	4,08-4,09	4,09 ± 0,00^{A}		-	-	
	Dam	1	2,47-2,67	2,54 ± 2,54^{C}		2,72-3,66	3,29 ± 0,29^{C}	
		2	3,36-3,92	3,67 ± 0,16^{B}		4,02-4,16	4,09 ± 0,04^{B}	
	Irrigation pivot	1	-	-		3,16-3,72	3,43 ± 0,16^{C}	
		2	3,45-3,72	3,56 ± 0,08^{B}		4,61-4,90	4,76 ± 0,08^{A}	
Processing water	Washwater	1	0,00-0,00	0,00 ± 0,01^{D}		1,63-1,69	1,65 ± 0,02^{D}	
		2	0,00-0,00	0,00 ± 0,01^{D}		0,00-0,00	0,00 ± 0,00^{E}	
Lettuce	Lettuce at harvest	1	3,61-3,74	3,71 ± 0,02^{A}	0.1487	3,81-4,61	4,26 ± 0,14^{A}	0.2069
		2	3,94-4,61	4,34 ± 0,16^{A}		4,58-4,65	4,62 ± 0,01^{A}	
	Lettuce at receive	1	3,59-3,74	3,68 ± 0,05^{A}		3,78-4,54	4,05 ± 0,24^{A}	
		2	4,31-4,58	4,41 ± 0,13^{A}		4,62-4,66	4,64 ± 0,01^{A}	
	Lettuce after wash	1	2,79-3,48	3,15 ± 0,19^{A}		3,46-4,54	4,15 ± 0,35^{A}	
		2	2,26-3,79	3,06 ± 0,44^{A}		4,04-4,39	4,17 ± 0,11^{A}	
	Lettuce after pack	1	3,08-3,31	3,16 ± 0,07^{A}		3,73-4,55	4,01 ± 0,27^{A}	
		2	2,77-3,38	3,01 ± 0,19^{A}		4,05-4,26	4,17 ± 0,07^{A}	
	Lettuce at retail	1	2,55-3,48	3,06 ± 0,27^{A}		3,28-3,85	3,55 ± 0,16^{A}	
		2	3,65-4,00	3,88 ± 0,11^{A}		4,09-4,63	4,43 ± 0,17^{A}	
Spinach Production								
Irrigation water	River	1	4,08-4,09	4,09 ± 0,00^{A}	0.1336	-	-	0.1529
		2	3,67-4,29	3,89 ± 0,20^{A}		-	-	
	Dam	1	3,36-3,91	3,67 ± 0,16^{A}		4,09-4,27	4,16 ± 0,06^{A}	
		2	3,94-4,04	3,99 ± 0,03^{A}		4,02-4,16	4,09 ± 0,04^{A}	
	Irrigation pivot	1	3,45-3,71	3,56 ± 0,08^{A}		4,54-4,72	4,62 ± 0,05^{A}	
		2	3,51-4,01	3,81± 0,16^{A}		4,08-4,50	4,30 ± 0,12^{A}	
Processing water	Washwater	1	0,00-0,00	0,00 ± 0,00^{A}		0,00-0,00	0,00 ± 0,00^{A}	
		2	0,00-0,00	0,00 ± 0,00^{A}		0,00-0,00	0,00 ± 0,00^{A}	
Spinach	Spinach at harvest	1	3,24-4,09	3,73 ± 0,16^{A}	0.4133	3,56-3,97	3,76 ± 0,07 ^{CD}	0.0090
		2	4,07-4,58	4,19 ± 0,10^{A}		4,65-4,71	4,68 ± 0,01^{A}	
	Spinach at receival	1	3,35-3,98	3,77 ± 0,21^{A}		2,89-3,80	3,28 ± 0,27^{DE}	
		2	3,38-3,83	3,62 ± 0,13^{A}		3,99-4,64	4,39 ± 0,20 ^{AB}	
	Spinach after wash	1	2,89-3,24	3,09 ± 0,11^{A}		2,65-2,93	2,78 ± 0,08^{F}	
		2	3,00-3,26	3,15 ± 0,08^{A}		4,64-4,69	4,67 ± 0,02 ^{AB}	
	Spinach after pack	1	3,32-3,58	3,47 ± 0,08^{A}		2,74-3,61	3,04 ± 0,28 ^{EF}	
		2	3,29-4,00	3,62 ± 0,21^{A}		4,65-4,67	4,66 ± 0,01 ^{AB}	
	Spinach at retail	1	4,51-4,59	4,54 ± 0,03^{A}		3,54-3,81	3,64 ± 0,09^{D}	
		2	4,52-4,64	4,57 ± 0,04^{A}		4,16-4,23	4,19 ± 0,02 ^{BC}	

[a]SE: Standard error

[b]Means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

Table 45 Total *Escherichia coli* counts from irrigation water, washwater, lettuce and spinach sampled from Farm A and Farm B

Source	Sample point	Trip	Farm A			Farm B		
			Range	Mean ± SE[a,b]	P value	Range	Mean ± SE[a,b]	P value
Lettuce Production								
Irrigation water	River	1	0,00-2,00	0,67 ± 0,67 [A]		-	-	
		2	3,53-3,50	3,51 ± 0,01 [A]		-	-	
	Dam	1	0,00-1,30	0,87 ± 0,43 [A]		0,00-0,00	0,00 ± 0,00 [C]	
		2	0,00-2,71	1,73 ± 0,87 [A]	0.0892	2,30-2,93	2,72 ± 0,21 [A]	0.0095
	Irrigation pivot	1	-			0,00-2,71	1,57 ± 0,81 [B]	
		2	0,00-2,61	1,64 ± 0,82 [A]		2,00-2,49	2,26 ± 0,14 [AB]	
Processing water	Washwater	1	0,00-0,00	0,00 ± 0,00 [A]		0,00-0,00	0,00 ± 0,00 [C]	
		2	0,00-0,00	0,00 ± 0,00 [A]		0,00-0,00	0,00 ± 0,00 [C]	
Lettuce	Lettuce at harvest	1	2,38-2,68	2,52 ± 0,05 [A]		0,00-0,00	0,00 ± 0,00 [A]	
		2	0,00-0,00	0,00 ± 0,00 [C]		0,00-1,66	0,33 ± 0,33 [A]	
	Lettuce at receival	1	1,00-2,33	1,62 ± 0,39 [B]		0,00-0,00	0,00 ± 0,00 [A]	
		2	0,00-0,00	0,00 ± 0,00 [C]		0,00-1,66	1,11 ± 1,11 [A]	
	Lettuce after wash	1	0,00-0,74	0,49 ± 0,25 [C]	<0.0001	0,00-0,00	0,00 ± 0,00 [A]	0.1505
		2	0,00-0,00	0,00 ± 0,00 [C]		0,00-0,00	0,00 ± 0,00 [A]	
	Lettuce after pack	1	0,00-0,00	0,00 ± 0,00 [C]		0,00-0,00	0,00 ± 0,00 [A]	
		2	0,00-0,00	0,00 ± 0,00 [C]		0,00-0,00	0,00 ± 0,00 [A]	
	Lettuce at retail	1	0,00-0,00	0,00 ± 0,00 [C]		0,00-0,00	0,00 ± 0,00 [A]	
		2	0,00-2,70	1,55 ± 0,81 [B]		0,00-0,00	0,00 ± 0,00 [A]	
Spinach Production								
Irrigation water	River	1	3,49-3,53	3,51 ± 0,01 [A]		-	-	
		2	0,00-3,28	1,76 ± 0,95 [A]		-	-	
	Dam	1	0,00-2,72	1,73 ± 0,87 [A]		0,00-3,30	1,10 ± 1,10 [AB]	
		2	2,61-2,93	2,72 ± 0,11 [A]	0.1162	2,30-2,93	2,72 ± 0,21 [A]	0.0556
	Irrigation pivot	1	0,00-2,61	1,64 ± 0,82 [A]		0,00-3,00	2,00 ± 1,00 [AB]	
		2	0,00-2,79	1,70 ± 0,86 [A]		0,00-0,00	0,00 ± 0,00 [B]	
Processing water	Washwater	1	0,00-0,00	0,00 ± 0,00 [A]		0,00-0,00	0,00 ± 0,00 [B]	
		2	0,00-0,00	0,00 ± 0,00 [A]		0,00-0,00	0,00 ± 0,00 [B]	
Spinach	Spinach at harvest	1	0,00-2,14	1,15 ± 0,48 [BC]		0,74-1,71	1,36 ± 0,20 [B]	
		2	0,00-3,16	1,65 ± 0,71 [ABC]		0,00-2,36	1,69 ± 0,44 [B]	
	Spinach at receival	1	0,00-0,00	0,00 ± 0,00 [D]		0,00-0,00	0,00 ± 0,00 [C]	
		2	0,00-2,14	0,71 ± 0,71 [CD]		0,00-1,96	1,31 ± 0,65 [B]	
	Spinach after wash	1	1,67-3,39	2,75 ± 0,54 [A]	0.0011	0,00-0,00	0,00 ± 0,00 [C]	0.0419
		2	0,00-0,00	0,00 ± 0,00 [D]		1,67-4,02	2,93 ± 0,68 [A]	
	Spinach after pack	1	0,00-2,14	1,27 ± 0,65 [BC]		0,00-0,00	0,00 ± 0,00 [C]	
		2	0,00-0,00	0,00 ± 0,00 [D]		0,00-2,80	1,81 ± 0,90 [AB]	
	Spinach at retail	1	1,67-2,74	2,12 ± 0,32 [AB]		0,00-0,00	0,00 ± 0,00 [C]	
		2	0,00-0,00	0,00 ± 0,00 [D]		1,96-2,36	2,15 ± 0,11 [AB]	

[a]SE: Standard error

[b]Means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$)

Table 46 Total Enterobacteriaceae counts from irrigation water, washwater, lettuce and spinach sampled from Farm A and Farm B

Source	Sample point	Trip	Farm A			Farm B		
			Range	Mean ± SE[a,b]	P value	Range	Mean ± SE[a,b]	P value
Lettuce Production								
Irrigation water	River	1	3,59-3,64	3,63 ± 3,63 [A]		-	-	
		2	3,46-3,60	3,54 ± 0,04 [A]		-	-	
	Dam	1	2,53-2,71	2,61 ± 2,61 [C]		3,39-4,04	3,66 ± 0,19 [AB]	
		2	2,83-3,16	2,97 ± 0,09 [B]	0.0270	3,37-3,62	3,50 ± 0,07 [BC]	0.0272
	Irrigation pivot	1	-	-		3,81-4,06	3,89± 0,08 [A]	
		2	2,26-2,66	2,53 ± 0,13 [C]		3,14-3,33	3,25± 0,06 [C]	
Processing water	Washwater	1	0,00-0,00	0,00 ± 0,01 [D]		0,00-0,00	0,00 ± 0,00 [D]	
		2	0,00-0,00	0,00 ± 0,01 [D]		0,00-0,00	0,00± 0,00 [D]	
Lettuce	Lettuce at harvest	1	3,69-3,74	3,73 ± 0,00 [A]		3,12-4,53	4,03 ± 0,30 [BC]	
		2	0,00-4,56	2,52 ± 1,03 [A]		4,17-4,29	4,23 ± 0,02 [ABC]	
	Lettuce at receive	1	3,62-3,74	3,68 ± 0,03 [A]		4,47-4,62	4,54 ± 0,04 [AB]	
		2	3,86-4,59	4,22 ± 0,21 [A]		4,19-4,32	4,28 ± 0,04 [ABC]	
	Lettuce afterwash	1	3,52-3,68	3,58 ± 0,05 [A]	0.1957	4,00-4,67	4,40 ± 0,20 [ABC]	0.0229
		2	1,67-2,44	2,50 ± 0,50 [A]		3,95-4,02	3,97 ± 0,02 [C]	
	Lettuce afterpack	1	3,48-3,52	3,49 ± 0,01 [A]		4,56-4,65	4,60 ± 0,03 [A]	
		2	1,67-2,44	2,08 ± 0,22 [A]		3,89-3,95	3,93 ± 0,02 [C]	
	Lettuce at retail	1	2,80-3,20	2,97 ± 0,12 [A]		2,89-3,54	3,29 ± 0,20 [D]	
		2	3,23-4,07	3,77 ± 0,27 [A]		3,89-3,95	3,92 ± 0,02 [C]	
Spinach Production								
Irrigation water	River	1	3,46-3,60	3,54 ± 0,04 [A]		-	-	
		2	3,06-3,12	3,08 ± 0,02 [B]		-	-	
	Dam	1	2,83-3,16	2,97 ± 0,09 [B]		2,53-2,86	2,71 ± 0,09 [B]	
		2	2,50-2,98	2,81 ± 0,15 [B]	0.0099	3,37-3,62	3,50 ± 0,07 [A]	0.0077
	Irrigation pivot	1	2,26-2,66	2,53 ± 0,13 [C]		2,90-2,98	2,96 ± 0,03 [B]	
		2	2,74-2,91	2,85 ± 0,06 [B]		3,29-3,63	3,41 ± 0,11 [A]	
Processing water	Washwater	1	0,00-0,00	0,00 ± 0,00 [D]		0,00-0,00	0 ,00 ± 0,00 [C]	
		2	0,00-0,00	0,00 ± 0,00 [D]		0,00-0,00	0,00 ± 0,00 [C]	
Spinach	Spinach at harvest	1	2,89-3,53	3,24 ± 0,11 [DE]		2,90-3,84	3,51 ± 0,17 [B]	
		2	3,85-4,07	3,96 ± 0,06 [AB]		4,17-4,29	4,21 ± 0,02 [A]	
	Spinach at receival	1	2,66-4,06	3,55 ± 0,45 [BCD]		1,78-2,49	2,21 ± 0,22 [D]	
		2	2,91-3,57	3,19 ± 0,19 [DE]		4,14-4,37	4,28 ± 0,07 [A]	
	Spinach afterwash	1	2,80-3,39	3,01 ± 0,19 [DEF]	0.0125	2,20-2,65	2,40 ± 0,13 [DC]	0.0032
		2	2,14-2,77	2,47 ± 0,18 [F]		4,02-4,09	4,05 ± 0,02 [A]	
	Spinach afterpack	1	2,86-3,80	3,31 ± 0,27 [CDE]		2,32-3,57	2,79 ± 0,39 [C]	
		2	2,50-3,39	2,84 ± 0,28 [EF]		4,08-4,53	4,29 ± 0,13 [A]	
	Spinach at retail	1	4,50-4,52	4,51 ± 0,00 [A]		2,43-3,06	2,67 ± 0,19 [C]	
		2	3,46-4.00	3,87 ± 0,15 [C]		4,02-4,27	4,15 ± 0,07 [A]	

[a]SE: Standard error

[b]Means (based on the trip x source interactions) followed by the same letters are not significantly different ($p < 0,05$).

Confirmation of Enterobacteriaceae identities using MALDI-TOF MS analysis. From the 239 samples, 28 *E. coli* isolates were isolated from irrigation water sources (17.5%; 11/63), spinach samples (14.7%; 10/68), lettuce samples (8.8%; 6/68) and of soil samples (2.5%; 1/40). *Salmonella* spp. and *L. monocytogenes* were not detected in any of spinach and lettuce samples from both Farm A and Farm B. However, *Salmonella* spp. was detected in three (3/18) irrigation pivot point samples from Farm A, resulting in 4.8% (n=63) of all water samples and 16.7% in irrigation pivot point alone. Similarly, *L. monocytogenes* was detected in two (2/18) processing water samples from Farm A, resulting in 3.2% (n=63) of all water samples and 11.1% in processing water samples alone.

Discussion

The microbiological safety of leafy greens is an emerging global concern. This study evaluated the microbiological quality of irrigation water (river, dam, irrigation pivot) used to irrigate leafy greens from two commercial farms and the potential impact on safety of lettuce and spinach from the farm, through processing to the retailer. The *E. coli* levels in river water did not often exceed the maximum limit of <1000 *E. coli*/100 ml set by the Department of Water Affairs (DWAF) for safe irrigation water (DWAF, 1996). Similarly, a study carried out in the North West Province in South Africa showed that the *E. coli* counts of irrigation water samples from the Skeerpoort River did not often exceed the guideline limits (Aijuka *et al*., 2015). This is in contrast to a number of other studies that evaluated irrigation water quality in SA (Jongman and Korsten, 2016; Du Plessis *et al*., 2015, Van Dyk *et al*., 2016) where unacceptably high hygiene indicator bacteria levels were reported. The enumeration of *E. coli* from the source water used for irrigation on both farms indicate faecal contamination which might be from a sewage treatment plant upstream of Farm A and from informal settlements along the Jukskei river which feeds into the canal on Farm B (Decol *et al.,* 2017; Abakpa *et al*., 2013; Espigol *et al*., 2018). In addition, *E. coli* was isolated from 17.5% of the samples following enrichment of the irrigation water samples (river, dam and irrigation pivot), which was lower than the 84.8% and 59% reported by Decol *et al.* (2017) and Holvoet *et al.* (2014) respectively. Similar to Jongman and Korsten (2016), *L. monocytogens* was not detected in the irrigation water samples from commercial lettuce and spinach production farms in the current study. However, *Salmonella* spp. was detected in irrigation pivot water from Farm A at a level of 4.8% (3/63) of all irrigation water samples and may pose a health risk when leafy greens are eaten raw, since contaminated water can reach leaves during irrigation (Bourn *et al*., 2002). Although *E. coli* and *Salmonella* spp. were not detected in washwater, *L. monocytogens* was detected in

washwater from the onsite packhouse on Farm A. According to Aijuka *et al*. (2015). The presence of *L. monocytogenes* may increase the potential risk to consumers following consumption of the contaminated ready-to-eat (RTE) leafy greens.

The microbial quality (coliforms) of all leafy greens in the current study were above the acceptable limits of <200 CFU/g for coliforms allowed for RTE fresh fruit and vegetables according to the national guidelines by the Department of Health (NDOH, 2000), currently under revision. Additionally, the coliform levels on retail lettuce and spinach remained high with an average of > 3 log CFU/g, this is regardless of rinsing in hypochlorite water (at 75-80 ppm). Similarly, other studies also found that washing vegetables with hypochlorite did not significantly decrease the coliform counts (Falomir *et al.*, 2013; Bencardino *et al*., 2018). In contrast, Espigol *et al.* (2018) observed a 31% reduction of coliform counts on vegetables (lettuce) after washing in chlorinated water. However, a study by Merlini et al. (2018) suggested that high levels of coliforms on leafy greens may not necessarily represent a health risk but suggest poor hygiene conditions (Merlini *et al*., 2018). Several other studies enumerated >3 log CFU/g of coliforms from lettuce samples (Espigol *et al.*, 2018; Benti *et al*., 2014) which indicates that coliforms are naturally high on leafy green commodities such as lettuce and spinach (Abias *et al*., 2008). Consequently, microbiological guidelines for total coliforms on RTE fresh produce were not established by other countries which included Hong Kong [Centre for Food Safety (CFS), 2014], Australia [New South Wales (NSW) Food Authority, 2009] and Canada (Health Canada, 2002). The Canadian guidelines indicated that high coliforms are expected on leafy greens (Health Canada, 2002). Taking the fact that coliform levels are expected to be high (exceeding >3 log CFU/g) on leafy greens, the Department of Health should consider excluding the coliform guidelines of <200 log CFU/g for RTE fruits and vegetables, currently under review, in order to align with other international guidelines. The European microbiological criteria for Enterobacteriaceae on leafy greens states that levels higher than >4 log CFU/g were unsatisfactory [Health Protection Agency (HPA), 2009], therefore with 36.76% of leafy greens in the current study exceeding the stipulated limit, this showed poor hygiene of leafy greens.

In contrast to coliform levels, leafy greens were not all positive for *E. coli* regardless of rinsing in hypochlorite water (at 75-80 ppm). The *E. coli* levels in 63.97% of the leafy green sample exceeded zero *E. coli* CFU/g for raw fresh produce which would have been acceptable according to the previous NDOH guidelines and Additionally, *E. coli* colonies enumerated

from spinach (50%) and lettuce (22%) samples in the current study were higher than 18% and 20% reported on baby spinach and lettuce respectively, in a study by Jongman and Korsten (2016). However, *E. coli* levels were enumerated at 2.9% and 8.8% from retail lettuce and spinach samples, with an average range of 2.70 and 2.74 CFU/g, respectively. This was lower than 0-73.3% reported on retail spinach samples (Du Plessis et al., 2017). According to the suggested *E. coli* limit which requires zero *E. coli* CFU/g on raw fresh produce, the presence of *E. coli* in eight of the retail samples were unacceptable at >1.66 log CFU/g (NDOH guidelines currently under review), thus suggesting microbiological quality of ready to eat fresh produce. However, when *E. coli* levels were evaluated against the International guidelines; Hong Kong (20-100 CFU/g), United Kingdom (20-100 CFU/g) and Australia (3-100 CFU/g), 91.6% (11/12) and 75% (9/12) of retail lettuce and spinach respectively, would have been compliant (CFS, 2014; HPA, 2009; NSW Food Authority, 2009].

No *Salmonella* spp. and *L. monocytogenes* were detected on leafy greens (lettuce and spinach in field, packhouse and at retail) collected from both farms, similar to results reported in several other studies (Campos *et al*., 2013; Bencardino *et al*., 2018; Laubscher, MSc 2019; Merlini *et al.*, 2018). However, in another study *Salmonella* spp. and *L. monocytogenes* were detected at a low percentage (<1%) of leafy greens (lettuce and spinach) (Zhang *et al*., 2018). Another study by Espigol *et al*. (2018) also detected *Salmonella* spp. in freshly harvested vegetables (romaine lettuce) at market level. Although no *Salmonella* or *L. monocytogenes* were detected, the presence of *E. coli* indicates faecal contamination which could be a potential health risk. Following enrichment, *E. coli* was detected in 14.7% and 8.8% of the spinach and lettuce samples respectively. The latter was lower than 38.3% and 36% prevalence reported by Decol *et al*. (2017) and Aijuka *et al.* (2015). Farm animals grazing near the growing field were observed during the sampling trips which might have contributed to the presence of E. coli on leafy greens at pre-harvest stage (Liu *et al*., 2013; Pahl *et al*., 2013). No *E. coli* was enumerated from soil samples in this study, similar to a study by Van Dyk *et al*. (2016). However, after enrichment a low prevalence of *E. coli* (2.5%) was observed, contrary to another study by Holvoet *et al.* (2014) where *E. coli* was isolated from 31.9% of the soil samples. However, *Salmonella* spp. and *L. monocytogenes* were not detected in soil samples in the current study.

Conclusion

Although the consumption of ready-to-eat vegetables (RTE) are known to be healthy and therefore provide the nutrients and vitamins required, the consumption of leafy greens contaminated with foodborne bacterial pathogens may expose consumers to gastrointestinal diseases. The presence of *E. coli*, *Salmonella* spp. and *L. monocytogenes* in irrigation water sources and washwater raises a concern about the microbiological safety of ready-to-eat leafy greens in this study and may signify a route of foodborne outbreaks. Therefore, care should be taken to address the faecal contamination issue. Based on the findings from this study, it is important that good agricultural practices must be implemented and followed as required by the Global G.A.P, which might contribute to reducing the microbial loads and prevalence of foodborne pathogens in leafy green production systems. By minimizing the prevalence of bacterial pathogens, it also minimize risks of exposure as a result of consumption of ready-to-eat vegetables. In addition, the government should continuously monitor and manage the sewage plants specifically during rainy seasons when most spillage is experienced. There is a need to promote consumer awareness on food safety and the health hazard that come with consumption of leafy greens without properly disinfecting.

4.2.6 Occurrence, identification and characterization of multidrug resistant extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae through lettuce and spinach supply chains.

Authors: Muneiwa Ratshilingano, Erika du Plessis, Stacey Duvenage and Lise Korsten

Specific aim: This study aimed to evaluate the occurrence, identify and characterise multidrug resistant extended spectrum and AmpC β-lactamase-producing Enterobacteriaceae through lettuce and spinach supply chains.

Experimental procedures

Details of ESBL and AmpC-producing Enterobacteriaceae detection, isolation, identification and antimicrobial resistance characterisation (phenotypic and genotypic) were described in Chapter 3, section 3.4.3, 3.4.4 and 3.4.5.

Results

Isolation and identification of presumptive ESBL/ AmpC- producing Enterobacteriaceae. MALDI-TOF analysis showed that 48 (57.8%) of 83 presumptive ESBL/AmpC isolates were Enterobacteriaceae which belonged to six different genera (Figure 16). The Enterobacteriaceae isolates (n=48) were identified as *Escherichia coli* (47.9%), *Klebsiella pneumoniae* (27%), *Serratia fonticola* (10.4%), *Citrobacter freundii* (6.3%), *Enterobacter cloacae* (4.2%) and *Raoultella ornithinolytica* (4.2%). The remaining ESBL/AmpC isolates which did not belong to the Enterobacteriaceae family were identified as *Aeromonas* spp. *Pseudomonas* spp., but were not further characterised.

Antimicrobial resistance profiles. All isolates (n=48) identified as Enterobacteriaceae were tested to confirm the production of extended-spectrum and AmpC β-lactamases and the results were summarised in Figure 15. Using the DDST 85.4% (41/48) isolates tested positive for ESBL production. Additionally, resistance to cefpodoxime (100%), cefotaxime (95.8%) and ceftazidime (45.8%) was observed. Additionally, 70.8% (34/48) were ESBL/ AmpC producers. Of the 48 ESBL/ AmpC isolates, (n=30) were isolated from irrigation water sources which included *E. coli* (33.3%), *K. pneumoniae* (25%), *C. freundii* (4.2%), (n=16) from leafy greens with (n=10) isolates from spinach which included *S. fonticola* (10.4%), *E. coli* (8.3%), *C. freundii* (2.1%) and (n=6) from lettuce included *E. coli* (6.3%), *E. cloacae* (4.2%), *K. pneumoniae* (2.1%) and (n=2) were isolated from soil which included *R. ornithinolytica* (4.2%).

The results of the antimicrobial resistance analysis of all the presumptive ESBL/AmpC-producing Enterobacteriaceae isolates summarised in Table 47, while the antimicrobial resistance profiles were summarized in Table 48. Multidrug resistance was observed in 47/48 (97.9%) of all isolates; while susceptibility to carbapenemase (imipenem) was also observed in 43/48 (89.6%) of the isolates. Further resistance of isolates against the β-lactams was observed at a high rate of 93.8% against amoxicillin, with 56.3% of the isolates from irrigation water sources, 33.3% from leafy greens and 4.2% from soil. Additionally, 89.6% of the isolates were resistant to ampicillin, with 58.3% of the isolates from irrigation water source, 27.1% from leafy greens and 4.2% from soil.

Antibiotics from additional classes to which the ESBL/AmpC-producing Enterobacteriaceae isolates were resistant included neomycin (89.6%), tetracycline (60.4%), trimethoprim/ sulfamethoxazole (54.1%), chloramphenicol (25%) and gentamicin (18.8%).

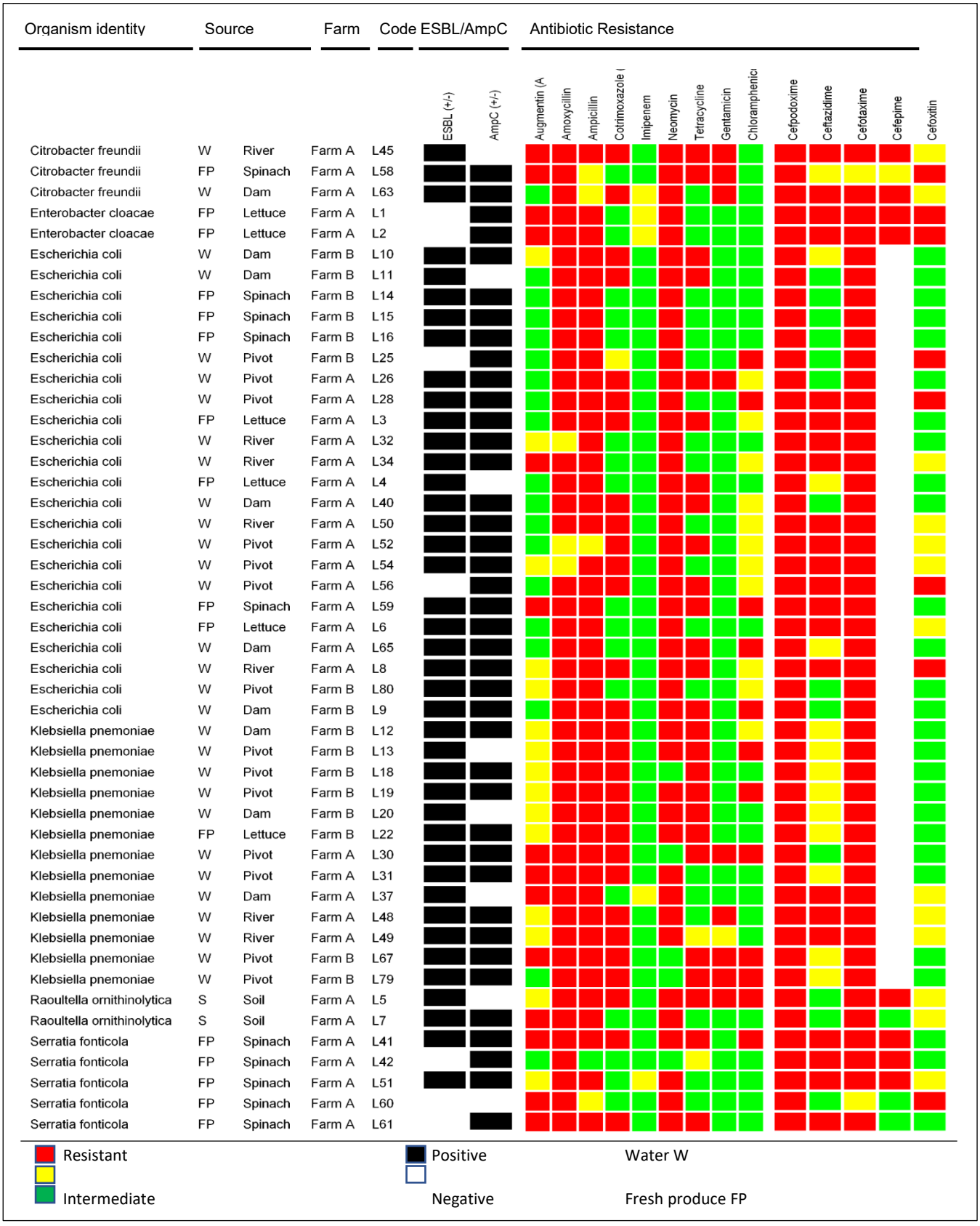


Figure 15: Summary of species isolated and tested for the detection of extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae.

Table 47: Summary of antibiotic resistance of presumptive ESBL/AmpC-producing Enterobacteriaceae isolates from irrigation water, soil, spinach and lettuce samples

	No. (%) of resistant isolates from:						
Antibiotic(s)	**River (n=9)**	**Storage Dam (n=18)**	**Irrigation pivot (n=18)**	**Soil (n=40)**	**Spinach (n=68)**	**Lettuce (n=68)**	Total
AUG30C	2(22.2)	1 (5.6)	3 (16.6)	1 (2.5)	5 (7.6)	2 (2.9)	14 (29)
A10C	6 (66.7)	9 (50)	12 (66.7)	2 (5)	10 (14.7)	6 (8.8)	45 (93.8)
AP10C	7 (77.8)	8 (44.4)	13 (72.2)	2 (5)	7 (10.3)	6 (8.8)	43 (89.6)
T30C	5 (55.6)	7 (38.9)	12 (66.7)	1 (2.5)	2 (2.9)	2 (2.9)	29 (60.4)
IMI10C	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NE10C	7 (77.8)	9 (50)	10 (55.6)	2 (5)	9 (13.2)	6 (8.8)	43 (89.6)
TS25C	2 (22.2)	7 (38.9)	9 (50)	1 (2.5)	4 (5.9)	3 (4.4)	26 (54.1)
GM10C	2 (22.2)	1 (5.6)	4 (22.2)	1 (2.5)	1 (1.5)	0 (0)	9 (18.8)
C30C	0 (0)	2 (11.1)	7 (38.9)	1 (2.5)	2 (2.9)	0 (0)	12 (25)
CPD10C	7 (77.8)	9 (50)	14 (77.8)	2 (5)	10 (14.7)	6 (8.8)	48 (100)
CAZ30C	7 (77.8)	2 (11.1)	4 (22.2)	0 (0)	5 (7.6)	4 (5.9)	22 (45.8)
CTX30C	7 (77.8)	9 (50)	14 (77.8)	2 (5)	8 (11.8)	6 (8.8)	46 (95.8)
CPM30C	1 (11.1)	1 (5.6)	0 (0)	1 (2.5)	3 (4.4)	2 (2.9)	8 (16.7)
FOX30C	1 (11.1)	0 (0)	3 (16.7)	0 (0)	2 (2.9)	2 (2.9)	8 (16.7)

Antibiotics abbreviations: AUG30C-augmentin, A10C-amoxycillin, AP10C-ampicillin, T30C-tetracycline, IMI10C-Imipenem, NE10C-neomycin, TS25C-cotrimoxazole, GM10C-gentamycin, C30C-chloramphenicol, CPD10C-cefpodoxime, CAZ30C-ceftazidime, CTX30C-cefotaxime, CPM30C-cefepime, FOX30C-cefoxitin,

Table 48: Summary of antibiotic resistance patterns and antibiotic classes to which ESBL/AmpC-producing Enterobacteriaceae isolates from irrigation water, soil, spinach and lettuce samples were resistant

No. of antibiotic isolates were resistant to	No. of isolates	No. of isolates with specific pattern	Antibiotic resistance pattern	No of antibiotic classes	Antibiotic classes
5	7	4	A10C-AP10C- NE10C-CPD10C-CTX30C	3	Penicillin, Aminoglycosides, Cephalosporin
		1	AP10C-NE10C-CPD10C-CAZ30C-CTX30C	3	Penicillin, Aminoglycosides, Cephalosporin
		1	A10C-CPD10C-CAZ30C-CTX30C-CPM30C	2	Penicillin, Cephalosporin
		1	AUG30C-A10C-NE10C-CPD10C-FOX30C	3	Penicillin, Aminoglycosides, Cephalosporin
6	6	1	A10C-AP10C-NE10C-T30C-CPD10C-CTX30C	4	Penicillin, Aminoglycosides, Tetracycline, Cephalosporin
		1	A10C-AP10C-NE10C-CPD10C-CAZ30C-CTX30C	3	Penicillin, Aminoglycosides, Cephalosporin
		1	TS25C-NE10C-T30C-CPD10C-CAZ30C-CTX30C	4	Sulfonamides, Aminoglycosides, Tetracycline, Cephalosporin
		1	AP10C-TS25C-NE10C-CPD10C-CAZ30C-CTX30C	4	Penicillin, Sulfonamides, Aminoglycosides, Cephalosporin
		1	AUG30C-A10C-AP10C-NE10C-CPD10C-CTX30C	3	Penicillin, Aminoglycosides, Cephalosporin
		1	A10C-AP10C-TS25C-T30C-CPD10C-CTX30C	4	Penicillin, Sulfonamides, Tetracycline, Cephalosporin
7	15	6	A10C-AP10C-TS25C-NE10C-T30C-CPD10C-CTX30C	5	Penicillin, Sulfonamides, Aminoglycosides, Tetracycline, Cephalosporin
		1	A10C-AP10C-NE10C-C30C-CPD10C-CTX30C-FOX30C	4	Penicillin, Aminoglycosides, Chloramphenicol, Cephalosporin,
		2	AUG30C-A10C-AP10C-NE10C-CPD10C-CAZ30C-CTX30C	3	Penicillin, Aminoglycosides, Cephalosporin
		2	A10C-AP10C-TS25C-NE10C-CPD10C-CAZ30C-CTX30C	4	Penicillin, Sulfonamides, Aminoglycosides, Cephalosporin
		1	A10C-AP10C-NE10C-T30C-C30C-CPD10C-CTX30C	5	Penicillin, Aminoglycosides, Tetracycline, Chloramphenicol, Cephalosporin
		1	AUG30C-A10C-AP10C-TS25C-NE10C-CPD10C-CTX30C	4	Penicillin, Sulfonamides, Aminoglycosides, Cephalosporin
		1	A10C-AP10C-NE10C-CPD10C-CAZ30C-CTX30C-CPM30C	3	Penicillin, Aminoglycosides, Cephalosporin
		1	AUG30C-A10C-NE10C-GM10C-T30C-CPD10C-FOX30C	4	Penicillin, Aminoglycosides, Tetracycline, Cephalosporin

Antibiotics abbreviations: AUG30C-augmentin, A10C-amoxycillin, AP10C-ampicillin, TS25C-cotrimoxazole, NE10C-neomycin, T30C-tetracycline, GM10C-gentamycin, C30C-chloramphenicol, CPD10C-cefpodoxime, CAZ30C-ceftazidime, CTX30C-cefotaxime, CPM30C-cefepime, FOX30C-cefoxitin.

Discussion

The current study documents the prevalence of ESBL and/or AmpC-producing Enterobacteriaceae throughout the lettuce and spinach supply chains. Six different genera of the Enterobacteriaceae family including *E. coli*, *K. pneumoniae*, *C. freundii*, *E. cloacae*, *S. fonticola* and *R. ornithinolytica* were detected in irrigation water, leafy greens (spinach and lettuce) and soil samples; more than the four genera (*S. fonticola*, *R. aquatilis*, *Citrobacter* and *Enterobacter* species) were also detected in irrigation water, fresh produce (lettuce) and soil samples in a study carried out in Netherlands (Blaak *et al*., 2014). Recent studies carried out in South Africa also detected *E. coli*, *S. fonticola*, *C. freundii* and *K. pneumoniae* bacteria in vegetables (spinach) samples, river water samples (Richter *et al*., 2020) and the *E. coli*, *K. pneumoniae*, *C. freundii* and *E. hormaechei* reported in irrigation water sources, vegetables (lettuce) and soil (Said *et al.*, 2015) but at lower levels than recorded in the current study.

Irrigation water sources have been reported as the main source of fresh produce contamination (Gekenidis *et al*., 2018; Ortega-Paredes *et al*., 2018). Only 4.2% prevalence was observed for ESBL/AmpC-producing R. ornithinolytica from soil in this study, different from the 1.3% prevalence of *S. fonticola* observed in growing medium in the Netherlands (Blaak *et al*., 2014). Interestingly, no ESBL/ AmpC-producing Enterobacteriaceae isolates were detected in soil from a study conducted in South Africa (Richter *et al*., 2020).

The current study found prevalence of ESBL/AmpC-producing *E. coli*, *Enterobacter cloacae*, *K. pneumoniae* in lettuce samples at 6.3%, 4.2% and 2.1%, respectively; which was more than 1.3% prevalence previously reported in Netherlands (Blaak *et al*., 2014). A corresponding study in South Korea also found prevalence of ESBL/AmpC-producing *E. coli* and *Klebsiella pnemoniae* in lettuce samples at 10.1% (Kim *et al*., 2015); lower than 20% and 60% prevalence of *Enterobacter* spp. and *Serratia* spp. observed in iceberg lettuce, respectively (Van Hoek *et al*., 2015). Additionally, the current study found prevalence of ESBL/AmpC-producing *S. fonticola*, *E. coli*, *C. freundii* in spinach samples at 10.4%, 8.3% and 2.1%, respectively; similar to a study conducted in South Africa which showed varied prevalence of *S. fonticola*, *K. pneumoniae*, *Rahnella aquatilis* and *E. coli* at 16.67%, 4.17%, 4.17% and 2.08%, respectively (Richter *et al*., 2020).

The presence of ESBL-producers and AmpC-producers in this study calls for a concern. This study observed an overall 95.8% 3GC cefotaxime resistance on ESBL/AmpC-producing Enterobacteriaceae

isolated from irrigation water sources, leafy greens and soil; similar to previous study that reported 91.4% resistance (Blaak *et al*., 2014). Additionally, 29.2% resistance against cephalosporin cefotaxime was observed in this study on isolates from leafy greens (lettuce and spinach) alone, lower than 100%, 88.3% and 86.0% resistance reported by Zurfluh *et al*. (2015), Bhutani *et al*. (2015) and Kim *et al.* (2015), respectively.

Phenotypic characterisation of ESBL/AmpC-producing Enterobacteriaceae isolates from this study showed that 97.9% were MDR with a MARI > 0.2; similar to other recent studies that reported 98.0%, 94.8% and 93.3% MDR (Richter *et al*., 2020; Ben Said *et al*., 2015; Ye *et al*., 2017). Additionally, a 100% MDR phenotype was recorded for isolates from irrigation water sources (river), similar to Richter *et al.* (2020) and Li *et al*. (2010). In contrast, 33.3% MDR observed in irrigation water sources (river) isolates in another study carried out in South Africa (Aijuka *et al.,* 2015). Ortega-Paredes *et al*. (2018) reported a 100% MDR phenotype of ESBL/AmpC Enterobacteriaceae isolates from municipal market vegetables in Quito-Ecuador; higher than 96.1% MDR phenotype reported in raw vegetables (spinach) retailed at selected sites in South Africa (Richter *et al*., 2019) and less than 90% MDR reported on spinach in the current study.

The current study also showed higher resistance of ESBL/AmpC Enterobacteriaceae isolates against the non-β-lactams aminoglycosides (18.8% to 89.6%), tetracycline (60.4%), trimethoprim/ sulfamethoxazole (54.1%) and chloramphenicol (25%), although an alternative study found varied resistance on aminoglycosides (94.8%), tetracycline (53.2%) and chloramphenicol (85.7%) on retailed vegetable samples (Richter *et al*., 2019). Similarly, resistance against aminoglycosides (gentamicin) (33.3%), tetracycline (65%), trimethoprim/ sulfamethoxazole between (73% and 75%) and chloramphenicol (46.7%) on imported fresh vegetables was also reported (Zurfluh *et al*., 2015). Additionally, this study recorded 89.6% susceptibility to imipenem; similar to other previous published studies (Zekar *et al.,* 2017; Kim *et al*., 2015; Zhang *et al*., 2018).

The high prevalence of MDR exhibited in all the isolates from the leafy green supply chain may pose a potential route of human exposure to these MDR strains. The findings in this study highlights the need for further evaluation on the prevalence of MDR ESBL/AmpC-producing Enterobacteriaceae not only in leafy greens linking actual organisms with clinical isolates using source tracking but also on isolates from irrigation water sources. Further research should consider assessing the level of risks

associated with the route of transmission of 3GC's resistant bacteria due to the increased consumption of leafy greens.

Conclusion

Results from this study confirmed that leafy green supply chains may pose a potential route of human exposure to MDR *E. coli* strains. The findings in this study highlight the need for further evaluation on the prevalence of MDR ESBL/AmpC-producing Enterobacteriaceae from source to final product, since isolates from irrigation water was clearly linked with isolates from the leafy greens. Further research should focus on determining the level of risk associated with this route of human exposure to 3GC's resistant bacteria due to increased consumption of leafy greens and the number of associated foodborne disease outbreaks globally.

4.2.7 Microbiological analysis of fresh produce from informal vendors

Microbiological quality of fresh produce purchased from street vendors in the informal settlements of Gauteng Province

Authors: Tintswalo Baloyi, Stacey Duvenage, Erika du Plessis, Germán Villamizar-Rodríguez and Lise Korsten

A manuscript entitled "Multidrug resistant *Escherichia coli* from fresh produce sold by street vendors in South African informal settlements" has been accepted for publication in the International Journal of Environmental Health Research.

Specific aim: This study aimed to assess the prevalence of foodborne pathogens (*Escherichia coli*, *Salmonella* spp. and *Listeria monocytogenes*), quality indicator organisms (coliforms, *E. coli* and Enterobacteriaceae) as well as extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, virulence genes and antibiotic resistance profiles of fresh produce samples sourced from informal street vendors (SVs).

Experimental procedures

Details of, street vendor sampling sites, fresh produce sampled, collection, processing and microbiological analysis were described in Chapter 3, section 3.2.3, 3,3.3 and 3.4.

Results

Coliform, Enterobacteriaceae and *Escherichia coli* enumeration. The hygiene indicator bacteria counts were summarised in Table 48. Significant differences were observed for all quality indicator organisms relative to the products, greengrocers and the area ($P<0.0001$) (Table 49).

The distribution of *E. coli* counts amongst the samples tested was low, with enumeration only possible in 44 out of 375 samples (11.73%) (Table 49). From the 44 samples, 35 had *E. coli* counts above the acceptable limit of 2 log CFU/g as recommended in the microbiological quality guidelines of other countries [Health Protection Agency (HPA), 2009; New South Wales (NSW) Food safety Authority, 2009]. The *E. coli* counts were predominately from spinach (20 out of 44) and carrots (14 out of 44) with a mean count of 0.73 and 0.57 log CFU/g respectively. The highest number of spinach samples contaminated with *E. coli* were from Mamelodi (40%) followed by Atteridgeville (32%).

Pathogen and ESBL-Enterobacteriaceae detection. Seven samples were positive for *Salmonella* spp. and six were positive for *Listeria* spp. However, only one spinach sample from Ivory Park resulted in the isolation of viable *Salmonella* spp. (Figure 16). *E. coli* was detected from 66 (17.6%) of fresh produce samples. Twelve samples were positive for ESBL-producing *E. coli* (Figure 17). *Escherichia coli* contamination of spinach (24 out of the 75 samples) was found to be significantly higher than observed on the other fresh produce ($p<0.001$). ESBL Enterobacteriaceae were detected from 63 (16.80%). The prevalence of presumptive ESBL Enterobacteriaceae on spinach (30.67%) were significantly higher than that found on apples (25.33%), cabbage (14.67%), carrots (8.00%) and tomato (5.33%). The confirmed ESBL Enterobacteriaceae isolates were belonging to seven genera including *Enterobacter* spp. (37.3%), *Rahnella* spp. (6.0%), *Proteus* spp. (10.4%), *Serratia* spp. (16.4%), *Citrobacter* spp. (26.9%) and *Hafnia alvei* (3%) (Figure 17).

Table 49: Total coliforms, *Escherichia coli* and Enterobacteriaceae loads in fresh produce purchased from informal street vendors in Gauteng Province

AREA Product (n)	Total coliforms (log CFU/g)			*Escherichia coli* (log CFU/g)			Enterobacteriaceae (log CFU/g)		
	Mean	Range	% positive	Mean	Range	% positive	Mean	Range	% positive
AREA 1 Mamelodi									
Apple (25)	2.27 ± 0.41^{D}	0.00-4.57	68.00	0.00 ± 0.00^{D}	0.00-0.00	0.00	2.14 ± 0.25^{D}	0.00-4.42	84.00
Cabbage (25)	4.61 ± 0.45^{C}	3.27-8.02	92.00	0.28 ± 0.16^{D}	0.00-2.80	12.00	5.57 ± 0.21^{B}	3.18-7.00	100.00
Carrots (25)	5.75 ± 0.29^{B}	0.00-7.30	96.00	0.85 ± 0.34^{BA}	0.00-5.30	28.00	5.52 ± 0.27^{B}	0.00-6.74	96.00
Spinach (25)	5.70 ± 0.44^{B}	0.00-9.06	96.00	0.91 ± 0.25^{BA}	0.00-4.48	40.00	5.81 ± 0.37^{B}	0.00-7.12	92.00
Tomato (25)	4.83 ± 0.34^{C}	0.00-6.30	96.00	1.19 ± 0.46^{A}	0.00-7.34	24.00	3.22 ± 0.39^{C}	0.00-5.65	80.00
Mean	**4.64 ± 0.21^{B}**			**0.65 ± 0.13^{A}**			**4.45 ± 0.19^{B}**		
AREA 2 Atteridgeville									
Apple (25)	2.51 ± 0.23^{D}	0.00-4.65	92.00	0.00 ± 0.00^{D}	0.00-0.00	0.00	1.64 ± 0.28^{D}	0.00-3.57	64.00
Cabbage (25)	5.62 ± 0.26^{B}	0.00-7.26	96.00	0.00 ± 0.00^{D}	0.00-0.00	0.00	5.36 ± 0.18^{B}	3.70-7.61	100.00
Carrots (25)	5.87 ± 0.10^{B}	4.60-6.50	100.00	0.39 ± 0.27^{DC}	0.00-5.30	8.00	5.45 ± 0.12^{B}	4.38-6.27	100.00
Spinach (25)	6.90 ± 0.14^{A}	5.60-8.36	100.00	1.13 ± 0.36^{A}	0.00-5.24	32.00	7.05 ± 0.20^{A}	4.70-9.20	100.00
Tomato (25)	4.16 ± 0.21^{C}	2.12-6.67	100.00	0.16 ± 0.16^{D}	0.00-4.08	4.00	3.04 ± 0.30^{C}	0.00-5.43	92.00
Mean	**5.01 ± 016^{A}**			**0.34 ± 0.10^{B}**			**4.51 ± 0.20^{B}**		
AREA 3 Ivory Park									
Apple (25)	2.43 ± 0.19^{D}	1.32-4.63	100.00	0.00 ± 0.00^{D}	0.00-0.00	0.00	ND	ND	ND
Cabbage (25)	4.79 ± 032^{C}	0.00-6.28	92.00	0.00 ± 0.00^{D}	0.00-0.00	0.00	ND	ND	ND
Carrots (25)	5.60 ± 0.27^{B}	3.00-7.78	100.00	0.47 ± 0.20^{BDC}	0.00-3.28	20.00	ND	ND	ND
Spinach (25)	5.65 ± 0.20^{B}	3.97-7.12	100.00	0.15 ± 0.11^{D}	0.00-2.10	8.00	5.53 ± 0.23^{B}	0.70-6.50	100.00
Tomato (25)	4.84 ± 0.29^{C}	2.30-8.51	100.00	0.00 ± 0.00^{D}	0.00-0.00	0.00	5.33 ± 0.19^{B}	3.34-6.78	100.00
Mean	**4.66 ± 0.16^{B}**			**0.13 ± 0.05^{B}**			**5.43 ± 0.15^{A}**		

[a] Within the same Column, means with different letters are significantly different ($p<0.0001$)

[ND] Enumeration not done

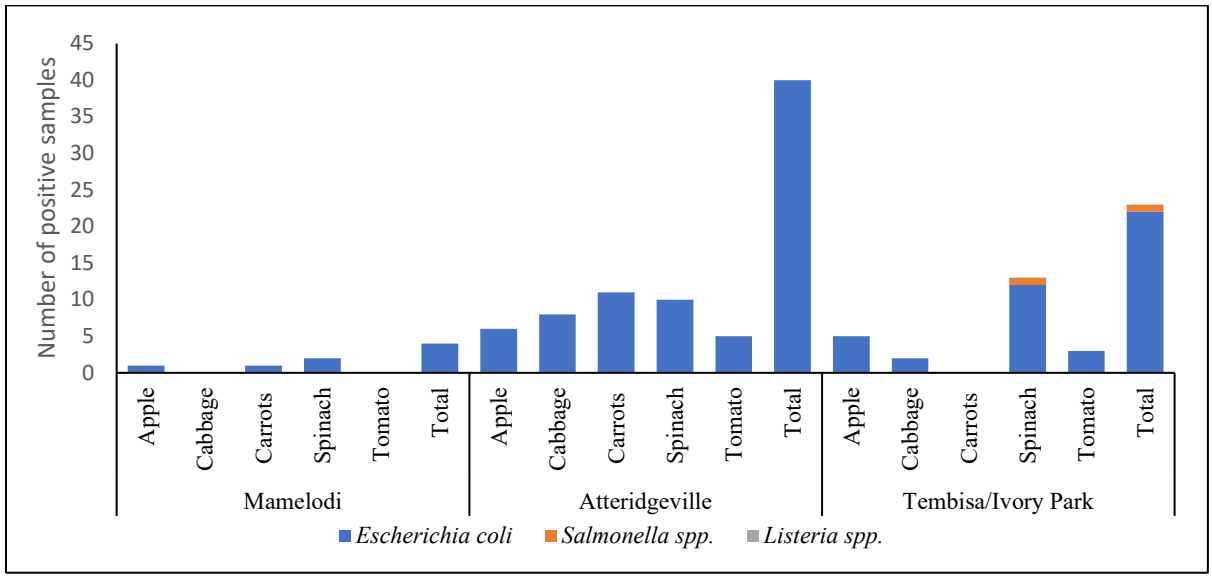


Figure 16: Prevalence of foodborne pathogens (*Escherichia coli*, *Salmonella* spp. and *Listeria* spp.) on fresh produce purchased from street vendors in the informal settlements of Gauteng Province.

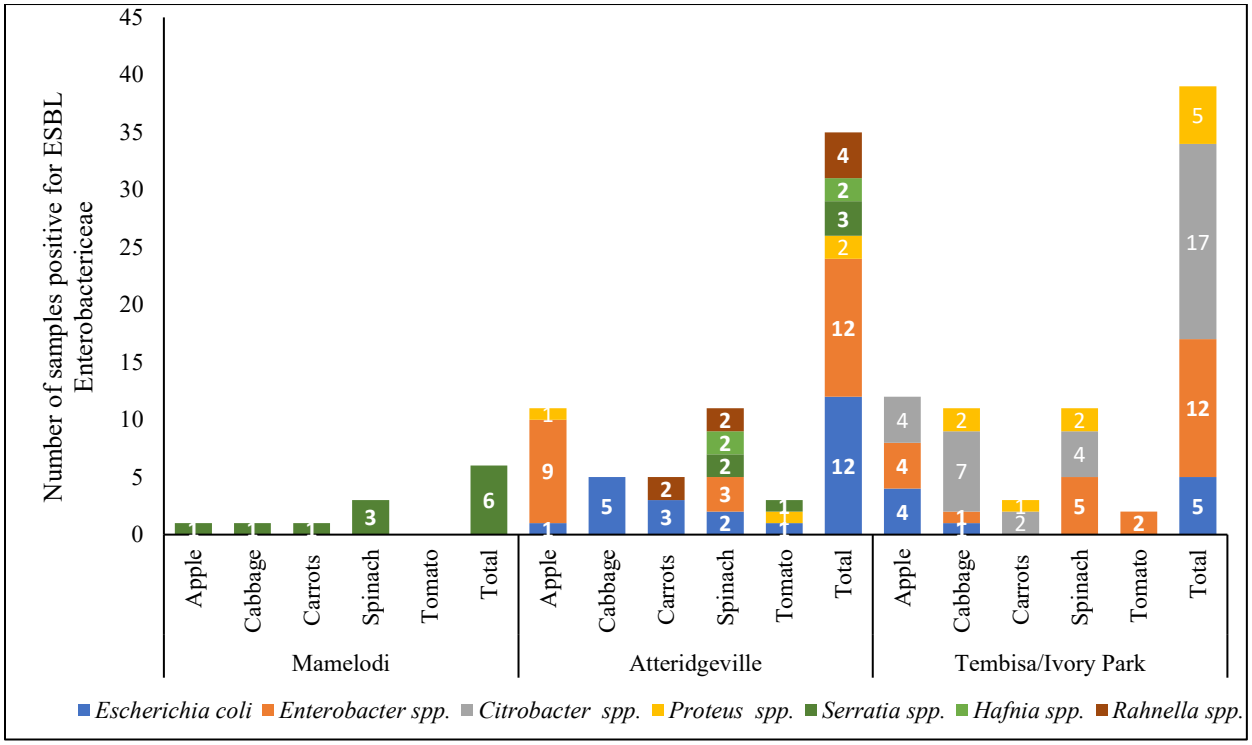


Figure 17: Different genera of ESBL-Enterobacteriaceae detected from fresh produce purchased from street vendors in the informal settlements of Gauteng Province.

Characterisation of *Escherichia coli.* Out of the 56 *E. coli* isolates screened, only one retrieved from a tomato sample was positive for the eae virulence factor; no other virulence genes were detected. The main phylogenetic groups identified were E (28.57%, n=16), C (26.79%, n=15) and B1 (21.43%; n=12), with 16.07% of isolates belonging to phylogenetic group A and 7.14% of isolates not grouped into a phylogenetic group and classified as unknown (Figure 18).

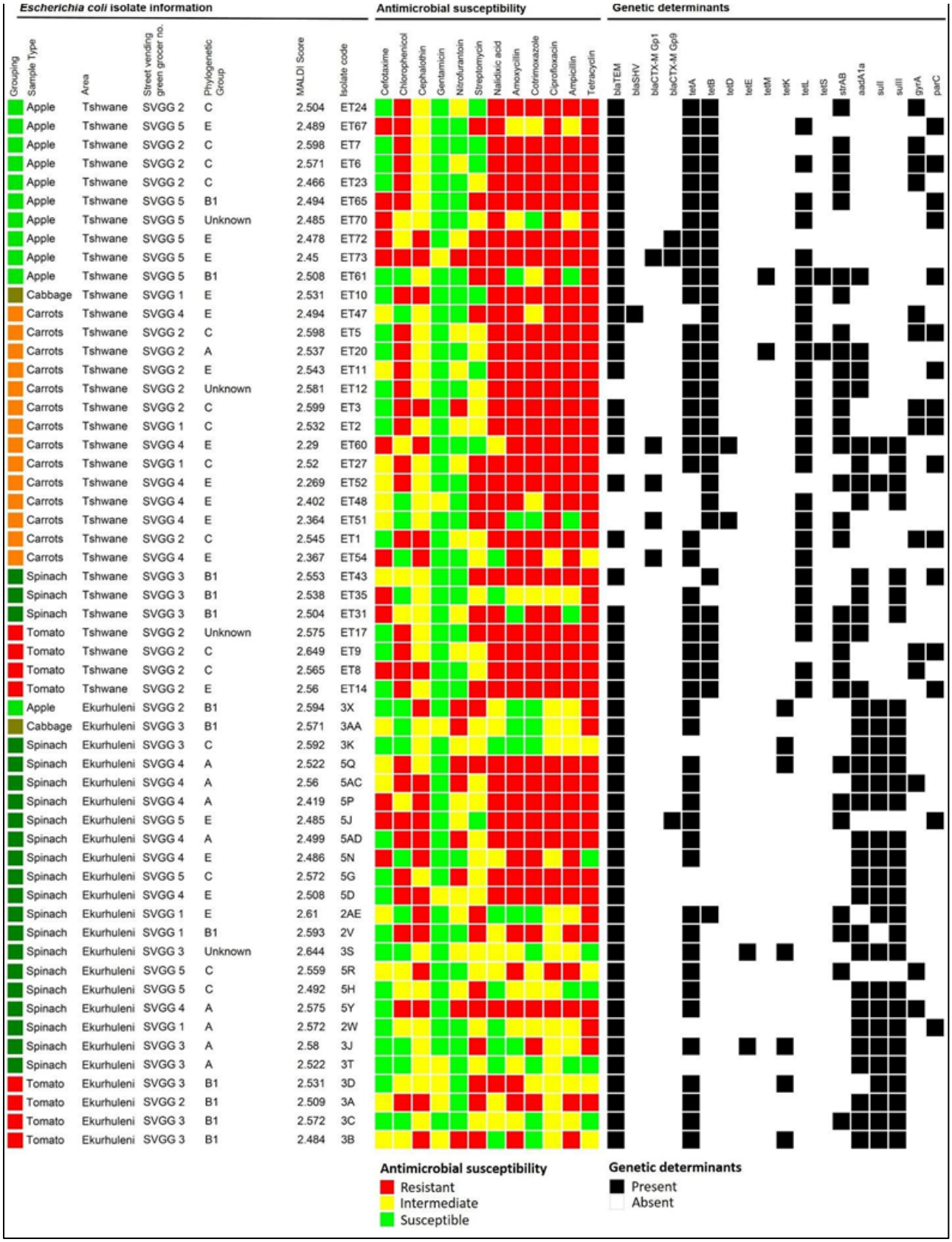

Escherichia coli isolate information
Antimicrobial susceptibility
Genetic determinants
Grouping
Sample Type
Area
Street vending green grocer no.
Phylogenetic Group
MALDI Score
Isolate code
Cefotaxime
Chloramphenicol
Cephalothin
Gentamicin
Nitrofurantoin
Streptomycin
Nalidixic acid
Amoxycillin
Cotrimoxazole
Ciprofloxacin
Ampicillin
Tetracyclin
blaTEM
blaSHV
blaCTX-M Gp1
blaCTX-M Gp9
tetA
tetB
tetD
tetE
tetM
tetK
tetL
tetS
strAB
aadA1a
sull
sulII
gyrA
parC
Apple Tshwane SVGG 2 C 2.504 ET24
Apple Tshwane SVGG 5 E 2.489 ET67
Apple Tshwane SVGG 2 C 2.598 ET7
Apple Tshwane SVGG 2 C 2.571 ET6
Apple Tshwane SVGG 2 C 2.466 ET23
Apple Tshwane SVGG 5 B1 2.494 ET65
Apple Tshwane SVGG 5 Unknown 2.485 ET70
Apple Tshwane SVGG 5 E 2.478 ET72
Apple Tshwane SVGG 5 E 2.45 ET73
Apple Tshwane SVGG 5 B1 2.508 ET61
Cabbage Tshwane SVGG 1 E 2.531 ET10
Carrots Tshwane SVGG 4 E 2.494 ET47
Carrots Tshwane SVGG 2 C 2.598 ET5
Carrots Tshwane SVGG 2 A 2.537 ET20
Carrots Tshwane SVGG 2 E 2.543 ET11
Carrots Tshwane SVGG 2 Unknown 2.581 ET12
Carrots Tshwane SVGG 2 C 2.599 ET3
Carrots Tshwane SVGG 1 C 2.532 ET2
Carrots Tshwane SVGG 4 E 2.29 ET60
Carrots Tshwane SVGG 1 C 2.52 ET27
Carrots Tshwane SVGG 4 E 2.269 ET52
Carrots Tshwane SVGG 4 E 2.402 ET48
Carrots Tshwane SVGG 4 E 2.364 ET51
Carrots Tshwane SVGG 2 C 2.545 ET1
Carrots Tshwane SVGG 4 E 2.367 ET54
Spinach Tshwane SVGG 3 B1 2.553 ET43
Spinach Tshwane SVGG 3 B1 2.538 ET35
Spinach Tshwane SVGG 3 B1 2.504 ET31
Tomato Tshwane SVGG 2 Unknown 2.575 ET17
Tomato Tshwane SVGG 2 C 2.649 ET9
Tomato Tshwane SVGG 2 C 2.565 ET8
Tomato Tshwane SVGG 2 E 2.56 ET14
Apple Ekurhuleni SVGG 2 B1 2.594 3X
Cabbage Ekurhuleni SVGG 3 B1 2.571 3AA
Spinach Ekurhuleni SVGG 3 C 2.592 3K
Spinach Ekurhuleni SVGG 4 A 2.522 5Q
Spinach Ekurhuleni SVGG 4 A 2.56 5AC
Spinach Ekurhuleni SVGG 4 A 2.419 5P
Spinach Ekurhuleni SVGG 5 E 2.485 5J
Spinach Ekurhuleni SVGG 4 A 2.499 5AD
Spinach Ekurhuleni SVGG 4 E 2.486 5N
Spinach Ekurhuleni SVGG 5 C 2.572 5G
Spinach Ekurhuleni SVGG 4 E 2.508 5D
Spinach Ekurhuleni SVGG 1 E 2.61 2AE
Spinach Ekurhuleni SVGG 1 B1 2.593 2V
Spinach Ekurhuleni SVGG 3 Unknown 2.644 3S
Spinach Ekurhuleni SVGG 5 C 2.559 5R
Spinach Ekurhuleni SVGG 5 C 2.492 5H
Spinach Ekurhuleni SVGG 4 A 2.575 5Y
Spinach Ekurhuleni SVGG 1 A 2.572 2W
Spinach Ekurhuleni SVGG 3 A 2.58 3J
Spinach Ekurhuleni SVGG 3 A 2.522 3T
Tomato Ekurhuleni SVGG 3 B1 2.531 3D
Tomato Ekurhuleni SVGG 2 B1 2.509 3A
Tomato Ekurhuleni SVGG 3 B1 2.572 3C
Tomato Ekurhuleni SVGG 3 B1 2.484 3B
Antimicrobial susceptibility
Resistant
Intermediate
Susceptible
Genetic determinants
Present
Absent

Figure 18 (appearing on previous page): Antimicrobial resistance profiles, antimicrobial genes present in all *Escherichia coli* isolates. Red blocks represent an isolate is resistant to the specified antibiotic, a yellow block indicates intermediate resistance to the specified antibiotic and a green block represents susceptibility to the specified antibiotic. A black block represents the presence of the antimicrobial resistance genes screened for and a white block indicates the absence of the gene.

Overall, *E. coli* isolates retrieved in this study demonstrated high levels of antimicrobial resistance, with 85.71% of all 56 *E. coli* isolates demonstrated MDR (n=48) and 82.00% exhibited a MARI value of more than 0.2 (Krumperman, 1983). *Escherichia coli* retrieved demonstrated resistance to tetracycline (80.36%), amoxicillin (73.21%), ampicillin (71.43%, n=40), trimethoprim-sulfamethoxazole (66.07%), cephalothin (64.29%), nalidixic acid and ciprofloxacin (57.14%), chloramphenicol (50%), streptomycin (46.43%), nitrofurantoin (41.07%) and gentamicin (10.71%) (Figure 18, Table 50).

Table 50: Antibiotic resistance profiles of *Escherichia coli* associated with fresh produce sampled

Multidrug resistant *Escherichia coli* profiles	**No. Isolates**	**% with same profiles**
CTX30C-KF30C-C30C-GM10C-S10C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-C30C-GM10C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-S10C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	3	6.52%
C30C-S10C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	9	19.57%
KF30C-C30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	2	4.35%
C30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
C30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	2	4.35%
C30C-NI300C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
KF30C-C30C-GM10C-S10C-TS25C-A10C-AP10C-T30C	1	2.17%
KF30C-C30C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	3	6.52%
KF30C-S10C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
C30C-TS25C-NA30C-A10C-AP10C-CIP5C-T30C	1	2.17%
CTX30C-KF30C-C30C-GM10C-A10C-AP10C-T30C	1	2.17%
CTX30C-KF30C-NI300C-TS25C-NA30C-CIP5C-T30C	1	2.17%
KF30C-C30C-S10C-TS25C-A10C-AP10C-T30C	1	2.17%
CTX30C-KF30C-TS25C-A10C-AP10C-CIP5C	1	2.17%
CTX30C-KF30C-S10C-A10C-T30C	1	2.17%

Table 50 cont.

Multidrug resistant *Escherichia coli* profiles	No. Isolates	% with same profiles
CTX30C-KF30C-TS25C-A10C-AP10C	1	2.17%
CTX30C-KF30C-TS25C-A10C-AP10C-T30C	1	2.17%
GM10C-S10C-NA30C-A10C-AP10C-T30C	1	2.17%
KF30C-S10C-NI300C-A10C-AP10C	1	2.17%
S10C-TS25C-NA30C-CIP5C-T30C	1	2.17%
KF30C-A10C-AP10C-T30C	1	2.17%
KF30C-S10C-NI300C-T30C	1	2.17%
KF30C-S10C-NI300C-T30C	1	2.17%
S10C-NA30C-CIP5C-T30C	1	2.17%
KF30C-NI300C-T30C	1	2.17%
KF30C-S10C-T30C	1	2.17%
S10C-TS25C-T30C	1	2.17%

Table 51: Prevalence of antimicrobial resistance genes in *Escherichia coli* isolated from fresh produce

Antimicrobial resistance gene tested	Number of isolates positive for the gene	Percentage of isolates containing the gene
bla_{TEM}	50	89.29%
tetA	46	82.14%
aadA1a	33	58.93%
tetB	30	53.57%
strAB	29	51.79%
sulII	29	51.79%
tetL	26	46.43%
sulI	23	41.07%
parC	16	28.57%
gyrA	14	25.00%
tetK	7	12.50%
$bla_{CTX\text{-}M\ Gp1}$	5	8.93%
$bla_{CTX\text{-}M\ Gp9}$	3	5.36%
tetD	2	3.57%
tetE	2	3.57%
tetM	2	3.57%
tetS	2	3.57%
bla_{SHV}	1	1.79%

bla_{OXA}, $bla_{CTX\text{-}M\ Gp2}$, $bla_{CTX\text{-}M\ Gp8\text{-}25}$, VEB, PER, GES, blaZ, ACC, FOX, MOX, DHA, CIT, EBC, ampC, tetC, tetO, tetP, tetQ, tetX, aac(6')-Ib, qnrD, qnrS, catI, catII and catIII were not detected in the 56 isolates

The frequency of the detected antimicrobial resistance genes are shown in Table 51. The following β-lactamase encoding genes were detected from the 56 isolates: bla_{TEM} (89.29%), $bla_{CTX\text{-}M\ Gp1}$ (8.93%), $bla_{CTX\text{-}M\ Gp9}$ (5.36%) and bla_{SHV} (1.79%). The following tetracycline encoding genes were detected: *tetA* (82.14%), *tetB* (53.57%), *tetL* (46.43%), *tetK* (12.50%), *tetD* (3.57%), *tetE* (3.57%), *tetM* (3.57%) and *tetS* (3.57%). Gene conferring resistance to aminoglycosides were detected with *aadA1a* and *strAB* present in 58.93% and 51.79% of isolates, respectively (Table 51). Genes *sulI* and *sulII* conferring resistance to Sulfonamides were detected from 41% and 51.79% of isolates, respectively (Table 51). No AmpC β-lactamase, ampC, Fluoroquinolones (qnrD and qnrS) and Phenicol (catI, catII and catIII) resistance encoding genes tested for were detected.

Discussion

Street vendors in the informal settlements are confronted with lack of infrastructure such as potable water, ablution, and storage and cooling facilities, that can greatly impact the microbiological quality of fresh produce (Marutlulle, 2017; Du Plessis *et al.*, 2017). Moreover, due to the unregulated system in informal street vending, implementation of a food safety standards can be challenging. According to the previously used microbiological guidelines by the Department of Health in South Africa (NDOH, 2000) no *Salmonella spp.* or *Listeria* spp. should be detected on 25 g of fresh produce and coliforms should be < 2 log CFU/g (NDOH, 2000) on vegetables and fruit to be eaten raw.

Foodborne pathogens including *L. monocytogenes*, *Salmonella* spp. and *E. coli* have been reported in outbreaks associated with fresh produce consumption (CDC, 2020). Besides the well described foodborne pathogen presence on fresh produce and the resultant disease outbreaks associated with the formal economy, very little is known about the informal sectors *(*Denis *et al.,* 2016*;* Korir *et al*., 2016; du Plessis *et al*., 2017). *Listeria monocytogenes* was not detected in any of the samples. Similarly, *L. monocytogenes* was not detected from 45 spinach and cabbage sourced in informal retailers in Gauteng and 150 varying fresh produce from street vendors in the Western Cape.

The low prevalence of *Salmonella* spp. in the current study was similar to results reported by Du Plessis *et al.* (2017). All fresh produce had average coliform counts above the acceptable limit if the previous NDOH guidelines are used. The natural occurrence of coliforms on fresh produce

and the fact that they are not all pathogenic, limits the use of this group as accurate indicator system for faecal contamination (Graça *et al.*, 2017). In contrast to the high concentration and prevalence of coliforms and Enterobacteriaceae, *E. coli* counts in the present study were below detectable limits for 88.27% of the samples. Spinach samples found to have the highest prevalence of *E. coli* (44%) followed by carrots (22%), apples (22%), tomatoes (16%) and finally cabbage (8% ESV; 32% TSV). In addition, *E. coli* was isolated from 32% of the spinach in this study, which is higher than the 20 and 18% detected by Jongman and Korsten (2017) on lettuce and spinach from commercial farms in South Africa. However, with the lack of effective policies and regulation, as well as sector-specific food safety standards, it is difficult to evaluate how safe the fresh produce really is and what the actual level of risk to the consumer is.

Resistance of 85 *E. coli* isolates in this study to all classes tested were far higher than those in other similar studies (Du Plessis *et al*., 2017; Verma *et al*., 2018) and similar to other studies (Kilonzo-Nthenge *et al*., 2018; Corzo-Ariyama *et al.,* 2019). The presence of these antimicrobial resistant commensal and environmental *E. coli* strains is considered a high-risk (Krumperman, 1983) due to the potential that these organisms have to transmit antimicrobial resistance conferring genes to other environmental and human gut bacteria (Marshall *et al*., 2009). Therefore, the spread of antimicrobial resistant bacteria from plants to humans via the food chain as well as the potential spread of antimicrobial resistant genes requires a holistic "One-Health" approach in order to control and mitigate the risk of exposure (Jans *et al*., 2018).

The diversity of phylogenetic groups in this study were higher than found by Du Plessis *et al.* (2017) who found that *E. coli* isolated from spinach and cabbage sold at retailers and informal vendors in South Africa belonged mainly to phylogenetic group A (86%), followed by group E (7%). In this study, a total of 28.57% of *E. coli* retrieved from apples, cabbage, carrots, spinach and tomatoes, were phylogenetically grouped into group E, which has predominantly been associated with intra-intestinal infections (Clermont *et al*., 2011). A further 26.79% of *E. coli* isolates in this study were grouped into the phylogenetic group C, which is far rarer and has previously been shown to demonstrate the potential for gut colonization, transmission and virulence (Moissenet *et al.,* 2010). Interestingly, Du Plessis *et al*. (2017) found that 3% of *E. coli* isolates from informal vendors in South Africa were retrieved from cabbage and spinach samples

were grouped into this rare phylogenetic group. These two phylogenetic groups are present in all fresh produce samples and sites tested from selected informal vendors in South Africa.

The results from the study showed that fresh produce from the SVs had low prevalence of foodborne pathogens regardless of the hygiene status in which they are sold. However, the results show that to the produce contained high levels of MDR microorganisms. Multidrug resistant organisms can have a long-term effect in public health due to the possibility of the resistance transferred to the commensal *E. coli* in the human gut. Therefore, proper control of environmental conditions where fresh produce is sold in informal settlements is important as well as managing the supplier base in terms of good agricultural practices.

Conclusion

Although fresh produce from the street vendors had low prevalence of foodborne pathogens regardless of the hygiene conditions in which they are sold in this study, produce contained high levels of MDR microorganisms which is a serious is a serious concern. Interestingly, *E. coli* isolates from the different fresh produce sourced from a variety of SVs in this study clustered together which indicates close genetic relatedness. Multidrug resistant organisms can have a long-term effect in public health due to the possibility of the resistance transferred to the commensal *E. coli* in the human gut. Proper control of environmental conditions where fresh produce is sold in informal settlements is important as well as evaluating and managing the supplier base in terms of good agricultural practices. The need for proper regulation of the informal fresh produce supply chain, including transportation, handling, display and hygiene practices from the farm to the consumer was clearly demonstrated in this study.

4.2.8 Microbial quality and prevalence of *Escherichia coli*, *Listeria* spp. and extended-spectrum-β-Lactamase Enterobacteriaceae from small-scale farming supply chains

Authors: Degracious Kgoale, Stacey Duvenage, Erika du Plessis and Lise Korsten

Specific aim: This study aimed to codetermine the microbiological quality by determining the hygiene indicator bacteria (coliforms, *E. coli*) and Enterobacteriaceae counts, prevalence of foodborne enteric human pathogens (*E. coli*, *L. monocytogenes* and *Salmonella* spp.) in morogo cultivated in small-scale production systems in South Africa.

Experimental procedures

Details of the eight small-scale farm selected and associated retailers (formal and informal), sample collection, processing, hygiene indicator bacteria enumeration, foodborne pathogen isolation, identification and characterisation were described in Chapter 3, section 3.2.4, 3.3.4 and 3.4.

The small-scale farms selected included five farms (B, C, E, F and H) located in the Brits area and three farms located in the Delmas area (A, D, E). Farms supplying retailers (formal and informal) in the Tshwane Metropole (Tshwane Fresh Produce Market and Atteridgeville Bakkie Vendors) included Farm B, Farm C and Farm E were all located in the Brits area. A total of 426 samples, comprising of 100 water samples, 37 composite soil samples and 289 fresh produce (n=65 rape; n=106 chinensis; n=94 spinach; n=16 kale and n=8 chomolia) were collected.

Results

Enumeration of indicator microorganisms.

The coliform counts ranged from log 0.00 to 4.81 MPN/100 ml (Figure 19). Average *E. coli* counts in water ranged from log 0.00 to 3.03 MPN/100 ml with the highest counts obtained from irrigation water from Farm C which uses flooding irrigation water while the source water is from an open dam with water drawn from the Hartbeespoort dam river (Figure 20). Significant differences were observed in Farm B and C between the source water and irrigation water, however no significant

differences were observed amongst the Farms A, D and G located in the Delmas area (Figure 20). The average enterococci counts ranged from log 0.00 to 3.19 MPN/100 ml with Farm C obtaining the highest counts and no enterococci counts obtained from Farm D (Figure 21).

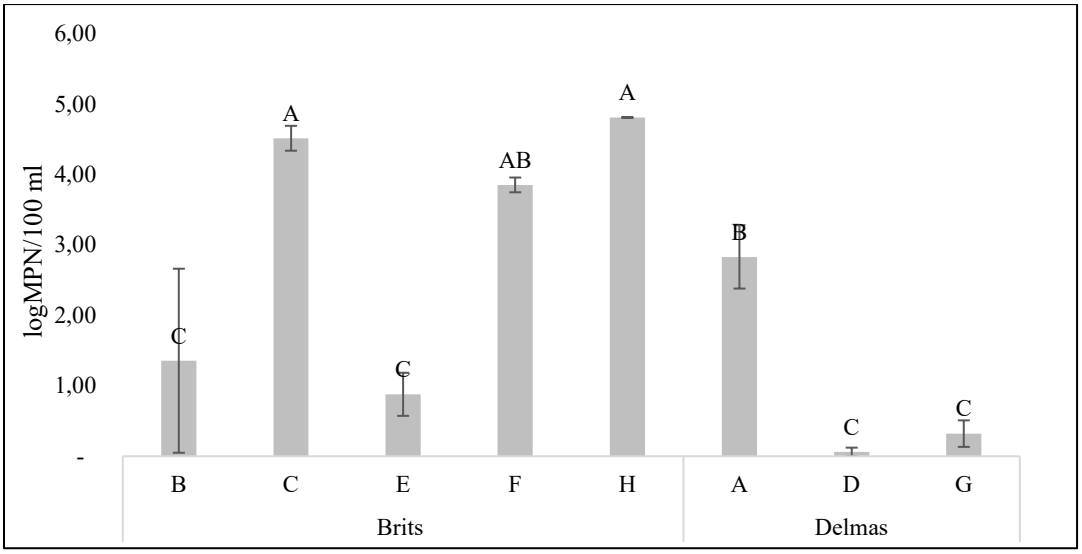


Figure 19: Coliform counts water in source water (boreholes, river) and irrigation points of the eight small-scale farms

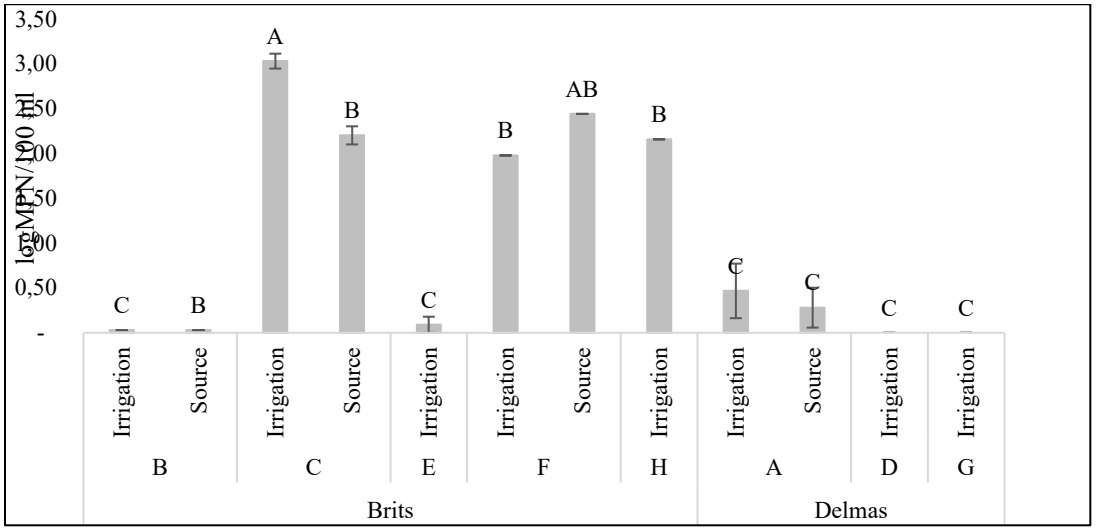


Figure 20: *Escherichia coli* counts in source water (boreholes, river) and irrigation points of the eight small-scale farms.

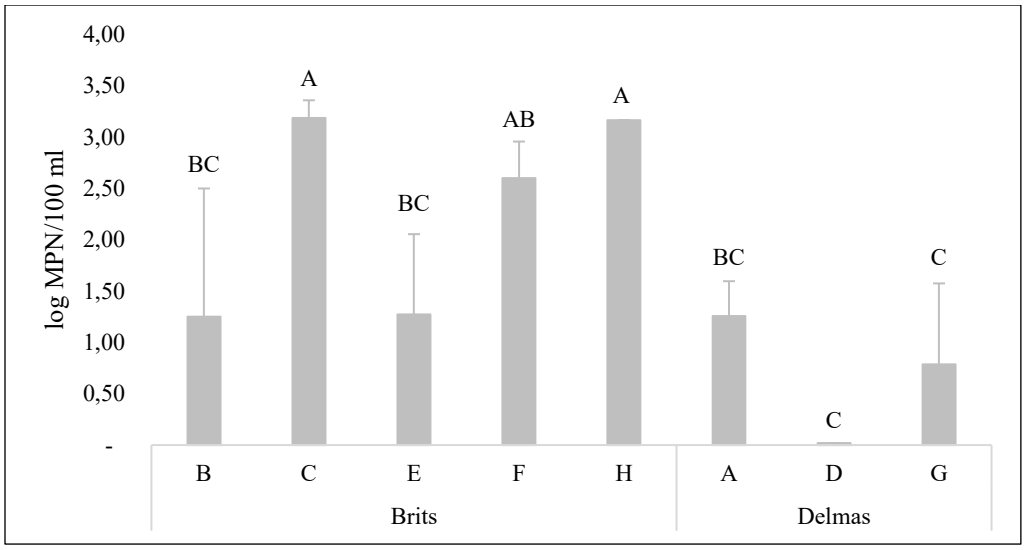


Figure 21: Enterococci counts in source water (boreholes, river) and irrigation points of the eight small-scale farms

Average Enterobacteriaceae counts in source water (river, borehole) and from the irrigation points ranged from log 0.00 to 4.4l CFU/100 ml with the highest counts obtained from Farm B located in the Brits area (Figure 22). The direct irrigation water typically showed higher counts than source water indicating that contamination of irrigation water is potentially enhanced downstream the source water.

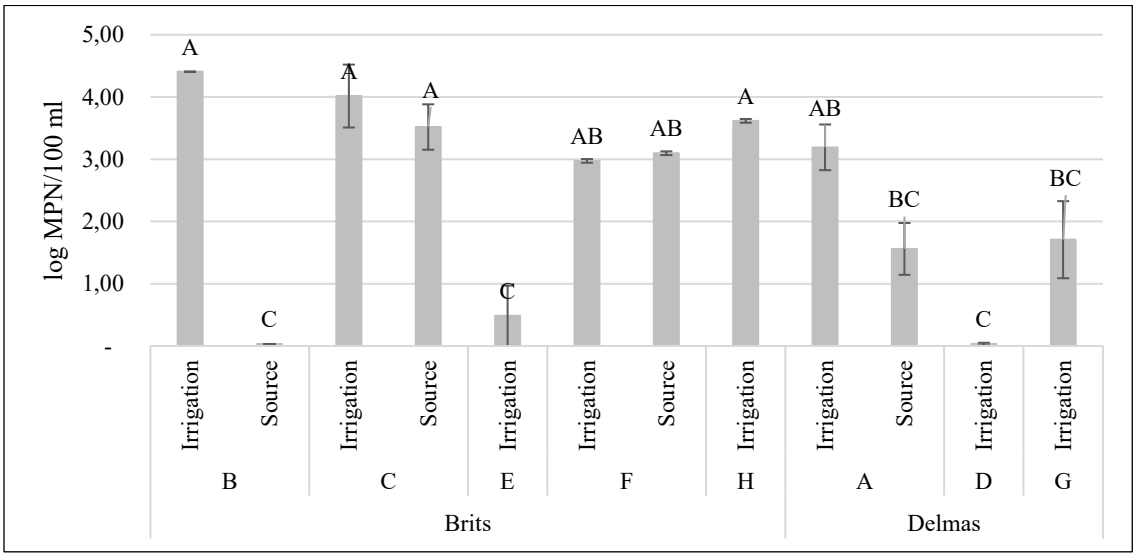


Figure 22: Enterobacteriaceae counts in source water (boreholes, river) and irrigation points of the eight small-scale farms

The average total coliform counts of the leafy greens ranged from log 1.63 to 4.57 CFU/g with the highest counts obtained from farm C point of sale spinach samples. Significant differences were observed between Brits and Delmas areas with Brits showing an average log 4.54 CFU/g and Delmas showing an average log 3.62 CFU/g in all fresh produce samples (P<0.0001) (Figure 23).

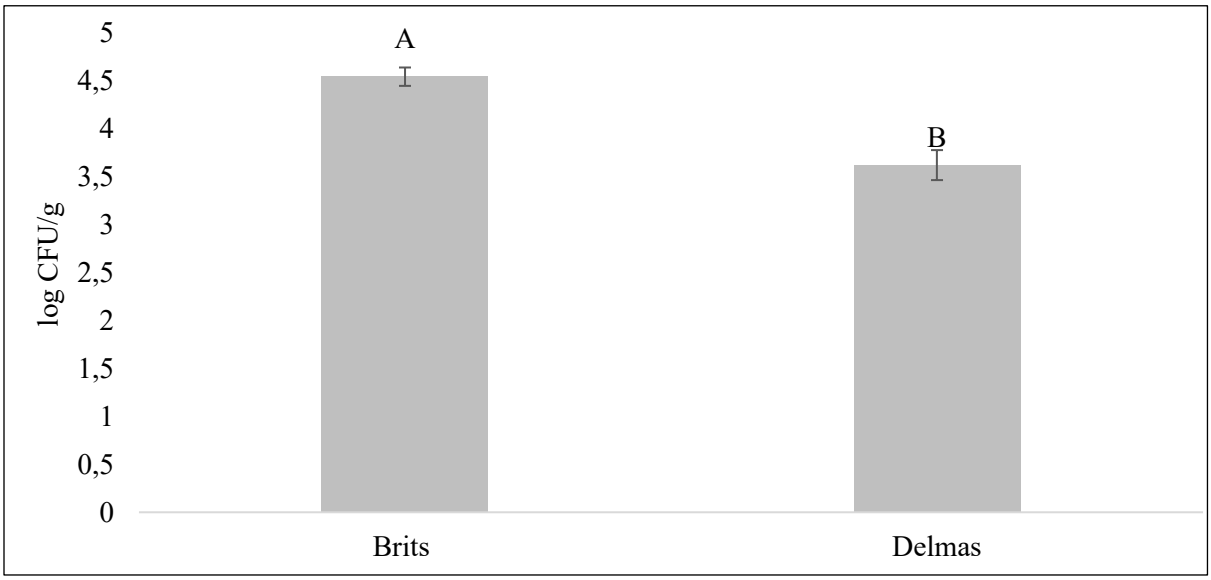


Figure 23: Total coliform counts enumerated from field and point of sale fresh produce samples from eight small-scale farms across geographical areas of Brits and Delmas. Capital letters represent LSD and error bars indicate standard deviation over the square root of the number of samples.

Average *E. coli* counts ranged from log 0.00 to 1.22 CFU/g with the highest counts obtained from field spinach samples obtained from farm F located in Brits (Figure 24). Significant differences were observed in counts between the two areas, with Brits showing higher counts than Delmas at average log 0.25 CFU/g over log 0.07 CFU/g at probability of 0.001 respectively. Furthermore, significant differences were observed at the level of area and farm where farm F showed higher *E. coli* counts of average log 0.80 CFU/g at probability of P< 0.0001. Significant differences were also observed between the different leafy greens with rape samples having the highest *E. coli* counts of average log 0.41 CFU/g (P<0.0034). Additionally, significant differences were observed at level of area, source and farm where retailer leafy green samples from farm F in Brits showed higher *E. coli* counts of average log 1.34 CFU/g with retailer samples from farm B (Brits) and G

(Delmas) showing no *E. coli* counts (Appendix). Retailer rape samples from farm F in Brits showed the highest *E. coli* counts of average log 2.02 CFU/g (Figure 24).

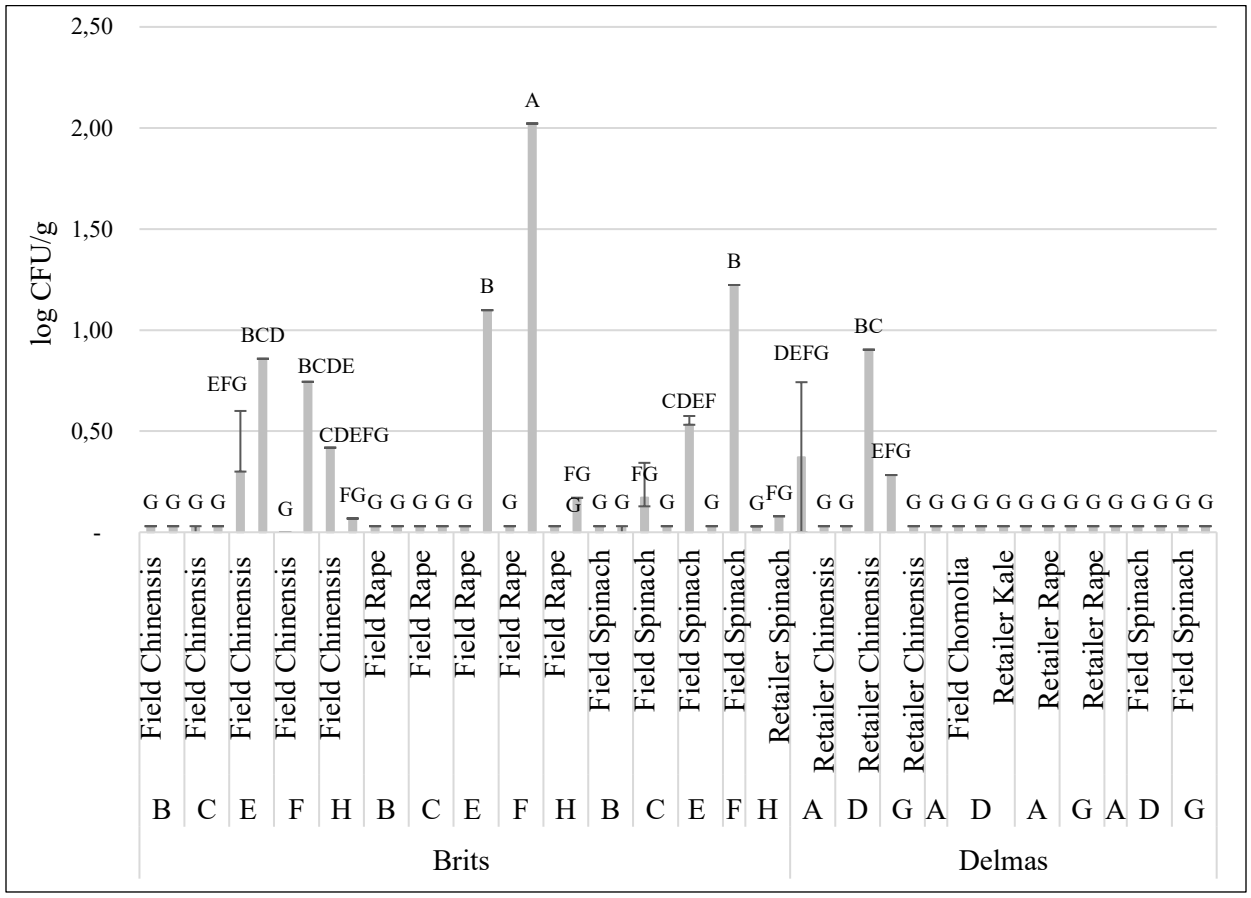


Figure 24: *Escherichia coli* counts enumerated from field and point of sale fresh produce samples from eight small-scale farms.

Average Enterobacteriaceae on fresh produce ranged from log 3.49 to 5.37 CFU/g with the highest counts observed in farm H located in Brits. However, no significant differences were observed between Brits and Delmas (Figure 25).

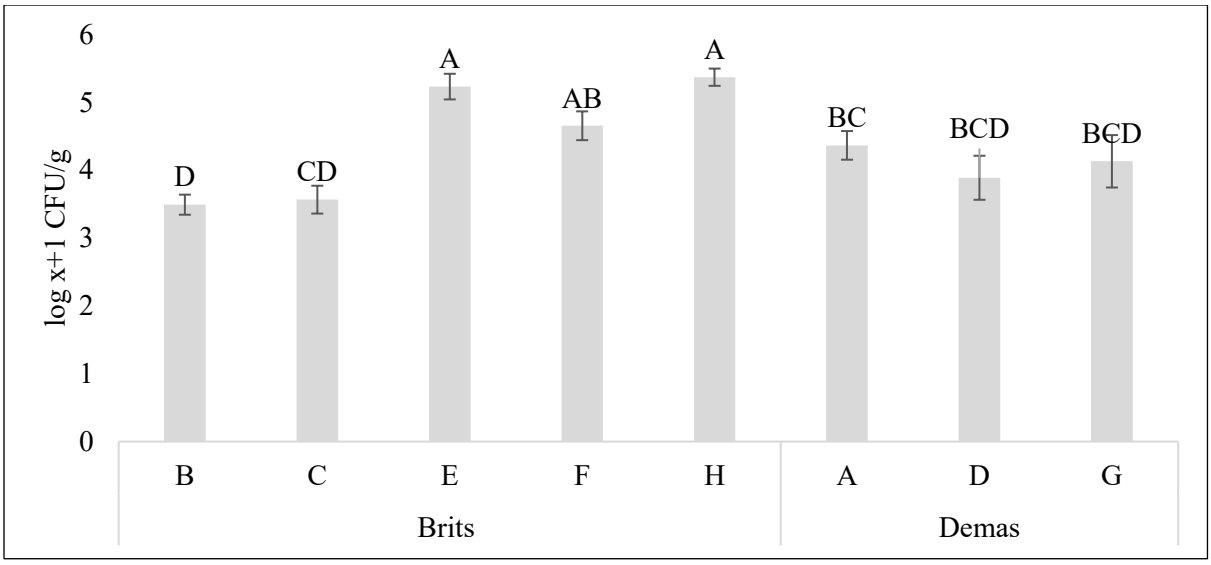


Figure 25: Enterobacteriaceae counts enumerated from field and point of sale fresh produce samples from eight small-scale farm across geographical areas of Brits and Delmas. Capital letters represent LSD and error bars indicate standard deviation over the square root of the number of samples.

Average total coliform counts ranged from log 2.43 CFU/g to log 3.74 CFU/g in soil, with the highest counts obtained from Farm F located in the Brits area (Figure 27). No significant difference was observed in the average total *E. coli* counts between the eight farms and across the two areas with counts ranging from log 0.00 to 1.33 CFU/g. No *E. coli* counts were obtained in farms B, C and H (all located in the Brits area). Although no significant difference, seemingly, the Delmas area had higher *E. coli* counts than the Brits area. Furthermore, Brits soil had significantly higher Enterobacteriaceae counts than Delmas ($p=0.0250$) with the average Enterobacteriaceae counts ranging from log 3.45-5.15 CFU/g for Brits and log 3.54-4.40 CFU/g for Delmas (Figure 26).

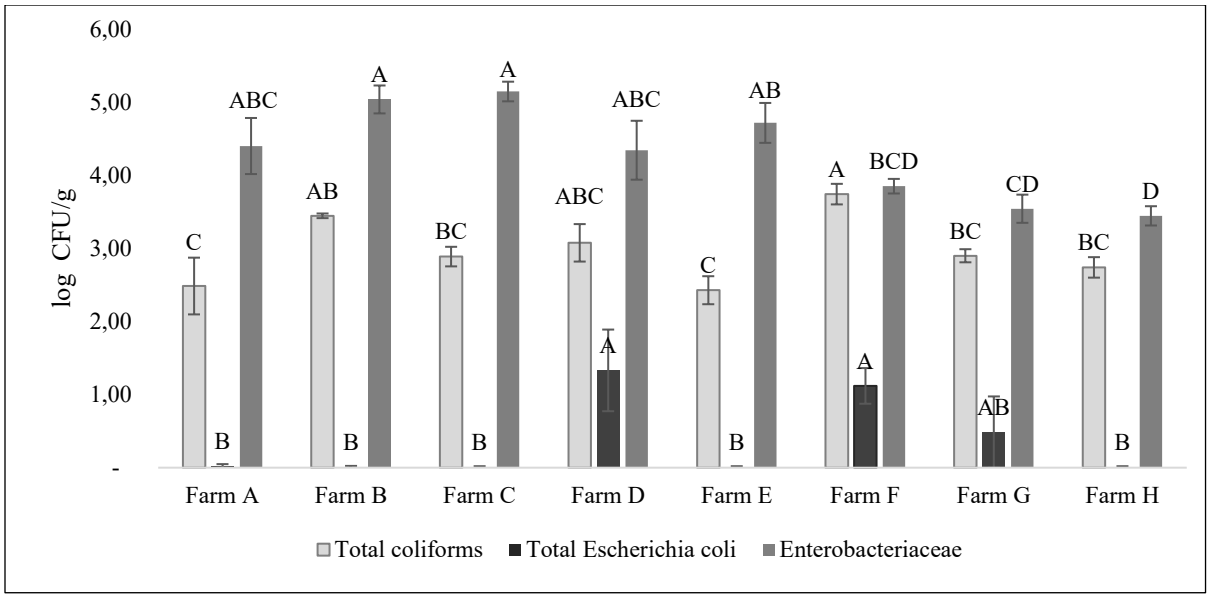
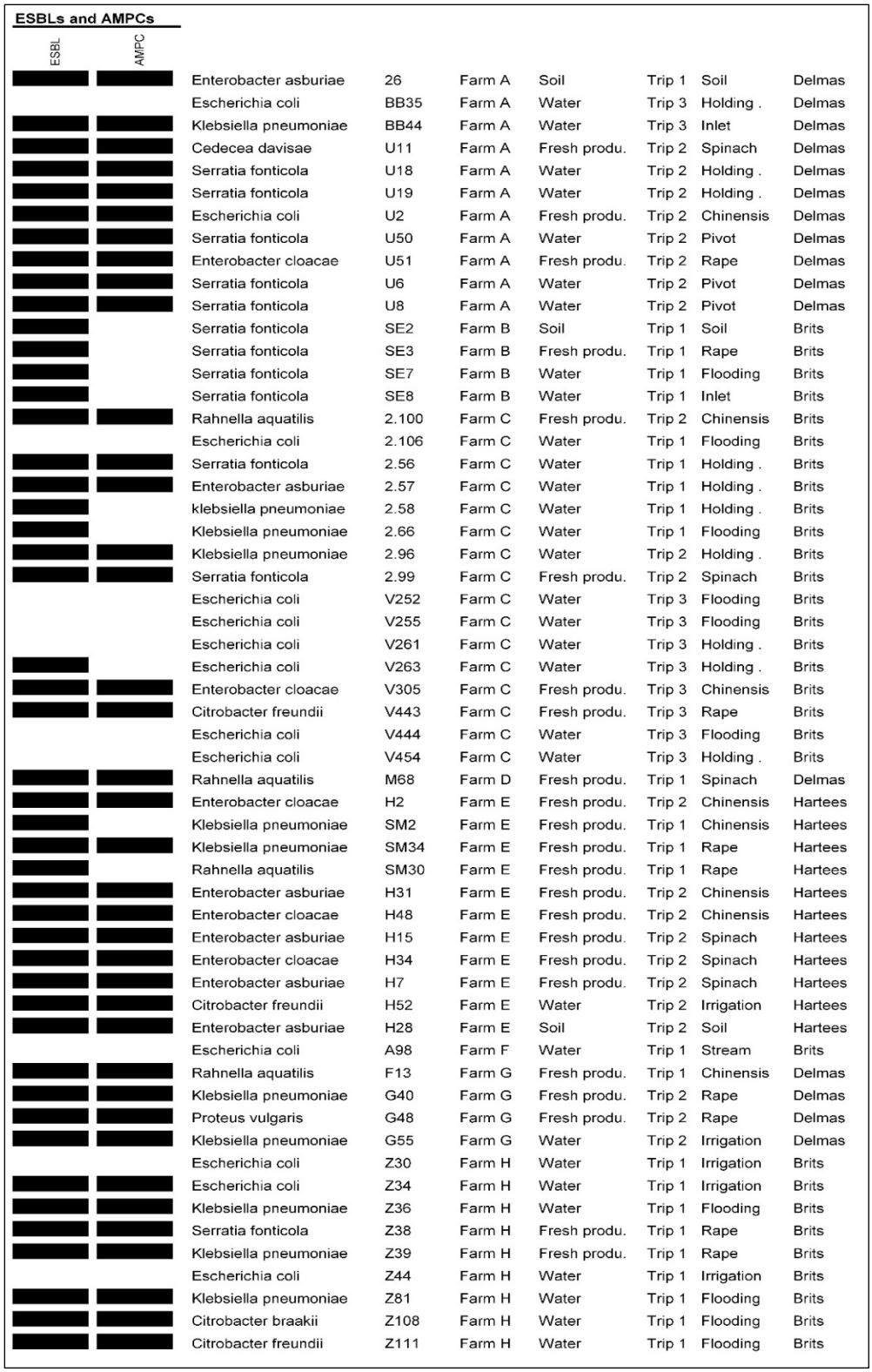


Figure 26: Coliform, *Escherichia coli* and Enteronbacteriaeae counts of soil from the eight small-scale farms

Prevalence of foodborne pathogens. No *Listeria* spp. were detected in soil and fresh produce across all eight farms. However, *Listeria* spp. occurrence was at a frequency of 0.94% (4/426) in water samples, with *Listeria monocytogenes* only detected from irrigation water from farm C and *Listeria ivanovii* was detected from irrigation water from Farm F. *Escherichia coli* was prevalent at 19.48% of all samples. Twenty-one (4.93%) samples were contaminated with *Salmonella* spp., including 17 water samples (17.00%; 17/426) and 4 leafy green vegetable samples (1.38%); mainly from Brits area.

Occurrence of Extended-spectrum β-lactamases. Presumptive ESBL Enterobacteriaceae isolates from the eight small-scale farms were isolated from 72 samples (16%) of the samples, while 79.17% isolates were confirmed ESBL-producing isolates while 78.95% of the confirmed ESBL-producing isolates also produced AmpC (Figure 27).



ESBL	AMPC							
■	■	Enterobacter asburiae	26	Farm A	Soil	Trip 1	Soil	Delmas
		Escherichia coli	BB35	Farm A	Water	Trip 3	Holding .	Delmas
■	■	Klebsiella pneumoniae	BB44	Farm A	Water	Trip 3	Inlet	Delmas
■	■	Cedecea davisae	U11	Farm A	Fresh produ.	Trip 2	Spinach	Delmas
■	■	Serratia fonticola	U18	Farm A	Water	Trip 2	Holding .	Delmas
■	■	Serratia fonticola	U19	Farm A	Water	Trip 2	Holding .	Delmas
■	■	Escherichia coli	U2	Farm A	Fresh produ.	Trip 2	Chinensis	Delmas
■	■	Serratia fonticola	U50	Farm A	Water	Trip 2	Pivot	Delmas
■	■	Enterobacter cloacae	U51	Farm A	Fresh produ.	Trip 2	Rape	Delmas
■	■	Serratia fonticola	U6	Farm A	Water	Trip 2	Pivot	Delmas
■	■	Serratia fonticola	U8	Farm A	Water	Trip 2	Pivot	Delmas
■		Serratia fonticola	SE2	Farm B	Soil	Trip 1	Soil	Brits
■		Serratia fonticola	SE3	Farm B	Fresh produ.	Trip 1	Rape	Brits
■		Serratia fonticola	SE7	Farm B	Water	Trip 1	Flooding	Brits
■		Serratia fonticola	SE8	Farm B	Water	Trip 1	Inlet	Brits
■	■	Rahnella aquatilis	2.100	Farm C	Fresh produ.	Trip 2	Chinensis	Brits
		Escherichia coli	2.106	Farm C	Water	Trip 1	Flooding	Brits
■	■	Serratia fonticola	2.56	Farm C	Water	Trip 1	Holding .	Brits
■	■	Enterobacter asburiae	2.57	Farm C	Water	Trip 1	Holding .	Brits
■		klebsiella pneumoniae	2.58	Farm C	Water	Trip 1	Holding .	Brits
■		Klebsiella pneumoniae	2.66	Farm C	Water	Trip 1	Flooding	Brits
■	■	Klebsiella pneumoniae	2.96	Farm C	Water	Trip 2	Holding .	Brits
■	■	Serratia fonticola	2.99	Farm C	Fresh produ.	Trip 2	Spinach	Brits
		Escherichia coli	V252	Farm C	Water	Trip 3	Flooding	Brits
		Escherichia coli	V255	Farm C	Water	Trip 3	Flooding	Brits
		Escherichia coli	V261	Farm C	Water	Trip 3	Holding .	Brits
■		Escherichia coli	V263	Farm C	Water	Trip 3	Holding .	Brits
■	■	Enterobacter cloacae	V305	Farm C	Fresh produ.	Trip 3	Chinensis	Brits
■	■	Citrobacter freundii	V443	Farm C	Fresh produ.	Trip 3	Rape	Brits
		Escherichia coli	V444	Farm C	Water	Trip 3	Flooding	Brits
		Escherichia coli	V454	Farm C	Water	Trip 3	Holding .	Brits
■	■	Rahnella aquatilis	M68	Farm D	Fresh produ.	Trip 1	Spinach	Delmas
■	■	Enterobacter cloacae	H2	Farm E	Fresh produ.	Trip 2	Chinensis	Hartees
■		Klebsiella pneumoniae	SM2	Farm E	Fresh produ.	Trip 1	Chinensis	Hartees
■	■	Klebsiella pneumoniae	SM34	Farm E	Fresh produ.	Trip 1	Rape	Hartees
■		Rahnella aquatilis	SM30	Farm E	Fresh produ.	Trip 1	Rape	Hartees
■	■	Enterobacter asburiae	H31	Farm E	Fresh produ.	Trip 2	Chinensis	Hartees
■	■	Enterobacter cloacae	H48	Farm E	Fresh produ.	Trip 2	Chinensis	Hartees
■	■	Enterobacter asburiae	H15	Farm E	Fresh produ.	Trip 2	Spinach	Hartees
■	■	Enterobacter cloacae	H34	Farm E	Fresh produ.	Trip 2	Spinach	Hartees
■	■	Enterobacter asburiae	H7	Farm E	Fresh produ.	Trip 2	Spinach	Hartees
■	■	Citrobacter freundii	H52	Farm E	Water	Trip 2	Irrigation	Hartees
■	■	Enterobacter asburiae	H28	Farm E	Soil	Trip 2	Soil	Hartees
		Escherichia coli	A98	Farm F	Water	Trip 1	Stream	Brits
■	■	Rahnella aquatilis	F13	Farm G	Fresh produ.	Trip 1	Chinensis	Delmas
■	■	Klebsiella pneumoniae	G40	Farm G	Fresh produ.	Trip 2	Rape	Delmas
■	■	Proteus vulgaris	G48	Farm G	Fresh produ.	Trip 2	Rape	Delmas
■	■	Klebsiella pneumoniae	G55	Farm G	Water	Trip 2	Irrigation	Delmas
		Escherichia coli	Z30	Farm H	Water	Trip 1	Irrigation	Brits
■	■	Escherichia coli	Z34	Farm H	Water	Trip 1	Irrigation	Brits
■	■	Klebsiella pneumoniae	Z36	Farm H	Water	Trip 1	Flooding	Brits
■	■	Serratia fonticola	Z38	Farm H	Fresh produ.	Trip 1	Rape	Brits
■	■	Klebsiella pneumoniae	Z39	Farm H	Fresh produ.	Trip 1	Rape	Brits
		Escherichia coli	Z44	Farm H	Water	Trip 1	Irrigation	Brits
■	■	Klebsiella pneumoniae	Z81	Farm H	Water	Trip 1	Flooding	Brits
■	■	Citrobacter braakii	Z108	Farm H	Water	Trip 1	Flooding	Brits
■	■	Citrobacter freundii	Z111	Farm H	Water	Trip 1	Flooding	Brits

Figure 27: Confirmed ESBL-producing and AmpC Enterobactericeae organisms from eight small-scale farms. Black rectangles indicate positive identification

The detection of diarrheagenic *Escherichia coli*. One *E. coli* isolate from Farm B from spinach at the point of sale contained the ST gene indicating that isolate was an Enterotoxigenic *E. coli* (ETEC) strain. Also, an isolate from flooding irrigation water from farm C had both the Eagg-pCVD and the AA PR genes indicating the isolate was an Enteroaggregative *E. coli* (EAEC) strain. An Enteropathogenic *E. coli* (EPEC) strain, Farm A isolated from field chinensis was also detected as indicated by the presence of the eaeA gene. No LT, ial, ipaH, bfpa, stx 1 and stx 2 genes were detected indicating that no Enterohaemorrhagic *E. coli* (EHEC) and Enteroinvasive *E. coli* (EIEC) were detected.

Phylotyping of *Escherichia coli* isolates. A total of 18 *E. coli* isolates belonged to the phylogenetic group A (11.76%), 80 isolates to B1 (52.29%), 8 isolates to B2 (8.00%), 8 isolates to C (8.00%), one isolate to Clade l or ll (0.65%), 1 isolate to D (0.65%), 8 isolates to E (8.00%) and 29 isolates were unknown (18.95%). At 70% homology, we observed 18 clades and 27 unclustered isolates. Clade 7 comprised of 42 isolates all belonging the different phylogroups excluding phylotype D and clade 1 /ll. Evidence of irrigation water contaminating fresh produce is observed in clade 3 where water isolates have the same homology isolated from one farm A also in clade 4.

Conclusion

This study highlights crucial information on microbiological quality of small-scale informal morogo supply chains primarily from two geographical areas, Brits and Delmas in South Africa. Enterobacteriaceae counts as way of determining microbial contamination of fresh produce in supply chains identified at approximately an average of log 4.34 CFU/g. Although, *E. coli* contamination on fresh produce was minimal, the risk is real. Furthermore, isolation of diarrheagenic *E. coli* in irrigation water and fresh produce highlight that the risk is real. The presence of ESBL and AmpC-producing Enterobacteriaceae microorganism further put an emphasis of the risk to food safety in the informal morogo supply chains. Educational workshops are necessary as mitigation strategies to put awareness on food safety and the risk of microbes with small-scale supply chains.

4.2.9 Genetic diversity of *Salmonella* isolated from dark leafy green production at small-scale farms in South Africa

Authors: Degracious Kgoale, Stacey Duvenage, Erika du Plessis and Lise Korsten

A manuscript "Occurrence, serotype distribution, virulence genes, antimicrobial resistance and genetic diversity of *Salmonella* isolated from dark leafy green production at small-scale farms in South Africa has been prepared

Main aim: The aim of this study was to investigate the prevalence, antibiotic resistance, virulence gene profiles and genetic diversity of *Salmonella* spp. associated with dark leafy green vegetables in South Africa, which are mainly cultivated by small-scale farmers and commercialized at local markets.

Experimental procedures

Salmonella isolates were obtained as described in the characterised as described in 3.4.9.
Details of the eight small-scale farm selected and associated retailers (formal and informal), sample collection, processing, hygiene indicator bacteria enumeration, foodborne pathogen isolation, identification and characterisation were described in Chapter 3, section 3.2.4, 3.3.4 and 3.4.

Results

Characterisation of *Salmonella* spp. isolates. Twenty-one (4.93%; 21/426) samples were contaminated with *Salmonella* spp., including 17 irrigation water samples (17.00%) and 4 leafy green vegetable samples (1.38%); mainly from only one growing region. Using Seroseq 1.0, seven different *Salmonella* spp. serotypes were identified; *Salmonella* IIIb 42 or II 42 subspecies (18/53; 33.96%), *Salmonella* Enteritidis (12/53; 22.64%) and *Salmonella* II 42:z29 or *Salmonella* Djama (9/53; 16.98%), *Salmonella* Havana (7/53; 13.21%) and *Salmonella* Typhimurium (5/53; 9.43%) were the most prevalent. All the isolates carried *hilA*, *invA*, *ssrA*, *sipA*, *pipD*, *misL* and *stn* virulence genes. Furthermore, 3.8% (2 of 53) of isolates carried at least 15 virulence genes tested (*hilA*, *invA*, *ssrA*, *sipA*, *pipD*, *misL*, *pefA*, *sefA*, *sifA*, *sopB*, *sopE*, *spvC*, *spvR*, *orfL and stn*). A total of 49 isolates

(92.45%) were found to be multidrug resistant. Isolates showed high rates of resistance to aztreonam (47/53; 88.68%), ceftazidime (46/53; 86.79%), nalidixic acid (41/53; 77.36%), cefotaxime (40/53; 75.47%), cefepime (38/53; 71.70%) and streptomycin (37/53; 69.81%). Ten clusters were observed from repetitive PCR analyses, demonstrating high diversity amongst the *Salmonella* spp. from mainly one farm, with isolates from fresh produce and water in the same cluster indicating possible contamination and further indicating a potential food safety risk to consumers.

***Salmonella* spp. occurrence.** A total of 53 *Salmonella* isolates from 21 of the 426 samples (4.93%) were isolated from 17 of the 100 irrigation water samples (17.00%) and from 4 of the 289 leafy green vegetable samples (1.38%); mainly from only one growing region situated in the Hartbeespoort district in South Africa from two farms, Farm C and Farm F. Farm C was visited twice in 2017 where 27 isolates were isolated from both the reservoir (n=12) and the flooding irrigation (n=17) water, with no isolates recovered from the fresh produce samples. In 2018, 26 isolates were isolated mainly from Farm C (n=17) where 4 of the isolates were recovered from reservoir, 7 from flooding irrigation water and 5 from fresh produce at point of sale. The remaining nine isolates from 2018 were recovered from Farm F with four isolates from fresh produce and five from irrigation water.

Antibiotic susceptibility testing. The antibiotic classes which isolates were predominantly resistant to, included Monobactam (88.68%), cephems (77.99%) and fluoroquinolone (70.75%), while susceptibility to antimicrobial agents phenicol (3.77%) and fosfomycin (7.55%) was seen (Figure 29). A total of 49 out of 53 *Salmonella* isolates (n=92.45%) were classified as MDR, with 50 different antimicrobial resistance patterns (Figure 28). The dominant antimicrobial resistance patterns were Ab1 and Ab2 which showed resistance to 16 different antibiotics. The most common resistance pattern was ATM-CAZ-NA-CTX-CPM-S-CIP-ETP-MEM-TN where at least 62.26% (n=33) of the isolates showed resistance to these 10 antibiotics. Only two isolates showed resistance to these 10 antibiotics and were assigned to Ab25. Antibiotypes Ab49 and Ab50 showed the lowest resistance to two and one antibiotics, respectively. Although antibiotypes were unique to most of the isolates, Ab18, Ab19 and Ab25 were observed in at least 3.8% (2/53) of the isolates, respectively (Figure 28).

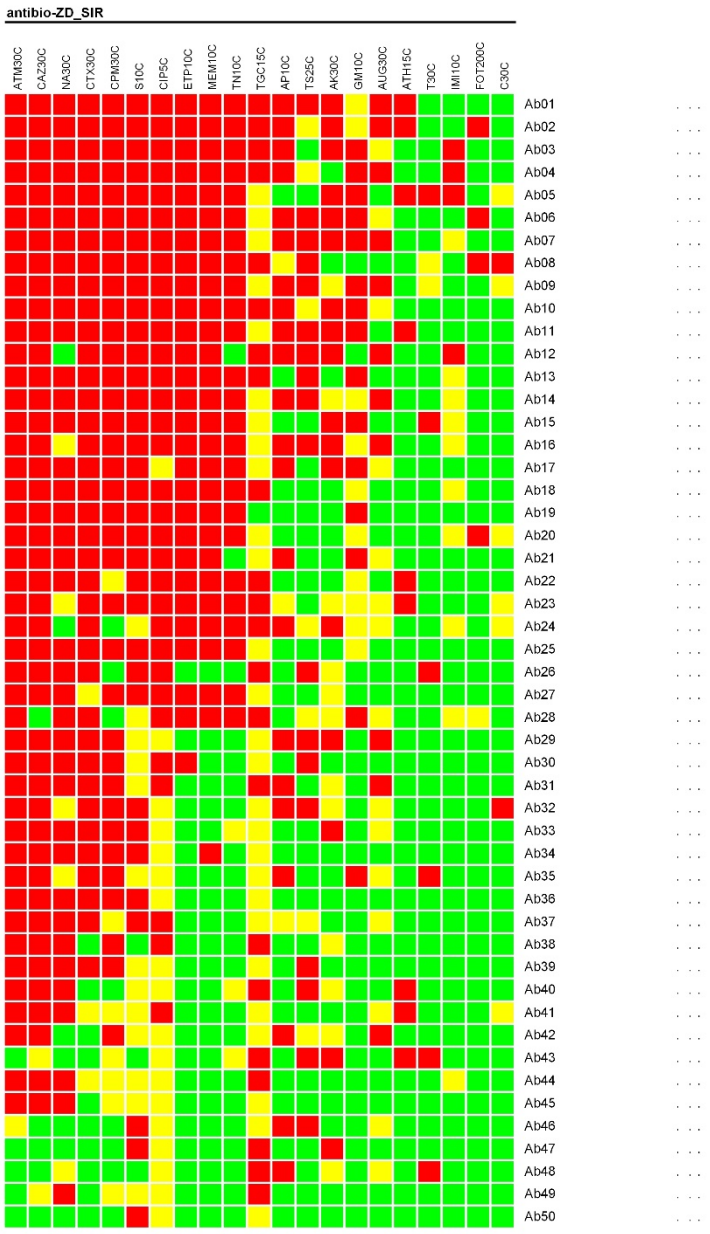


Figure 28: Antibiogram of *Salmonella* spp. isolates for 21 antibiotic showing antibiotic resistance pattern numbers (Ab). Red boxes indicate resistance, yellow intermediate and green susceptibility to specified antibiotics.

The antibiotic classes which isolates demonstrated the most resistance to, included Monobactam (88.68%), Cephems (77.99%) and Fluoroquinolone (70.75%), while susceptibility to antimicrobial agents Phenicol (3.77%) and Fosfomycin (7.55%) was seen (Figure 29).

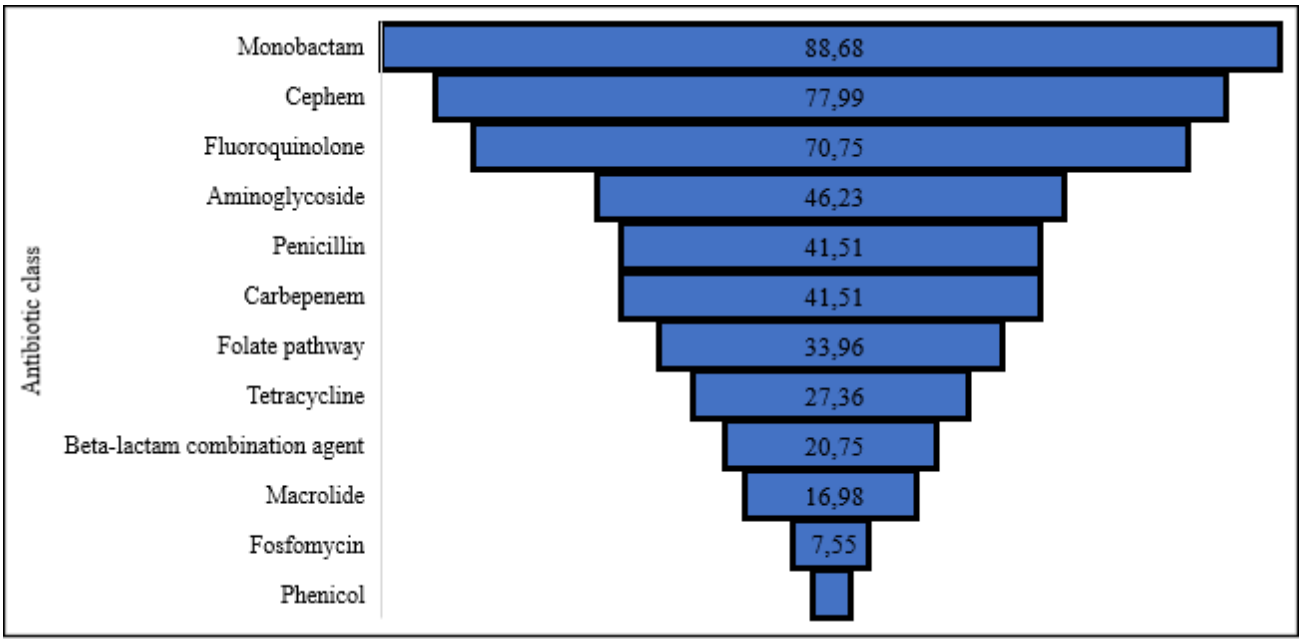


Figure 29: Antimicrobial resistance of *Salmonella* spp. isolates to twelve antibiotic classes. The numbers in the rectangles indicate the percentage of resistance to the specified antibiotic class. Phenicol (3.77%).

Chloramphenicol was determined to be the most effective antibiotic agent, with 96.22% (51/53) of the *Salmonella* isolates being susceptible and only 3.77% (2/53) demonstrating resistance (Figure 30). A total of 49 out of 53 isolates (92.45%) were susceptible to fosfomycin and imipenem. The following tested antibiotics demonstrated high resistance levels: aztreonam (47/53 isolates; 88.68%), ceftazidime (46/53 isolates; 86.79%), nalidixic acid (41/53 isolates; 77.36%), cefotaxime (40/53 isolates; 75.47%), cefepime (38/53 isolates; 71.70%), streptomycin (37/53 isolates; 69.81%), ciprofloxacin (34/53 isolates; 64.15%), ertapenem (31/53 isolates; 58.49%), meropenem (31/53 isolates; 58.49%), tobramycin (28/53 isolates; 52.83%) (Figure 31). Out of the 53 isolates tested less resistance was seen against tigecycline (23/53; 43.40%), ampicillin (22/53; 41.51%), trimethoprim/sulfamethoxazole (18/53; 33.96%), amikacin (17/53; 32.08%), gentamicin (16/53; 30.19%), amoxicillin /clavulanic acid (11/53; 20.75%), azithromycin (9/53; 16.98%), tetracycline (6/53; 11.32%) (Figure 30).

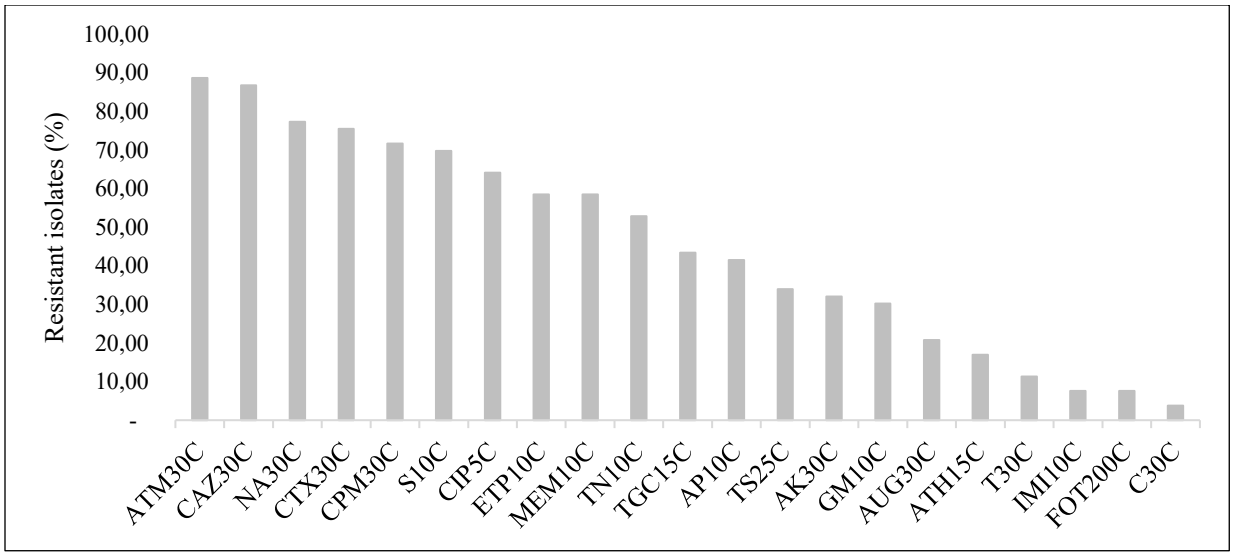


Figure 30: Descending antibiotic resistance of 53 *Salmonella* spp. to 21 antibiotics.
Aztreonam (ATM30C), ceftazidime (CAZ30C), nalidixic acid (NA30C), cefotaxime (CTX30C), cefepime (CPM30C), streptomycin (S10C), ciprofloxacin (CIP5C). etrapenen (ETP10C), meropenem (MEM10C), tobramycin (TN10C), tigeclyclin (TGC15C), ampicillin (AP10C), trimethoprim-sufamethoxazole (TS25C), amikacin (AK30C), gentamycin (GM10C), amoxicillin-clavulanic acid (AUG30C), azithromycin (ATH15C), tetracycline (T30C), imipenem (IMI10C), fosfomycin/trometamol (FOT200C) and chloramphenicol (C30C).

Detection of virulence genes. All 53 isolates carried the following virulence genes: hilA, invA, misL, pipD, sipA, ssrA and stn (Figure 31). Whilst, sifA was carried in 92.45% isolates, orfL in 52.83% of isolates, sopB in 49.06%, pefA in 37.73%, spvC and spvR in 33.96%, sefA and sopE in 26.42% of the total 53 isolates. IroN was detected in 9.43% of the isolates (Figure 32). Based on the presence of the selected virulence genes twelve different virulence profiles (virulotypes) were identified (Figure 31). VP11 which represented 37.73% of the isolates, followed by VP01 in 20.75% and VP08 in 11.32% of the isolates (n=53) (Figure 31).

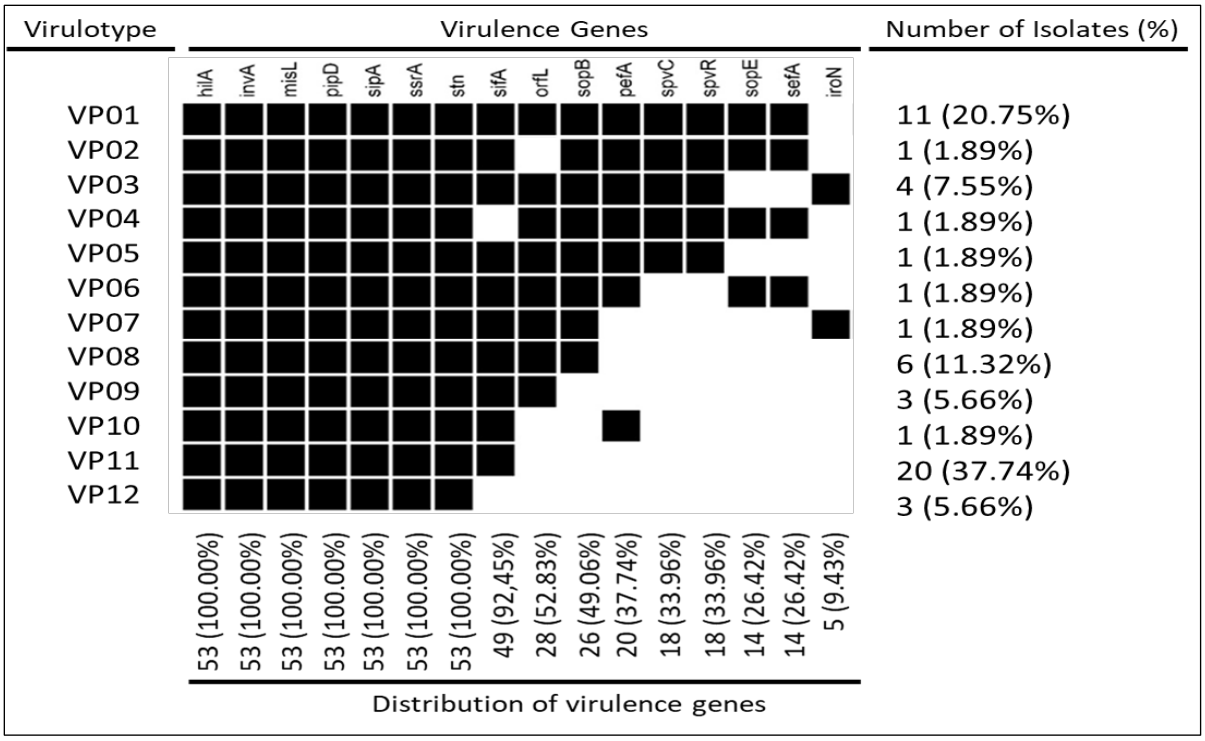


Figure 31: Distribution of virulence gene sin *Salmonella* isolates.

Serotype distribution. Mainly from Farm C, 29 isolates were isolated in 2017 and comprised of serotype Salmonella II 42:r (n=17), Salmonella II 42:z29 (n=8), *Salmonella* Enteriditis (n=1), *Salmonella* Typhimurium (n=2) and Salmonella IIIb 47:i:z (n=1). In 2018, 24 isolates were isolated were from Farm C (n=15) and Farm F (n=9). Serovars from Farm C comprised of *Salmonella* Typhimurium (n=3), *Salmonella* II 42:r (n=1) and *Salmonella* Enteritidis (n=11). The isolates from Farm F comprised of serotype *Salmonella* Havana (n=8) and *Salmonella* II 42:z29 (n=1) only. Overall results reveal *Salmonella* II 42:r as the dominant serotype (n=17) and was isolated in both sampling periods and are present in both Farm C and Farm F from irrigation water samples. Serotype IIIb 47:i:z was the only serotype isolated from flooding irrigation water from Farm C. *Salmonella* Havana isolates were isolated from Farm F from the water (n=4), field fresh produce (n-=2) and retail fresh produce (n=2). In addition, all *Salmonella* Typhimurium isolates were found in retailer fresh produce samples from Farm C.

Sequence type distribution. Of the 52 *Salmonella* isolates tested, six STs were identified in silico MLST using Enterobase. Seventeen isolates (32,7%) classified as ST1208, all isolated from water. A further 12 isolates (23.07%) from water classified into ST 11, 9 isolates (17.3%) from water

classified into ST 4395 and one isolate (0.19%) from water was classified into ST 7890. Five *Salmonella* isolates (9.61%) from retailer fresh produce classified into ST 19. Interestingly, four isolates from water, two from field fresh produce and two from retailer fresh produce all classified into ST 1524. These results corresponds with the identified six serovars identified from SeroSeq 1 and CRISPR-SeroSeq, where *Salmonella* Enteritidis denotes ST11 (n=12), *Salmonella* Typhimurium ST19 (n= 5), *Salmonella* II 42:r ST1208 (n=18), *Salmonella* Havana ST 1524 (n=8), *Salmonella* II42:r:z29 ST4395 and *Salmonella* IIIb 47:i:z ST7890.

Discussion

The occurrence of *Salmonella* spp. in animals (Mthembu et al., 2019) and animal-based products (Chen *et al*., 2020) has been widely studied. This is the first study to the authors' knowledge investigating the occurrence of *Salmonella* spp. on small-scale dark leafy greens (morogo) in production supply chains. Small-scale farming is characterized by the limitation or, in some instances, the abuse of agrochemicals as well as a lack of effective policies and regulation that govern the food safety standards and hygiene practices in the informal sector. The water samples used for irrigation on the selected small-scale farms were contaminated with *Salmonella* spp. and subsequently, the morogo produced on these small-scale farms is a potential route of human exposure to *Salmonella* spp. Although the prevalence of *Salmonella* spp. on morogo in this study was low (1.38%), the risk is real. The point of sale morogo samples were found to be contaminated with *Salmonella* Typhimurium and while spinach at harvest was contaminated with *Salmonella* Havana, both of which have been caused foodborne disease outbreaks. Furthermore, we report serovar Havana on spinach at harvest potentially distributed from irrigation water. *Salmonella* Havana has been implicated in an outbreak of Alfalfa sprouts in California where 18 cases were identified and one patient died and also in Australia in 2018 where hospitalization occurred often (Desk, 2019). In South Africa, occurrence of serovar Havana was reported in environmental samples (poultry houses, abattoirs and feed mills) at 7.5% in the period of 2012-2014 (Magwedere *et al*.,2015). Globally, the presence of *Salmonella* spp. in irrigation water is a known source of fresh produce contamination. To the authors knowledge this study is the first report of *Salmonella* Havana in irrigation water in South Africa. The current study also highlights the presence of S. enterica contamination in irrigation water, predominated by *Salmonella* serovars: II 42:r, Enteriditis, II 42:Z29 and IIIb 47:i:z. All these serovars are of clinical importance.

The various *Salmonella* serotypes isolated from irrigation water and fresh produce demonstrated 92.5% MDR, while similar serovars did not have the same antibiogram patterns Furthermore, we observed a rarely isolated serovar IIIb 47:i:z which showed no MDR phenotype and was only resistant to only streptomycin.

Pathogenicity is associated with both antibiotic resistance and the presence of virulence genes (Hai *et al*., 2020). All isolates contained the hilA, invA, misL, pipD, sipA, ssrA and stn virulence genes, the combination of these seven genes represents the potential for pathogenesis. The profile VP12, which represents the presence of these seven genes represents only 5.66% of isolates, and these were dominated by *Salmonella* II 42:r and II 42:z29 strains. Moreover, 37.74% of isolates harboured eight virulence genes which included hilA, invA, misL, pipD, sipA, ssrA, stn and sifA (VP11), and the serovar diversity included *Salmonella* II 42:r and II 42:z29. Overall, the twelve virulotypes belonged to a variety of *S. enterica* serovars and therefore highlights that specific possession of virulence genes is not restricted to a specific serotype. These findings corresponded with findings of (Nde and Logue, 2008), which showed that most *S. enterica* serovars harbour several of the tested virulence genes. The virulence gene sefA is known as *Salmonella* enteritidis fimbral gene and encodes an adhesion protein, as expected it was prominent in all *Salmonella* Enteriditis strains.

Conclusion

This study provides crucial information on the presence of *Salmonella* enterica at farm level to the point-of-sale which is serious concern because it shows the potential of the *Salmonella* to disseminate to the household via this route. Furthermore, these *Salmonella* isolates are of clinical importance as they harbour virulence genes and the majority are multidrug resistant. Mitigation strategies that will allow the reduction of *Salmonella* presence at the farm level and on fresh produce. Future research should include genotyping of the isolates to obtain information on their geographical and sequence types in order to elucidate their origin.

4.3 Formal supply chain fresh produce sampling and microbiological analysis in the Cape Town metropole

4.3.1 Commercial fresh vegetable processing/ packaging facility in Philippi in the Western Cape Province

Authors: Efaishe Kavela and Gunnar Sigge

Specific aim: This study aimed at enumerating the microbial indicators: Enterobacteriaceae, coliforms and *E. coli*, as well as to test for the presence of microbial pathogens: *Salmonella* spp. and Shoga-toxin-producing *E. coli* (STEC), ESBL-producing Enterobacteriaceae as well as antimicrobial susceptibility on fresh produce samples of broccoli, cabbage, carrot, and lettuce sampled from different processing steps in the packhouse, and the retail outlets.

Experimental procedures

Details of the commercial fresh vegetable processing facility, fresh produce collection, processing, hygiene indicator bacteria enumeration, foodborne pathogen and (ESBL) and AmpC-producing Enterobacteriaceae occurrence, identification and antimicrobial resistance characterisation were described in Chapter 3, sections 3.5.1, 3.6.1 and 3.7.

Results

Enumeration of hygiene indicator bacteria from fresh vegetables. *Escherichia coli*, coliform, and Enterobacteriaceae counts of broccoli, coleslaw and lettuce from different processing points (before washing, after peeling and washing. and after shredding) as well as at the retailers were summarised in Table 52. A total of 45 broccoli stem, 45 carrot, 18 red cabbage, and 75 lettuce samples were collected. Enterobacteriaceae was recovered from all broccoli stem, carrot, and cabbage samples in average levels ranging from 2.1 to 5.13 log CFU/g. Coliforms were also recovered from all samples in average counts ranging from 1.62 to 4.81 log CFU/g *E. coli* were only found on a few samples: 2 of 45 (4%) untreated broccoli stem samples, 1 of 45 (2%) untreated carrot samples, and 6 of 45 (13%) shredded carrot samples. *No E. coli* was detected on cabbage samples. *E. coli* ranged from < 1 log CFU/g (undetected) to 2.035 log CFU/g. Samples peeled and washed in chlorine (150-200 ppm) water had significantly lower average counts than unwashed samples. The reduction levels of Enterobacteriaceae and coliform observed in this study ranged from 0. 94 to 1.17 log CFU/g and 0.83 to 0.95 log CFU/g respectively on broccoli and

carrot samples. On lettuce samples, Enterobacteriaceae and coliform reduction ranged from 0.89 to 2.35 CFU/g and from 0.69 to 2.27 CFU/g, respectively. An increase in microorganisms was observed in shredded samples. Therefore, this study identified shredding and packaging as potential contamination points.

Table 52 Summary of *Escherichia coli*, coliform and Enterobacteriaceae counts of broccoli coleslaw and lettuce from the commercial supply chain

Source	***Escherichia coli* (log CFU/g)**		**Coliforms (log CFU/g)**		**Enterobacteriaceae (log CFU/g)**	
(Packhouse)	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**
Untreated Broccoli stem (unpeeled-unwashed)	1.0-1.48	1.05	1.30-3.23	2.37	2.28-4.41	3.04
Treated Broccoli stem (peeled-washed)	1.0-1.0	1.00	1.0-2.43	1.63	1.0-2.869	2.16
Shredded Broccoli stem	1.0-1.0	1.00	2.04-4.91	2.85	2.10-5.15	3.28
Untreated Carrot (Unpeeled-unwashed)	1.0-1.30	1.02	3.65-4.32	4.01	3.71-5.26	4.39
Treated Carrot (peeled-washed)	1.0-1.0	1.00	1.78-4.28	3.07	2.18-4.39	3.29
Shredded Carrot	1.0-4.20	2.03	3.65-6.10	4.70	4.04-6.34	5.2
Untreated Cabbage (outer leaves removed-unwashed)	1.0-1.0	1.00	1.0-4.28	2.68	1.0-5.15	3.12
Treated Cabbage (outer leaves removed-washed)	1.0-1.0	1.00	1.78-3.28	2.69	2.69-3.62	3.18
Lettuce head (untreated)	1.0-1.0	1.00	3.10-5.85	4.48	3.18-5.96	4.77
Loose lettuce (Dipped in borehole chlorinated water)	1.0-1.0	1.00	3.12-4.23	3.88	3.64-5.21	4.44
whole lettuce (outer leaves removed- unwashed)	1.0-2.34	1.24	3.60-4.24	4.01	3.76-4.72	4.18
Pre-packaged lettuce (Outer leaves removed-dipped in borehole chlorinated water)	1.0-1.78	1.16	2.74-4.27	3.73	2.79-4.29	3.88
Pillow-packs (stem cut off-washed-spin-packaged)	1.0-1.0	1.00	1.30-2.68	2.17	1.85-2.89	2.43
(packhouse and shops)						
Untreated Broccoli stem (Packhouse)	1.0-2.79	1.70	2.41-4.79	3.27	2.60-4.90	3.41
Treated broccoli stem (Packhouse)	1.0-2.26	1.26	1.0-2.99	2.02	1.69-3.20	2.38
Untreated Carrot (Packhouse)	1.48-4.18	2.99	4.83-6.26	5.51	4.89-6.38	5.57
Treated Carrot (Packhouse)	1.0-1.30	1.07	1.0-3.86	2.41	1.0-3.92	2.55

Table 52 cont.

Source	*Escherichia coli* (log CFU/g)		Coliforms (log CFU/g)		Enterobacteriaceae (log CFU/g)	
Untreated Cabbage (Packhouse)	1.0-3.36	1.74	1.48-3.89	3.05	2.48-3.96	3.41
Treated Cabbage (Packhouse)	1.0-1.0	1.00	1.0-2.23	1.47	1.0-2.46	1.55
Coleslaw bag mix (packhouse)	1.30-3.49	2.47	4.08-5.95	5.22	4.11-5.98	5.31
Coleslaw bag mix (Shops)	2.11-2.76	2.42	5.68-6.77	6.12	6.08-6.85	6.33
Lettuce head (Packhouse)	1.0-3.89	2.63	3.72-4.36	4.08	4.53-4.86	4.70
Loose lettuce (Packhouse)	1.0-3.95	2.16	3.61-4.96	4.13	3.79-5.20	4.42
Pre-packaged lettuce (Packhouse)	1.0-2.23	1.22	2.94-3.97	3.54	3.0-4.83	3.61
Pre-packaged lettuce (Shops)	1.0-1.85	1.19	2.51-4.28	2.25	2.69-3.34	3.55
Pillow packs (Packhouse)	1.0-2.08	1.21	1.48-2.65	3.03	1.78-2.65	2.27
Pillow-Packs (packhouse retention)	1.0-1.0	1.00	1.60-2.60	2.18	2.28-3.10	2.71

Shiga toxin-producing *Escherichia coli* and *Salmonella* spp. Shiga toxin-producing *E. coli* (STEC) is an important food pathogen associated with diarrheal sickness, which in some cases develops into haemorrhagic colitis (HC) or into haemolytic uremic syndrome (HUS) (Baker *et al.*, 2016). Shiga toxin-producing *E. coli* cause diseases by producing one or more stx (*stx1*, *stx2*) genes and it also carries the chromosomal eae gene (Bryan *et al*., 2015). In this study the presence of STEC was screened from all 72 samples (lettuce, red cabbage, broccoli stem and carrots). None of the 72 samples was detected with STEC; however, one of the nine untreated lettuce samples was detected with the chromosomal gene eae, one of the nine untreated carrots were found with the stx gene. However, these two samples could not be described as STEC positive because neither contained all STEC virulence factors. These results are similar to results obtained by de Bruin *et al.* (2016), which did not detect *E. coli* O157:H7.

Salmonella was also screened from all 72 samples (lettuce, red cabbage, broccoli stem and carrots), and none was detected with Salmonella. Similar results were reported by Van Dyk *et al*. (2016) on commercially produced tomatoes, who found all sampled tomatoes free from *Salmonella* Typhimurium. *Salmonella* spp. have been implicated in numerous foodborne outbreaks associated with fresh produce (Murray *et al*., 2018). *Salmonella* was detected on fresh-cut organic vegetables in Nigeria by Nguz *et al.* (2005). *Salmonella* in fresh produce could potentially complicate

consumer's health. The South African Department of Health limits *Salmonella* to zero detection in food.

Isolation and detection of ESBL-producing Enterobacteriaceae. In this study all 144 samples were screened for ESBL-producing Enterobacteriaceae. Enriched samples where grown on ChromID Brilliance ESBL agar (bioMérieux, South Africa). Sixty four samples were identified to have produced presumptive ESBL colonies. However, the confirmation tests and the susceptibility testing are yet to be done.

Conclusion

The microbiological quality of broccoli, cabbage, carrot, and lettuce sampled from different processing steps in the packhouse, and the retail outlets was successfully determined. Peeling and washing were the most important processing steps which remove microorganisms from fresh produce. An increase in the level of microorganisms after washing and peeling was considered to be as a result of recontamination of fresh produce (from packers hands, surfaces, shredder, or packaging material), or exposure of fresh produce to high temperatures that support the growth of microorganisms already present. Unwashed samples in this study were found to have higher levels of Enterobacteriaceae and coliforms, than peeled and washed samples, which were significantly reduced after peeling and washing. The peeling and washing did however not completely eliminate the organisms from fresh produce, but only reduced to lower levels. The highest reduction was observed in lettuce pre-packs sampled from the packhouse. However, a significant increase in both microorganisms was observed after shredding, and the highest microorganism levels (Enterobacteriaceae and coliforms), were detected in the coleslaw bags collected from the retail outlets, which also had *E. coli*. The fact that the *E. coli* was only detected at such a late stage in the product cycle could either be due to E.coli growth from previously undetectable levels or through post-processing contamination. This is of concern because the coleslaw mix sold at retail outlets is consumed without heat treatment and sometimes with no further washing. In addition, these levels were higher than the guideline limits for coliform (< 200 CFU/g) and *E. coli* (0 CFU/g) for raw vegetables set by the South African Department of Health (NDOH), and *E. coli* levels were also higher than the European Union acceptable level (≤ 100 CFU/g). The presence of high levels of coliform and *E. coli* could indicate the presence of similar ecologically enteric

pathogens. Although Shiga toxin-producing *E. coli* was not detected, it does not really mean the produce was completely safe, other diarrheal strains might be present, considering that *E. coli* as an indicator was detected. Therefore, a further study to identify the type of *E. coli* present would be necessary. This study also suggests a study on effective disinfection of shredded samples since microorganisms were not effectively removed from the produce, and they proliferate in packaged bags intended to be consumed raw. This study has also concluded that shredding and packaging could be the pontential contamination points, and that inconsistencies in the cold chain could compromise the microbiological quality of fresh produce.

Results pathogen detection and characterisation

Shiga toxin-producing *E. coli* (STEC) is an important food pathogen associated with diarrheal sickness, which develops into hemorrhagic colitis (HC) and can eventually results into haemolytic uremic syndrome (HUS) (Baker *et al.*, 2016). STEC causes disease by producing one or more stx (stx1, stx2) toxins and it also carries the chromosomal eae gene which is responsible for intimate attachment to the intestinal surface (Bryan *et al.*, 2015). In this study all 72 different samples (lettuce, red cabbage, broccoli stem and carrots) were screened for the presence of STEC. None of the 72 samples tested positive for STEC. However, in two different occasions, stx gene and eae chromosomal gene were detected individually from two different samples. The *eae* gene was detected in unwashed lettuce head, and the stx gene was found in untreated carrot sample. However, these two samples could not be described as STEC positive because neither contained all STEC virulence factors (one or two stx and the eae gene) at the same time. These results are in agreement with results obtained by de Bruin *et al*. (2016) on microbial quality of fresh basil along the supply chain in Gauteng and Northwest province of South Africa. De Bruin *et al.* (2016) tested the fresh basil for *E. coli* O157:H7 (which is an STEC type) from production to the retail outlet, and none of the samples tested positive for *E. coli* O157:H7.

Salmonella spp. have been implicated in foodborne outbreaks associated with fresh produce (Jung *et al*., 2014; Murray *et al*., 2018). *Salmonella* was detected on fresh-cut organic vegetables in Nigeria by Nguz *et al.* (2005). *Salmonella* in fresh produce can potentially complicate consumer's health. The South African NDOH (2000) and the EU guidelines (EFSA, 2007) suggest that *Salmonella* should be absent in ready to eat fruits and vegetables. In this study, *Salmonella* was

screened from all 72 samples (lettuce, red cabbage, broccoli stem and carrots). None of these samples tested positive for *Salmonella* spp. Similar results were reported by Van Dyk *et al*. (2016) on commercially produced tomatoes, who found all sampled tomatoes free from *Salmonella* Typhimurium.

Detection and isolation of ESBL-producing Enterobacteriaceae. Of all samples screened for ESBL-producing Enterobacteriaceae, 56 produced presumptive ESBL-producing colonies. This included 26 lettuce samples and 30 broccoli coleslaw samples (7 broccoli coleslaw, 10 cabbages, 7 carrots, and 6 broccoli stems samples) (Figure 32).

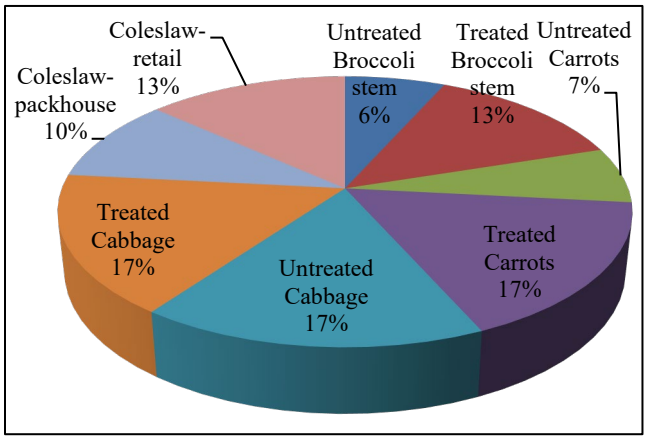


Figure 32: Percentage distributions of 30 coleslaw samples (broccoli stem, carrots, cabbage, and coleslaw bags samples) from which presumptive positive ESBL-producing colonies were isolated.

Most presumptive positive ESBL-producing isolates were from untreated cabbage (17%), treated cabbage (17%) and treated carrots (17%). Untreated carrot and untreated broccoli stem samples had the lowest number of positive isolates (7% and 6% respectively) (Figure 33). Some treated samples (treated carrots, treated broccoli coleslaw) had more presumptive positive ESBL-producing isolates than untreated samples. Most positive isolates were expected to come from untreated samples than treated samples. Owing to the fact that untreated samples have been open to possible microbial contaminations while in the field, as well as during handling and transportation. However, more percentages of presumptive ESBL-producing Enterobacteriaceae on treated than untreated could be a reflection of new contamination during processing, possibly from surfaces, and workers' hands.

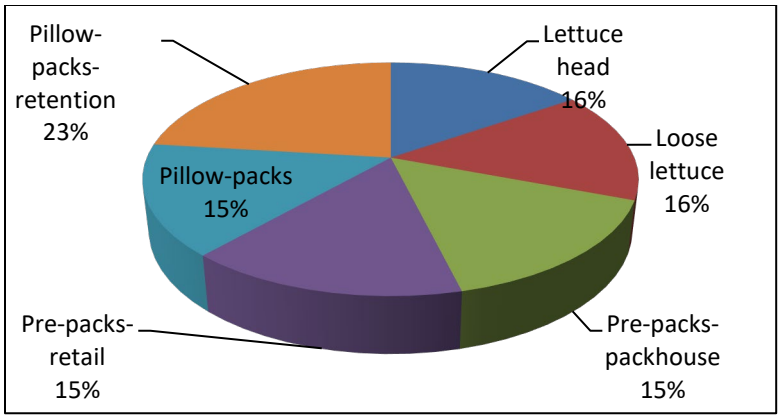


Figure 33 Percentage distributions of 26 lettuce samples (lettuce head, loose lettuce, pre-packs packs, and pillow-packs samples) from which presumptive positive ESBL-producing colonies were isolated.

The suspected isolates were more prevalent in retention pillow-packs (23%). In this study, retention pillow-packs were found carrying lower levels of Enterobacteriaceae and coliforms (2.71-2.25 log CFU/g respectively) compared to lettuce head (4.7 and 4.08 CFU/g respectively), loose lettuce (4.42-4.13 log CFU/g, respectively), and pre-packs (3.61-3.54 log CFU/g, respectively). Therefore, *pillow*-packs were expected to have the lowest presumptive ESBL-producing Enterobacteriaceae isolates. Lettuce head and loose lettuce individually were observed with 16% positive presumptive ESBL-producing organisms. The lowest isolates percentages (15%) were observed on pre-packs-retails, pre-packs-packhouse, and pillow-packs-packaging.

Identification of Extended spectrum β-lactamase-producing Enterobacteriaceae. All 56 isolates that were suspected to be presumptive ESBL-producing bacteria were identified using MALDI-TOF mass spectrometry. According to the identification results, 50 (89%) of 56 isolates were classified as members of Enterobacteriaceae. Six isolates of the 56 (11%) were identified as *Pseudomonas* spp., which are non-Enterobacteriaceae. The Enterobacteriaceae strains identified were Enterobacter cloacae (64%) *Klebsiella oxytoca* (18%), *E. coli* (7%). The *Pseudomonas* spp. strains identified were *Pseudomonas* spp. [2] (4%), *Pseudomonas putida* (4%) and *Pseudomonas geminis* (4%) (Table 53).

Table 53: Identification of isolates according to the MALDI-TOF mass spectrometry

Microorganism	Number of isolates	Percentage (%)
Enterobacter cloacae	36	64
Klebsiella oxytoca	10	18
Escherichia coli	4	7
Pseudomonas sp [2]	2	4
Pseudomonas putida	2	4
Pseudomonas geminis	2	4

Antimicrobial susceptibility (ESBL-producing Enterobacteriaceae confirmation). The Enterobacteriaceae strains producing ESBLs are becoming more prevalent in many environments other than clinical environments (Mesa *et al.*, 2006). Several studies done on fresh produce in Gauteng province, South Africa (Richter *et al.*, 2019) and elsewhere (Reuland *et al.,* 2014) have reported the prevalence of Enterobacteriaceae strains producing ESBL on fresh produce. In this study, the presence of ESBL-producing Enterobacteriaceae in fresh produce isolates, and the resistance to antibiotics was investigated. To confirm the ESBL-production, Enterobacteriaceae, isolates were subjected to a standard ESBL confirmatory disc diffusion test according to the EUCAST method (2017b). Amongst the 50 MALDI-TOF identified Enterobacteriaceae isolates in this study, 11 (22%) of them were confirmed as ESBL-producers (Table 54).

All 50 Enterobacteriaceae isolates were also tested for resistance against five additional antibiotics representing different classes: ampicillin, gentamicine, tetracycline, ciprofloxacin, and chloramphenicol, as described in the methods section. The results were interpreted according to the CLSI (2016) interpretive criteria. Most isolates (88%) were found resistant to Ampicillin, followed by Gentamicine (18%), Chloramphenicol (14%), and Tetracycline (6%). No isolate was found resistant to ciproflaxin (88% were intermediate and 12% was sensitive to ciprofloxacin.
High resistance to ampicillin by isolates from fresh produce has been reported in literature. Zurfluh *et al*. (2015) have reported similar results, where all isolate from the produce (100%) was resistant to ampicillin. Similarly, Laubscher (2019) in her study done on fresh produce samples collected from the informal markets in the Western Cape have also found all tested fresh produce isolates resistant to ampicillin. Ampicillin is a very important antimicrobial frequently used to fight against bacterial infections (Lode, 2008). Isolates that were co-resistant to more than two antimicrobials

were classified as multidrug resistant (Doyle *et al.*, 2013). Out of all 50 Enterobacteriaceae isolates only three (6%) isolates were classified as multidrug resistant (Table 55). However, these results are limited to the five of antibiotics used in this study. Isolates might also be resistant to other antibiotics classes like sulphonamides, cephamycins, and cephalosporins, which were not tested in this study.

Table 54 A summary of confirmed ESBL-producers strains from fresh produce isolates in this study

			Growth-inhibitory zone diameter (increase)			**ESBL producer (Yes/No)**
Code	**Source**	**Organisms**	**CTX**	**CAZ**	**CPM**	
21A2	Pillow-packs (lettuce)	*Escherichia coli*	26	16	10	Yes
22B	Untreated cabbage	*Enterobacter cloacae*	7	6	5	Yes
22D3	Loose lettuce	*Enterobacter cloacae*	13	6	6	yes
22D1	Loose lettuce	*Klebsiella oxytoca*	12	7	6	yes
21E3a	Treated-cabbage	*Klebsiella oxytoca*	19	13	10	Yes
21E3b	Treated-cabbage	*Klebsiella oxytoca*	19	12	8	Yes
24A1	Pillow-packs	*Klebsiella oxytoca*	20	18	8	Yes
05C1	Treated broccoli stem	*Enterobacter cloacae*	20	13	12	Yes
05C2	Treated broccoli stem	*Enterobacter cloacae*	19	13	12	yes
05F1	Untreated carrot	*Enterobacter cloacae*	19	13	11	yes
05F2	Untreated-carrot	*Enterobacter cloacae*	21	13	10	Yes

CTX = Cefotaxime, CAZ = Ceftadizime, CPM = Cefepime, treated = washed, untreated = unwashed

Table 55 Antimicrobial susceptibility of positive ESBL producer isolates to five additional antimicrobials

Code	Source	Organism	Antimicrobial susceptibility					
			Antimicrobial drugs					MDR
			AMP	TE	CIP	GM	C30	Yes/No
ATCC 35218		*Escherichia coli*	R	R	S	S	R	Yes
ATCC 29522		*Escherichia coli*	S	S	I	S	S	No
21A2	Pillow-packs lettuce)	*Escherichia coli*	R	R	I	R	S	Yes
22B	Untreated cabbage	*Enterobacter cloacae*	R	S	S	S	S	No
22D3	Loose lettuce	*Enterobacter cloacae*	R	S	I	S	S	No
22D1	Loose lettuce	*Klebsiella oxytoca*	R	S	I	S	S	No
21E3a	Treated-cabbage	*Klebsiella oxytoca*	R	S	I	I	S	No
21E3b	Treated-cabbage	*Klebsiella oxytoca*	R	S	I	R	S	No
24A1	Pillow-packs (lettuce)	*Klebsiella oxytoca*	R	S	I	S	I	No
05C1	Treated broccoli stem	*Enterobacter cloacae*	R	R	I	R	S	Yes
05C2	Treated broccoli stem	*Enterobacter cloacae*	R	R	I	R	S	Yes
05F1	Untreated carrot	*Enterobacter cloacae*	R	R	S	R	S	No
05F2	Untreated-carrot	*Enterobacter cloacae*	R	R	S	R	S	No

AMP = Ampicillin, TE = Tetracycline, CIP = Ciprofloxacin, GM = Gentamicine, C30 = Chloramphenicol, R = Resistant, S = sensitive, I = Intermediate, treated = washed, untreated = unwashed, MDR = multi-drug resistance

Genotypic confirmation (ESBL genes detection). It has been reported that Enterobacteriaceae strain are increasingly showing resistance to penicillin, and the broad-spectrum cephalosporins (Blaak *et al*., 2014). The resistance to the broad-spectrum cephalosporin results from the production of ESBLs (Van Hoek *et al*., 2015). The most prevalent related ESBL genes found in Enterobacteriaceae on fresh produce are *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ type (Reuland *et al.*, 2014; Richter *et al*., 2019).

In this study, all 50 isolates, including both non-ESBL-producers and the confirmed ESBL-producers, were analysed with PCR for genotypic confirmation. The targeted genes were bla$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$. Out of the 50 isolates, only seven isolates were carrying the ESBL genes

(Table 56). Six of these isolates were co-producers of bla_{CTX} and bla_{TEM}. While one isolate was carrying bla_{TEM} only. The dominant beta-lactamase (bla) gene detected in this study was bla_{TEM}, detected from seven (14%) of the 50 tested isolates, followed by bla_{CTX-M} detected from six (12%) isolates. The gene bla_{SHV} was not detected in any of the tested isolates. Jena *et al*. (2018) reported similar results, where bla_{TEM} was the most predominant ESBL gene present in 96%, of the tested isolates followed by bla_{CTX-M} with 75%, and bla_{SHV} (18%) was found in very few isolates. However, the strains were isolated from tertiary care hospital, not from fresh produce (Jena *et al*., 2018). In some studies, bla_{CTX-M} has been reported the most predominant beta-lactamase gene than bla_{TEM} and bla_{SHV}. Ojer-Usoz *et al*. (2014) have found 67% bla_{CTX-M}, 47% bla_{TEM} and 17% bla_{SHV} in wastewater treatment plant. Shahid *et al.* (2011) have also found bla_{CTX-M} (29%) dominating in clinical isolates, followed by SHV (14%) and bla_{TEM} (11%). Although the genes including bla_{CTX-M} and bla_{TEM}, are more common than SHV in many studies, the type and predominance of the ESBLs might be influenced by the geographical location (Shahid *et al*., 2011). The β-lactamase genes are said to be predominant in *K. pneumonia* and *E. coli* worldwide (Kim *et al*., 2015; Pitout and Laupland, 2008). In this study, there was no *K. pneumonia* identified. The organisms found carrying bla_{TEM} *and* bla_{CTX-M} in this study were *E. cloacae* (50%) and *K. oxytoca* (50%). A single bla_{TEM} was found in the isolate identified with *E. coli* Table 56.

Table 56 A summary of identified organisms with ESBL genes and the sources from which they were isolated

			Disc diffusion	**Molecular confirmation**
Isolate code	Source	Organism	ESBL producer	ESBL genes
21C3	Treated Broccoli stem	*Enterobacter cloacae*	No	TEM + CTX-M
21D1	Treated cabbage	*E. coli*	No	TEM
21E3	Treated carrot	*Klebsiella oxytoca*	Yes	TEM + CTX-M
22B	Untreated cabbage	*Enterobacter cloacae*	Yes	TEM + CTX-M
22D3	Loose lettuce	*Klebsiella oxytoca*	Yes	TEM + CTX-M
22D1	Loose lettuce	*Klebsiella oxytoca*	Yes	TEM + CTX-M
07bC2	Pre-packs (lettuce)	*Enterobacter cloacae*	No	TEM + CTX-M

Higher levels of microbial indicators in this study were found in untreated (unwashed and unpeeled samples) samples than in treated (washed in chlorine solution (150-200 ppm) and peeled) samples.

Therefore, isolates from untreated samples were expected to carry β-lactamases genes. However, organisms carrying the β-lactamases genes in this study were mostly identified from treated samples isolates. Only one untreated sample (unwashed cabbage) of the seven isolates was found carrying the ESBL genes in this study. The contaminated soils, irrigation water, surfaces, processing equipment, and animal droppings can be reservoir of the β-lactamases genes (Blaak *et al*., 2014). Therefore, the produce may acquire the β-lactamases genes during primary production (from contaminated soils, irrigation water, inadequately composited animal manure, and contaminated harvesting materials), transportation, and during processing (from contaminated surfaces, processing equipment, contaminated washwater, and also from workers) (Freitag *et al.,* 2018).

The fact that the β-lactamase genes were found in isolates from treated samples indicates that ESBL-producing Enterobacteriaceae might have been acquired during processing. This is worrisome, because most produce is consumed fresh without heat treatment which may degrade the DNA, and in some cases unwashed. As a result, consumers may acquire Enterobacteriaceae carrying the β-lactamase genes. Once ingested, the genes can be transferred to other organisms found in the intestinal tract of humans, causing resistance. The transfer of resistant genes may occur through integrons, which are mobile genetic elements carried in microbial plasmids and transposons (Weldhagen, 2004; Pirzaman and Mojtahedi, 2019). The β-lactamases are known responsible for bacterial resistance, against the activity of the β-'lactam antibiotics such penicillin, cephalosporin, cephamicins, and carbapenems (Shahid *et al*., 2011; Pitout and Laupland, 2008). This can result in failure to control infections with the β-lactam family antibiotics. The β-lactamase genes may interfere with clinical treatment by causing resistance to certain antibiotics like penicillin and the cephalosporins. On the other hand, the organisms carrying these genes may not cause infection in humans, but humans may disseminate β-lactamase genes in the environment through faecal contaminations (Hölzel *et al*., 2018).

The ESBL-production ability has been noted in South Africa to be most prevalent in *Klebsiella* and *Enterobacter* spp. (Brink *et al.*, 2006). *Escherichia coli* have also been increasingly developing resistant to many antibiotics used in South Africa (Brink *et al*., 2006). As indicated in this study, *K. oxytoca* and *E. cloacae* strains carried more ESBL genes than *E. coli. Klebsiella* species

including *K. oxytoca* have been isolated from fresh produce and other foods, and are also frequently found in clinical samples, (Lowe *et al.*, 2012; Richter *et al.,* 2019). *Klebsiella oxytoca* has emerged as a significant bacterial pathogen resulting in morbidity in humans, by mostly colonising the immunocompromised, patients and neonates (Lowe *et al*., 2012). An outbreak of ESBL-producing *K. oxytoca* has been reported between 2017 and 2018 in special care nursery neonates, however, the source was not identified (Vesey *et al*., 2018). Lowe *et al.* (2012) have also reported an outbreak associated with ESBL-producing *Klebsiella oxytoca* in Canada's Toronto Hospital (mainly in the "intensive care units, step down units, and medical care units") from 2006 to 2011. Handwashing sinks in the intensive care unit were found contaminated with ESBL-producing *K. oxytoca* and were indicated as having contributed to the prolonged outbreak (Lowe *et al*., 2012).

Enterobacter cloacae are found in the gastrointestinal tract of humans and warm blooded animals, and can be transmitted through contaminated environments, surfaces and hands (Bousquet *et al.*, 2017). *Enterobacter cloacae* is one of the pathogens mostly associated with the urinary tract infection (Xu and He, 2019). These bacteria have been reported frequently causing nosocomial infections especially in the intensive care unit (ICU) (Bousquet *et al*., 2017). Fresh produce contaminated with *K. oxytoca* and Enterobacter cloacae strains carrying the ESBL genes can be detrimental to consumer's health.

Discussion

The microbial qualities of fresh produce specifically broccoli stem, cabbage, carrot, lettuce and broccoli coleslaw collected in the packhouse (pre- and post-processing) as well as from the retailers, was successfully evaluated. All samples were tested for microbial indicators (Enterobacteriaceae, coliforms and *E. coli*) and the presence of pathogenic *E. coli* (STEC), *Salmonella* spp. They were also tested for the antimicrobial resistant strains as well as the presence of ESBL-producing Enterobactericaeae. The levels of microorganisms on fresh produce collected from different sampling points along the production chain (packhouse to retailer) were evaluated, in order to determine microbial changes along the production chain. Untreated samples were found with significantly high microbial levels than treated (washed in chlorine solution 150-200 ppm) samples. This is due to the fact that, untreated produce samples were exposed to potential

contamination while in the field, during harvest and transportation from the farms to the packhouse. Fresh produce used in this study were treated mainly by washing in chlorine solution (150-200 ppm), however, findings obtained in this study indicated that, the treatment was not effective enough. Microorganisms were not completely removed they were only reduced to certain levels for each produce. The effectiveness of washing produce in chlorine solutions can be affected by the contact time, pH of the solution or the reaction of chlorine with organic matter from the produce. The latter can form disinfection by-products and reduce chlorine efficacy. In this regard, a comprehensive study assessing the effectiveness of chlorine solution on different produce, as well as factors affecting the disinfection efficacy is recommended. Microbial levels were significantly higher on the mixed coleslaw samples than any other samples. The coliform average levels found on mixed coleslaw samples were higher than the guideline limits set by the NDOH (2000). *E. coli* found was also above the EC (2007) guideline limits for ready to eat fresh produce. These findings are worrisome because the coleslaw is eaten raw, without even further washing subsequently transferring microorganisms to the consumers. The mixed coleslaw samples might have been contaminated during shredding and packaging, as a result of contaminated surfaces, packaging materials or workers hands. Therefore, a further study to assess the impact of microorganisms present on workers' hands, packaging material, equipment and surfaces is hereby recommended. Mixed coleslaw samples collected from the retailers two days after packaging were found with significantly higher average levels of Enterobacteriaceae and coliforms than mixed coleslaw bag sampled from the packhouse the day of production. The increase in microbial average levels encountered in the mixed coleslaw samples collected from the retailer might have been induced by the breakdown in the cold chain from the packhouse to retailer point of sale. It is therefore recommended that the exact impact of transport and distribution on microbial numbers be examined in future studies.

In lettuce samples a gradual decrease in the average levels of microorganisms in samples was observed, of which in some samples, like loose lettuce, was not significantly different from the average level of lettuce head (unprocessed lettuce). Pillow-packs samples were observed with the lowest microbial levels compared to "loose lettuce" and pre-packaged lettuce samples. The level of coliforms on pillow-packs was below the guideline set by the NDOH (2000), and no *E. coli* was found in pillow-pack samples.

Salmonella and STEC were not detected in any of the produce samples. This does not rule out the possibility that pathogens other than *Salmonella* and STEC might be present. Pillow-packs samples were found with more positive presumptive ESBL-producing organism compared to other samples. This might have occurred as a result of post-processing contamination. Twenty percent of the isolates that were identified as Enterobacteriaceae members were found to be ESBL-producers. The findings have also indicated that some isolates were resistant to multiple antimicrobials. The genotypic confirmation findings have indicated that seven of the tested isolates carried ESBL genes (bla_{TEM}, and bla_{CTX-M}) only, bla_{SHV} was not found in any of the samples. These findings are worrisome because fresh produce is eaten raw, as a result, consumers may acquire resistant bacteria which interfere with treatment against bacterial infections.

Conclusion

Findings obtained in this study gave a limited indication of the microbial quality of some fresh produce sold in the Western Cape in some retailers. However, microbial quality of fresh produce can be different at other packhouses due to different processing methods used and workers with different understandings about hygiene. Future research should focus on investigating fresh produce safety, occurrence, identities and antimicrobial resistance characterisation from different packhouses in the Western Cape. Data generated during these studies will contribute to developing and implementing crop-specific guidelines in fresh produce production systems.

4.3.2 Informal supply chain fresh produce sampling and microbiological analysis in the Cape Town metropole

Authors: Anika Laubscher and Gunnar Sigge

Specific aim: This study aimed to determine the microbiological safety of fresh produce sold at the informal market in the Cape Town Metropolitan area, South Africa, by enumerating hygiene indicator systems such as coliforms, *Escherichia coli* and Enterobacteriaceae. Five informal vendors were selected to represent the informal market.

Results

Foodborne pathogens. No *Salmonella* or *Listeria monocytogenes* were detected in any of the fresh produce, however, one lettuce sample tested positive for STEC. Out of a total of 150 produce samples tested, 11.33% contained *E. coli* at average levels of 3,43 log CFU/g. There were no significant differences ($p < 0.05$) between the presence of *E. coli* in the different fresh produce samples tested. The presence of *E. coli* occurred sporadically suggesting that *E. coli* contamination could be linked to the post-harvest handling of fresh produce. Regardless of the high hygiene indicator counts and the sporadic presence of *E. coli*, no pathogens were detected (excluding one event). Therefore, there is no evidence supporting the assumption that the fresh produce tested is unsafe for consumption.

Coliform enumeration. The total coliform counts of all the fresh produce sampled at sites A-E during the three repetitions are presented in Figures 35, 36 and 37. In each figure, the coloured figure bars below the zero on the x-axes are indicative of produce types that were not tested at a particular time and site. The microbiological limit for coliforms, as advised by the NDOH (NDOH, 2000), is also indicated.

The coliform count for lettuce, spinach and green beans were well over the advised NDOH microbiological limit of < 200 CFU/g (NDOH, 2000) (Figure 34-35). The tomatoes and green pepper coliform results in Figure 35 showed variation. At site A, the average coliform count for green peppers was above the NDOH microbiological limit; however, at site B the counts were in the NDOH's limits. Because very little is known about the produce source or post-harvest handling of the product, the reasons for the results can only be speculated about. Similar results were observed for the tomatoes (Figure 34). Site D's coliform results were in the NDOH's microbiological limit whereas site E's coliform results for tomatoes was above the microbiological limit.

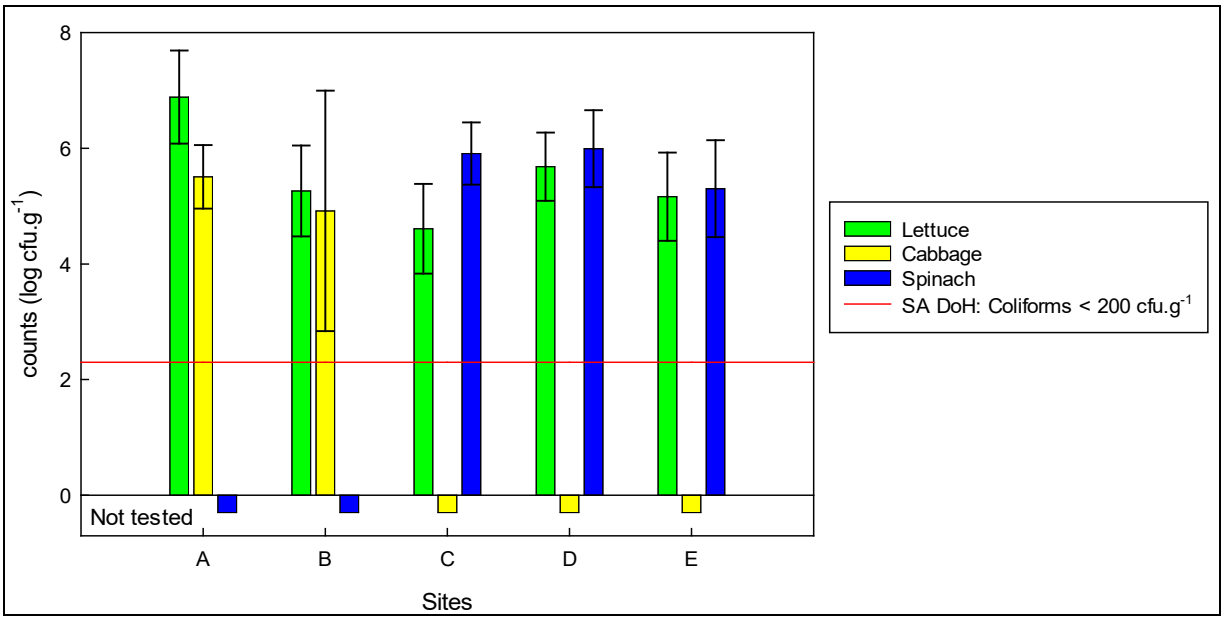


Figure 34: The total coliform counts of the selected fresh produce products sampled at sites A-E for the first repetition of a total of three.

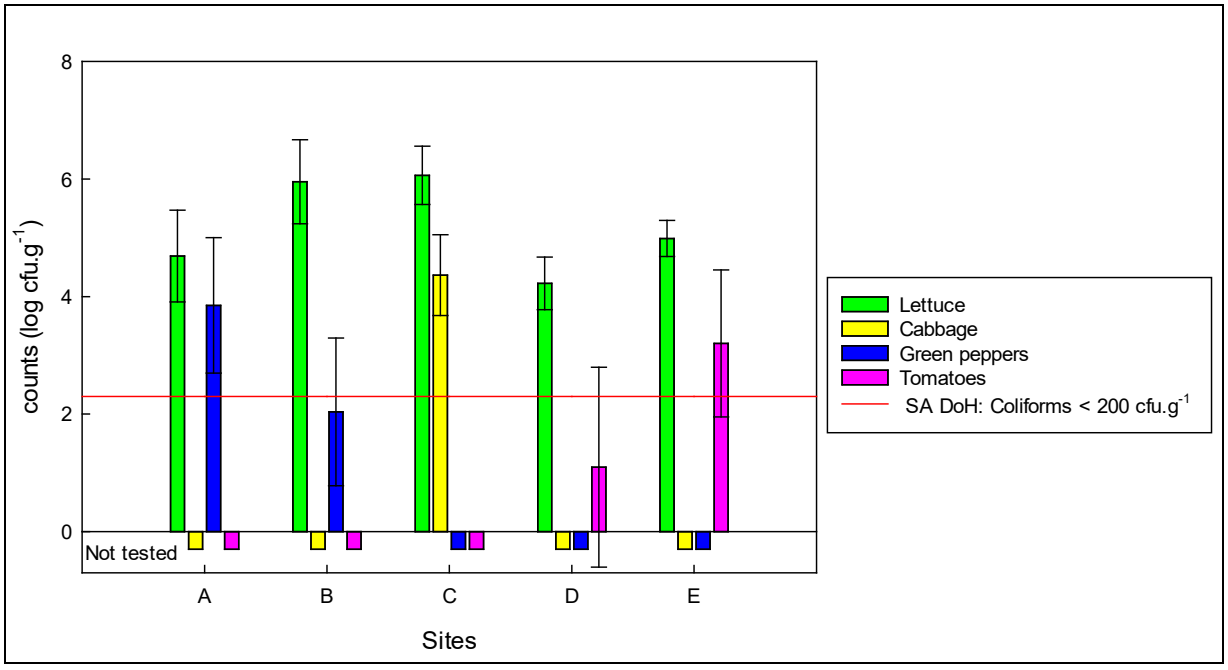


Figure 35: The total coliform counts of the selected fresh produce products sampled at sites A-E for the second repetition of a total of three.

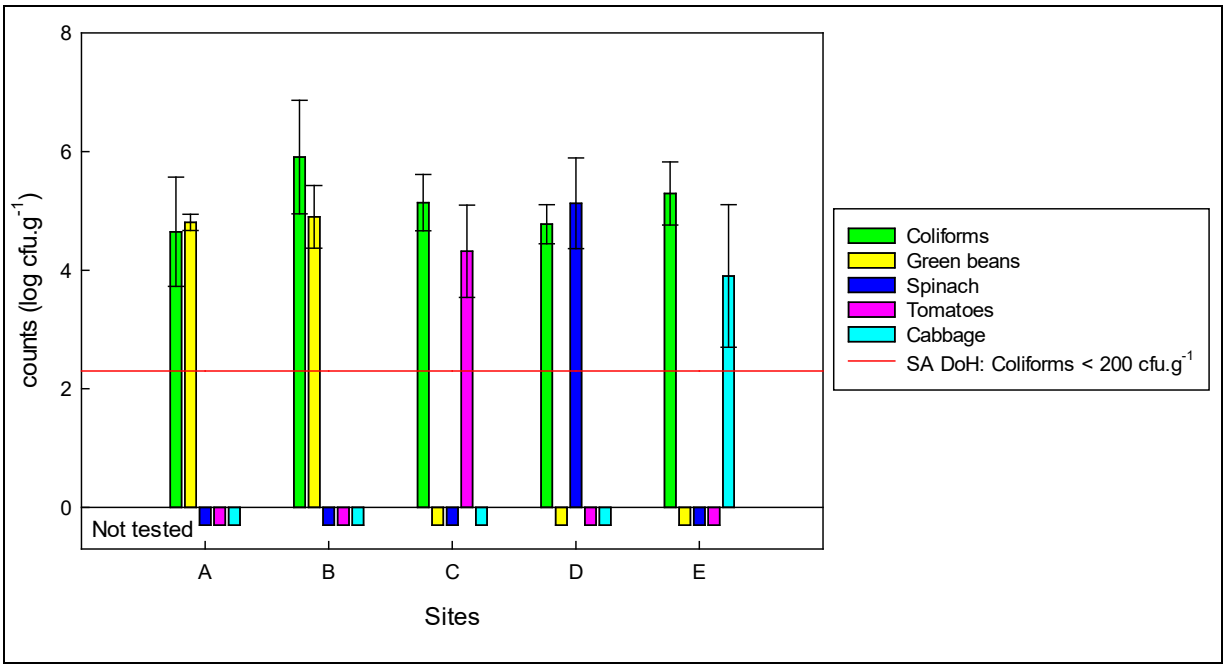


Figure 36: The total coliform counts of the selected fresh produce products sampled at sites A-E for the third repetition of a total of three.

The average coliform count range for spinach over all three repetitions was 5.13-5.99 log CFU/g (Figures 34, 35 and 36), whereas the results from a similar study conducted in Johannesburg of spinach sold at informal retailers reported an average coliform count range of 2.64-5.74 log CFU/g (Du Plessis *et al*., 2017). The spinach results from both studies are very similar except that this study's spinach coliform counts were more consistent, varying with less than one log CFU/g.

The average range for coliforms of cabbage over all three repetitions (Figures 34-36) were well above the suggested limit at 4.24-6.89 log CFU/g. A similar study completed in 2017 on fresh produce from the formal retailers and informal vendors in Johannesburg, South Africa, documented a range of 2.78-5.73 log CFU/g coliforms for cabbage sampled at six different informal retailers (Du Plessis *et al.*, 2017). This study's maximum average coliform count for cabbage was more than one log CFU/g higher.

The colony counts of the lettuce samples from all sites during all repetitions were used to compare the long-term microbiological quality of lettuce sold in the informal market and is presented in Figure 37.

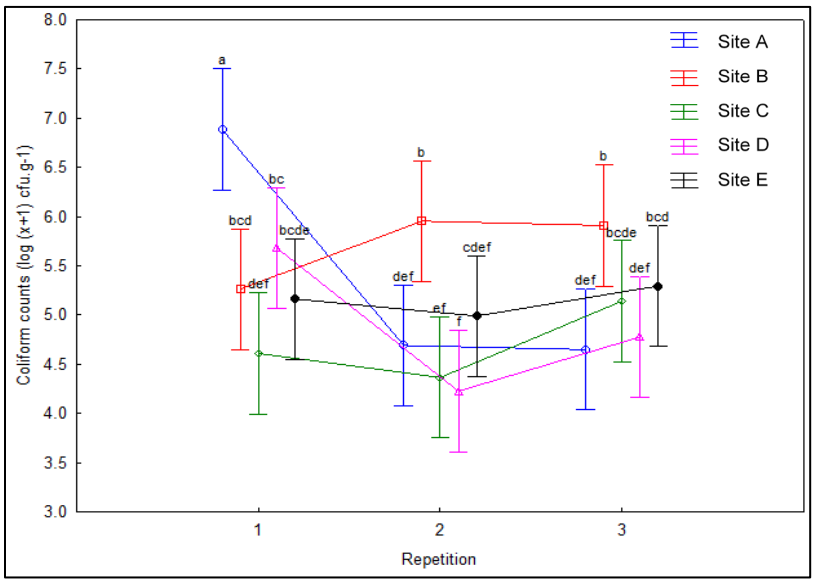


Figure 37: A general linear model illustrating significant differences for the coliform count from lettuce at sites A-E.

Error bars represent error at a 95% confidence interval.

According to the results (Figure 38), the quality of lettuce sold at sites B, C & E were more consistent during repetitions 1, 2 & 3, showing no significant differences between the coliform loads from samples in the three repetitions. However, sites A and D showed larger variation in coliform numbers. The coliform counts from the lettuce samples collected during the first repetition of both sites A and D was significantly higher than the coliform counts from the lettuce samples collected in repetitions 2 and 3 (Figure 38).

The coliform counts of all the produce tested in repetition 1-3 were pooled based on produce types and are presented in Figure 39. No significant differences were observed in the coliform loads of cabbage, lettuce, spinach and green beans. All four these produce types had significantly higher coliform loads than what was observed for green peppers and tomatoes.

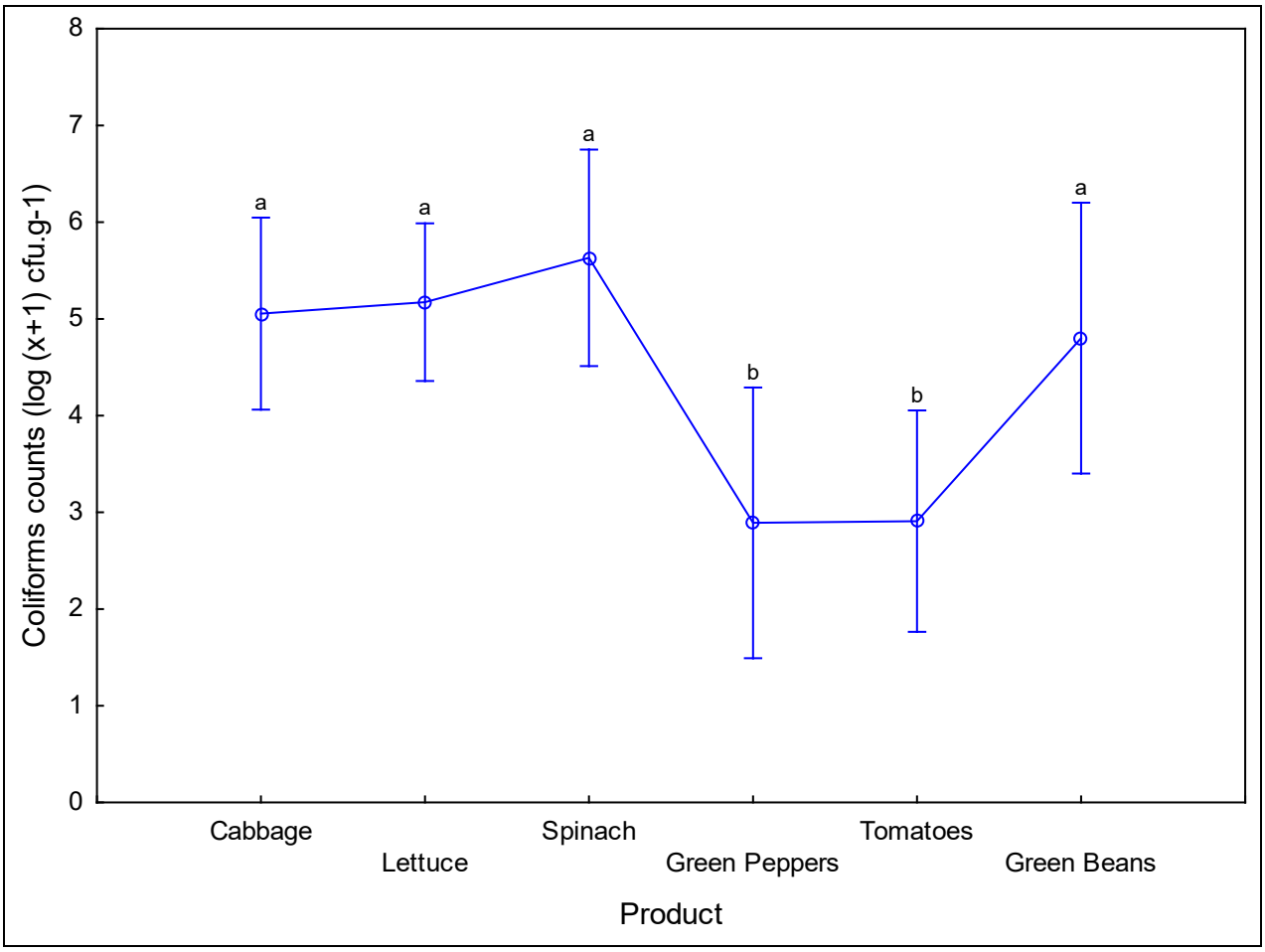


Figure 38: Distribution of coliforms loads based on produce types.

Error bars represent error at a 95% confidence interval.

Enterobacteriaceae loads were also determined for produce samples from all sites, and are presented in Figures 39, 40 and 41 for repetitions 1-3 respectively.

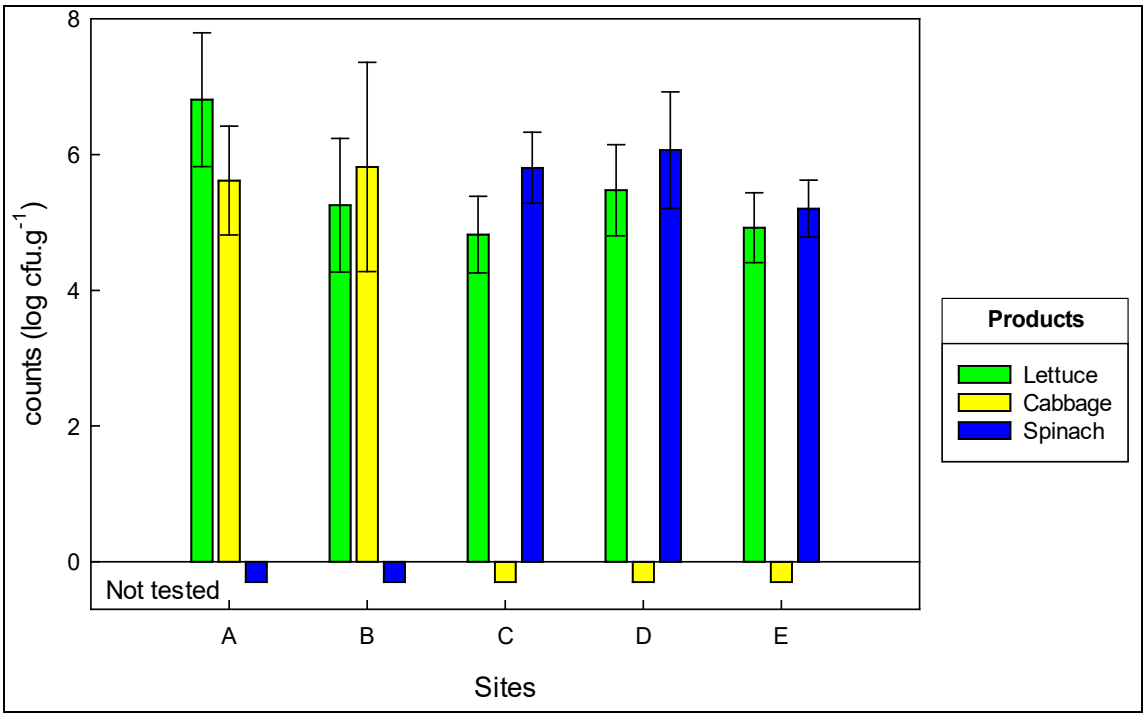


Figure 39: The Enterobacteriaceae counts of the selected fresh produce sampled at sites A-E for the first repetition of a total of three.

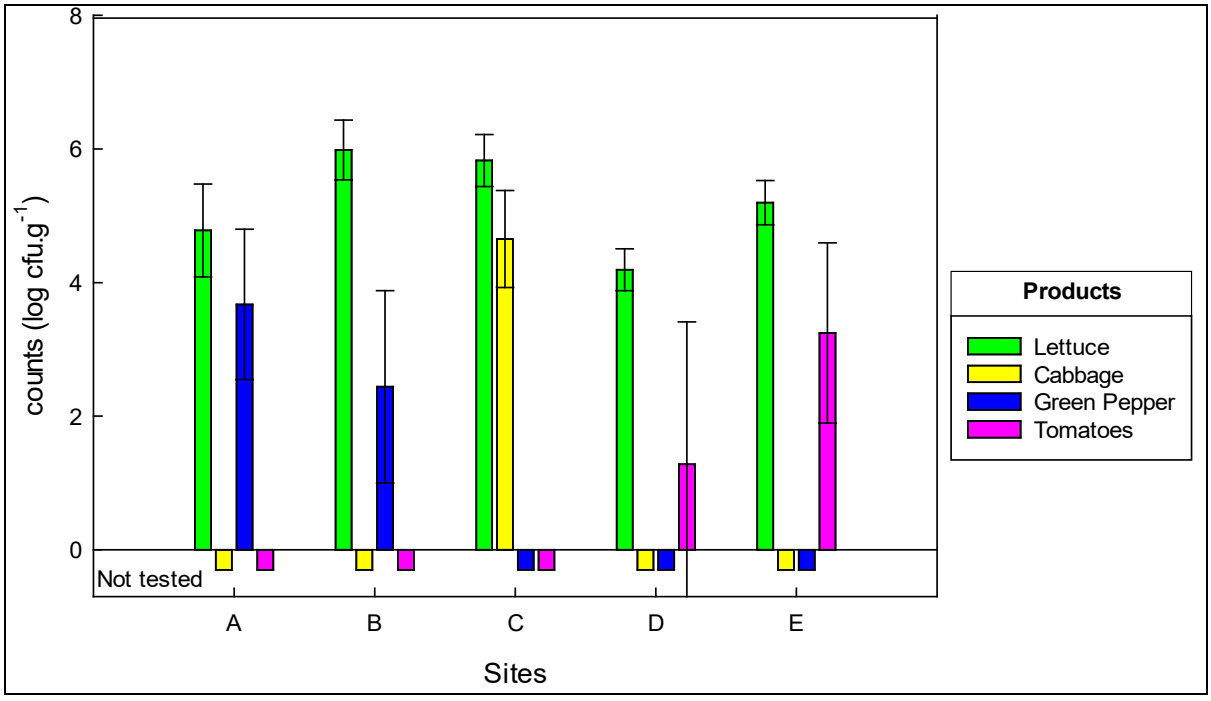


Figure 40: The Enterobacteriaceae counts of the selected fresh produce sampled at sites A-E for the second repetition of a total of three.

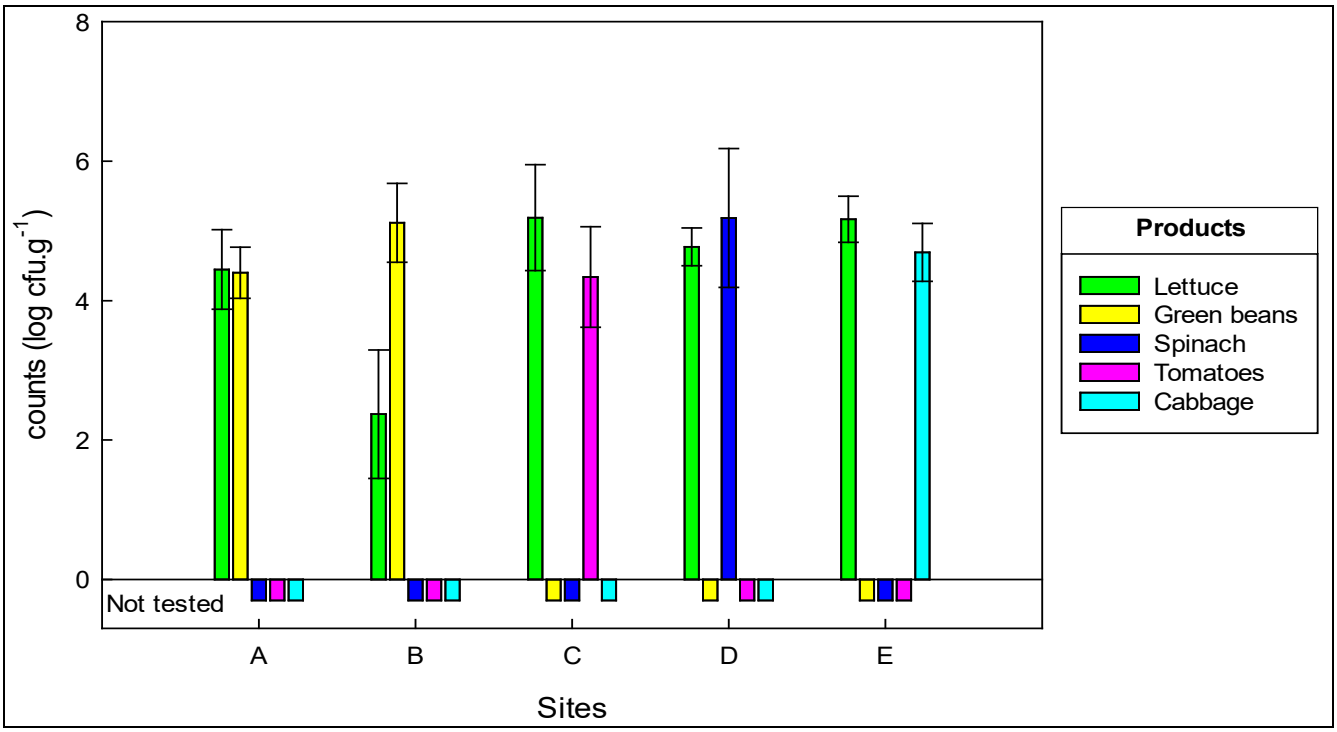


Figure 41: The Enterobacteriaceae counts of the selected fresh produce sampled at sites A-E for the third repetition of a total of three.

There was no significant difference counts ($P > 0.05$) between the Enterobacteriaceae and coliform loads of repetition 1 (figure 34 and figure 39 respectively). It can therefore be concluded that the majority of the Enterobacteriaceae population present on the produce samples in repetition 1 consisted of coliforms. This was the same for repetition 2 (figures 35 and 40) and repetition 3 (figures 36 and 41).

Enumeration of *Escherichia coli.* The same fresh produce samples that were tested for coliforms and Enterobacteriaceae were also tested for *E. coli* (determined as part of the coliform counting method using RAPID'E. coli 2 agar). The results are presented in Figures 42, 43 and 45. Two microbiological limit guidelines are included. The European Food Safety Authority (EFSA) advises a maximum *E. coli* limit of 1000 CFU/g (EFSA, 2007). The second advised microbiological limit indicated is from the NDOH who recommends a zero tolerance for *E. coli* (0 CFU/g) (NDOH, 2000).

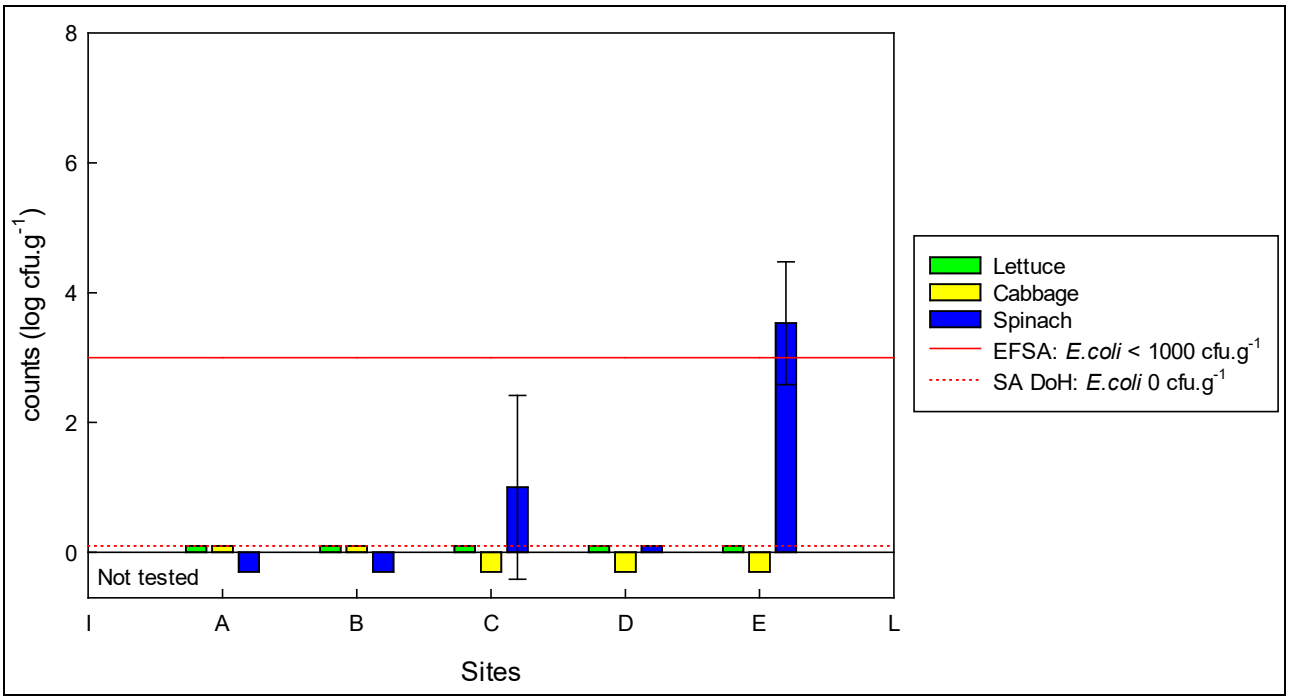


Figure 42 The average *E. coli* loads (n=5) of the selected fresh produce sampled at sites A-E for the first repetition of a total of three.

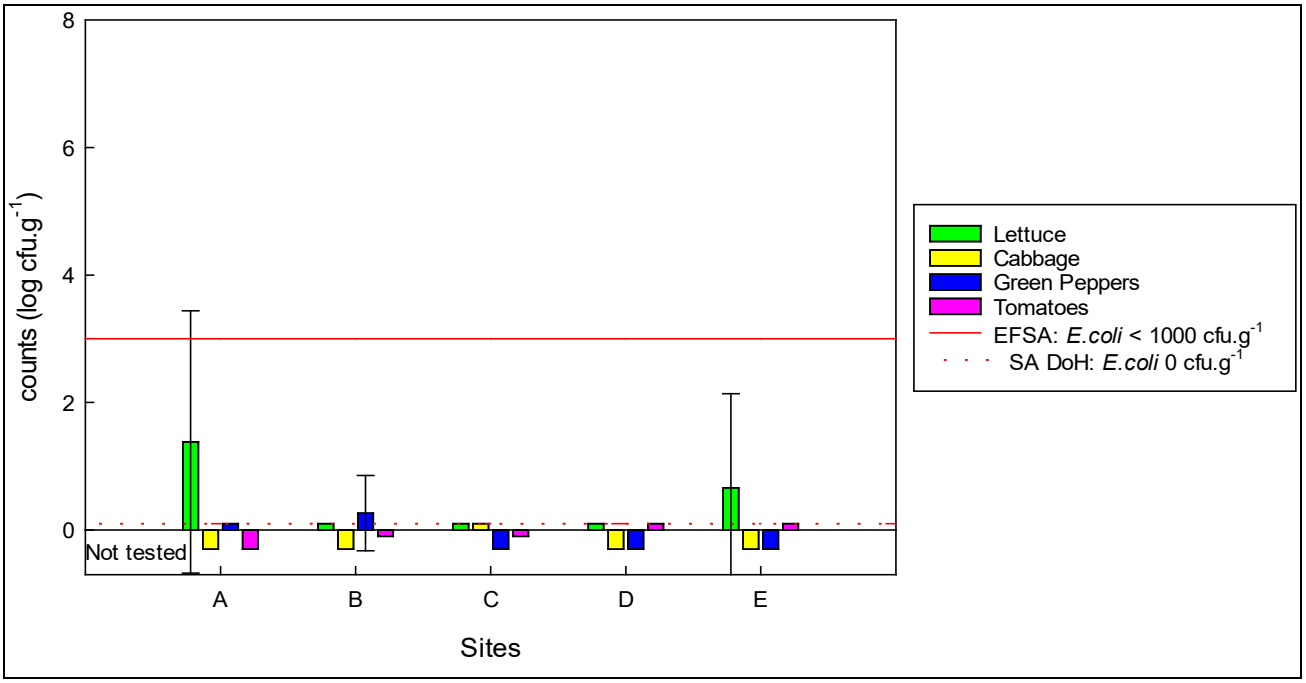


Figure 43 The average *E. coli* loads (n=5) of the selected fresh produce sampled at sites A-E for the second repetition of a total of three.

According to the South African NDOH's guidelines, any positive count for *E. coli* is regarded as unacceptable. In repetition 1 (Figure 42), which was conducted during the summer months

between November and December only the spinach samples at sites C and E tested positive *for E. coli*. Figure 42 only gives a representation of the average count (n=5) for *E. coli* per site. In reality, only two spinach samples tested positive for *E. coli* at Site C, which resulted in a large standard deviation of 1.00 ± 1.42. However, for site E all five samples of spinach were positive for E. coli, although levels varied from 2.48 to 5.00 log CFU/g. These results could be because of a combination of pre- and post-harvest factors that could include poor handling practices, exposed products, poor storage or transport.

In repetition 2, (Figure 43) other produce (lettuce and green peppers) and sites (A, B in addition to E) also tested positive for *E. coli*. The presence of *E. coli* during repetition 2 also occurred sporadically. Only two lettuce samples from site A tested positive whereas at Site E only one lettuce sample tested positive for *E. coli*. One green pepper from site B tested positive for E. coli at 1.322 log CFU/g.

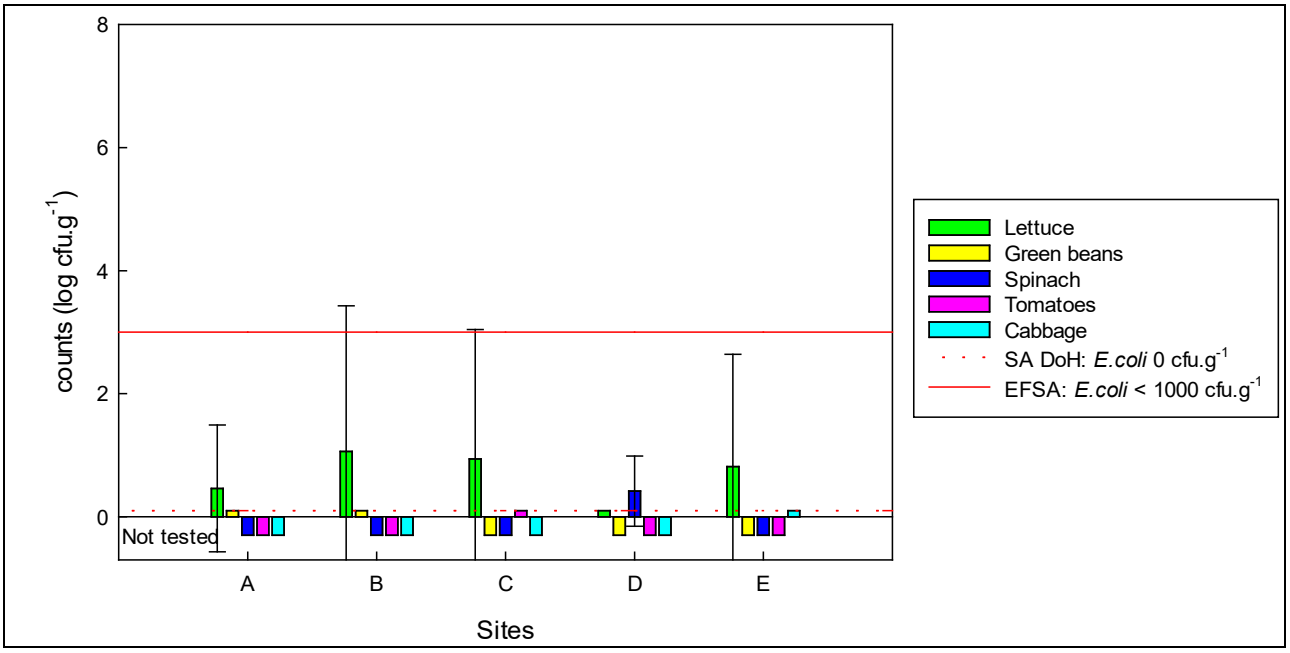


Figure 44 The average *E. coli* loads (n=5) of the selected fresh produce sampled at sites A-E for the third repetition of a total of three.

Figure 44 gives a representation of the *E. coli* results of repetition 3 from sites A-E. At most sites (except site D) only one of the five lettuce samples collected tested positive for *E. coli*. At site D, two spinach samples tested positive for *E coli.*

Although averaged values (n=5) are necessary for statistical comparison to other results, it should be noted that the *E. coli* counts presented in Figures 42, 43, and 44 do not represent the actual *E. coli* levels of the individual contaminated produce. These can be seen in Table 57.

Table 57 The results for individual produce samples that tested positive for *E. coli* in repetitions 1-3.

Sampling date	**Sampling site**	**Product**	**Sample no.**	***E. coli* counts log CFU/g**
Repetition 1				
05/12/2017	C	Spinach	435	3.00
05/12/2017	C	Spinach	438	2.00
28/11/2017	E	Spinach	381	2.48
28/11/2017	E	Spinach	189	3.70
28/11/2017	E	Spinach	345	3.00
28/11/2017	E	Spinach	080	5.00
28/11/2017	E	Spinach	658	3.48
Repetition 2				
08/01/2018	A	Lettuce	416	4.60
08/01/2018	A	Lettuce	075	2.30
15/01/2018	B	Green Peppers	073	1.32
05/02/2018	E	Lettuce	857	3.30
Repetition 3				
12/03/2018	A	Lettuce	525	2.30
19/03/2018	B	Lettuce	175	5.30
03/04/2018	C	Lettuce	691	4.70
26/03/2018	D	Spinach	708	1.04
26/03/2018	D	Spinach	274	1.04
16/04/2018	E	Lettuce	399	4.08

Pathogen detection. There were no pathogens detected in the majority of the samples collected at all sites during repetition 1-3. The only exception was that the STEC virulence genes eae and stx were detected in one lettuce sample isolated at site A (sample 416) during repetition 2. The overall *E. coli* count for this particular lettuce sample at site A during repetition 3 was well above both the NDOH and EFSA guideline limits at 4.6 log CFU/g (Table 57). It is unknown, however, what the concentration of STEC was that was present before enrichment. Although the presence of STEC is a concern, the results do not indicate that it is consistently present. Because of the seriousness

of the pathogen, it is, however recommended that more frequent and routine monitoring of STEC is conducted in future.

Isolation and identification of ESBL-producing Enterobacteriaceae. The screening for ESBL Enterobacteriaceae was conducted using ESBL ChromID agar (South Africa) after a two-step enrichment, as described in the EUCAST method (EUCAST, 2017a). The distribution of the produce samples from which presumptive positive ESBL-producing colonies were isolated at the respective sites are presented in Figures 45, 46 and 47.

Overall, an unexpectedly high number of presumptive positive ESBL-producing colonies were isolated from produce samples using ESBL ChromID agar. During all three repetitions, presumptive ESBL-producing colonies were isolated from all the lettuce and spinach samples (100%) (Figures 45, 46 and 47). The prevalence of presumptive positive ESBL-producing colonies in green peppers and tomatoes were slightly lower. After screening for ESBL-producers a total of 416 isolates was isolated from the 150 fresh produce samples. Only 38% (158) of the total number of isolates were selected for further identification using MALDI-TOF spectrometry. A summary of the species identification results are presented in Figure 48. Each species is expressed as a percentage of the total isolates tested (n=158). Only 9% of the isolated strains were identified as Enterobacteriaceae. Four strains of *Klebsiella pneumoniae* were isolated from cabbage and spinach. One *E. coli* was isolated from lettuce and three Enterobacter strains were isolated from lettuce and tomatoes. Five *Enterobacter absuriae* strains were isolated from lettuce, spinach and green beans.

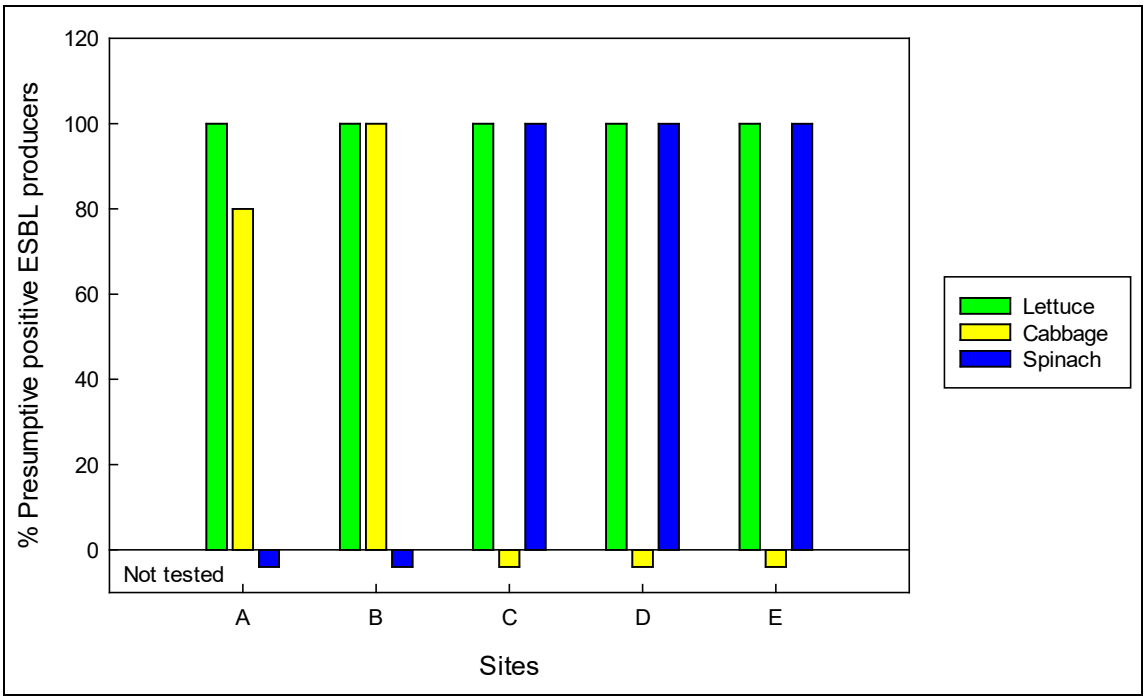


Figure 45 The distribution of the produce samples from which presumptive positive ESBL-producing colonies were isolated during repetition 1.

The produce types not tested at a particular site are indicated in a bar under the x-axis

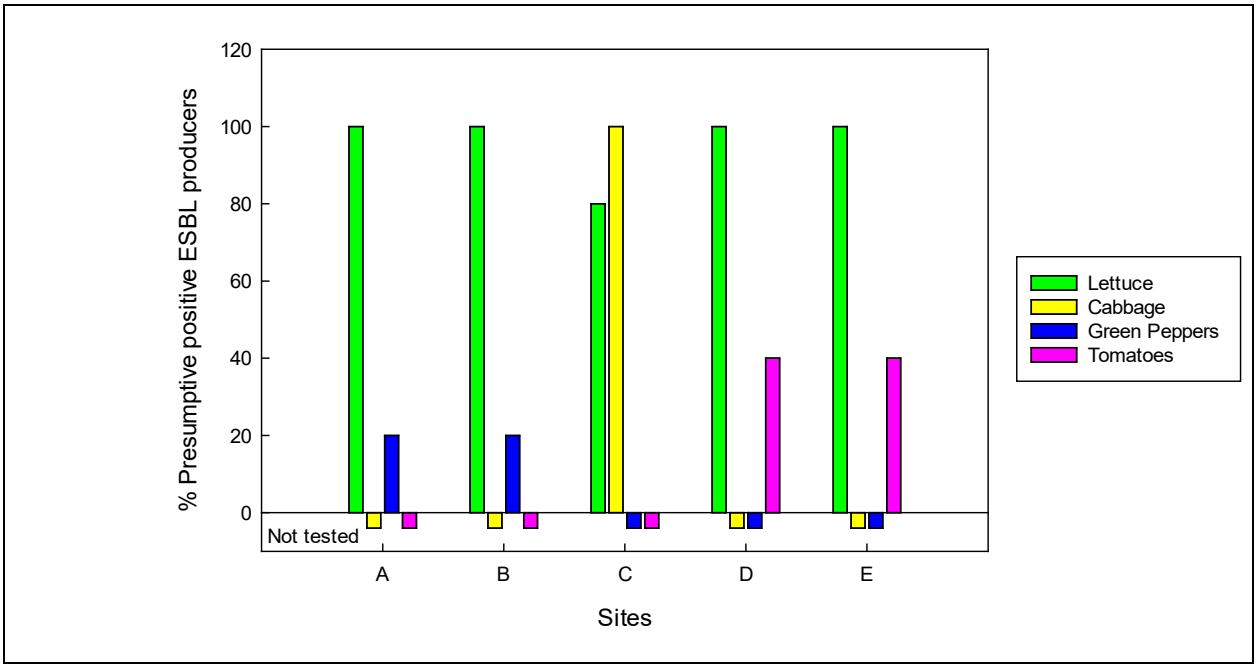


Figure 46 The distribution of the produce samples from which presumptive positive ESBL-producing colonies were isolated during repetition 2.

The produce types not tested at a particular site are indicated in a bar under the x-axis.

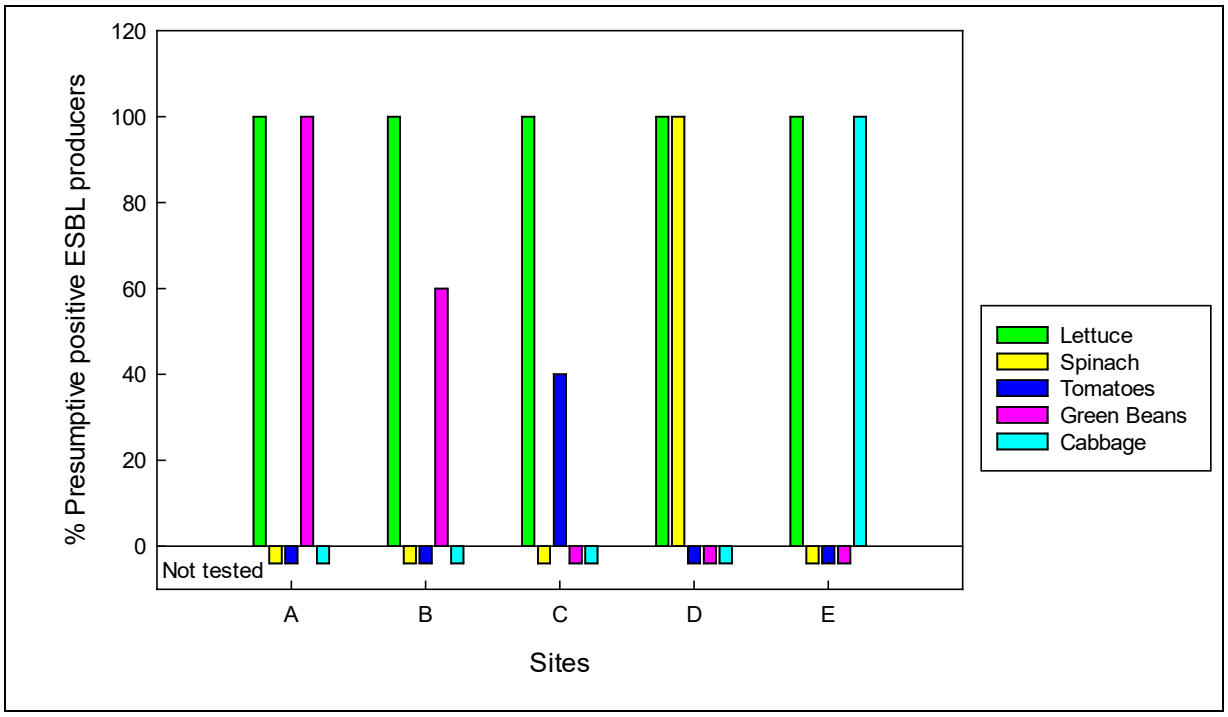


Figure 47 The distribution of the produce samples from which presumptive positive ESBL-producing colonies were isolated during repetition 3.

The produce types not tested at a particular site are indicated in a bar under the x-axis.

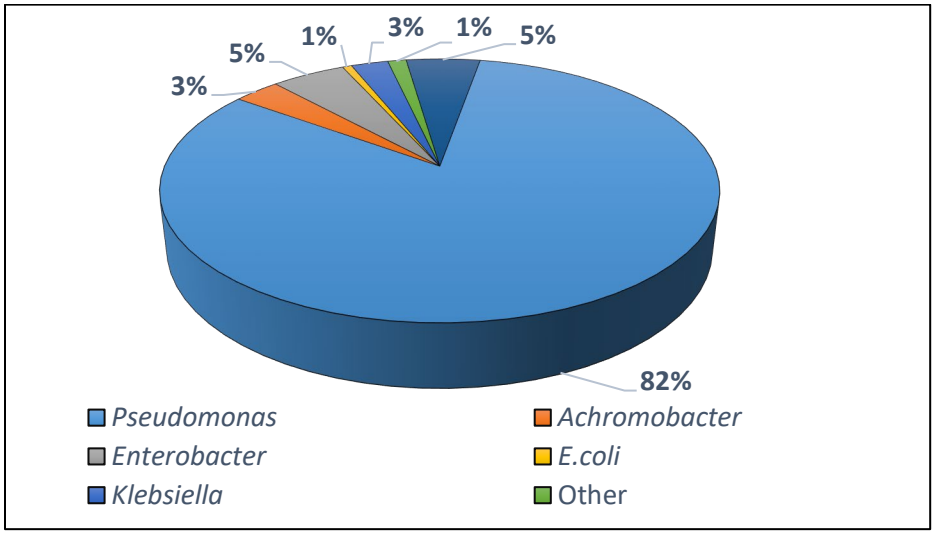


Figure 48 The species identification distribution of the 158 presumptive positive ESBL-producing isolates subjected to MALDI-TOF spectrometry identification expressed as a percentage of the total (n=158).

After MALDI-TOF identification, the 13 Enterobacteriaceae strains were subjected to a range of different antibiotics from different classes using the EUCAST disk diffusion method (EUCAST, 2017a). To confirm ESBL status these strains were also subjected to the specific antibiotic combination diffusion disk test (EUCAST, 2017a). Each isolate was exposed to Ceftazidime, Cefotaxime or Cefepime in combination with and without clavulanic acid. If the zone diameter of the Ceftazidime, Cefotaxime or Cefepime with clavulanic acid was 5 mm larger than the zone of the same agents without clavulanic acid, the isolate was confirmed as an ESBL-producing Enterobacteriaceae strain. Only seven strains were confirmed as ESBL-producing Enterobacteriaceae in this manner. These strains were also subjected to the PCR-confirmation method of Monstein *et al.* (2007) to determine which of three ESBL genes (bla $_{SHV}$, bla $_{CTX-M,}$ and bla $_{TEM}$) might be present. These results are presented in Table 58. The results of the broader antimicrobial resistance testing is presented in Table 59.

Table 58 The confirmed ESBL-producing Enterobacteriaceae results using the combination disk diffusion test (EUCAST, 2017b)

					Disk diffusion	**Molecular confirmation**		
Sample date	**Site**	**Product**	**Species**	**Isolate no.**	**ESBL confirm**	**bla SHV**	**bla CTX-M**	**bla TEM**
06/11/2017	B	Cabbage	*Klebsiella pneumoniae*	294	+	+	+	+
06/11/2017	B	Cabbage	*Klebsiella pneumoniae*	160	+	-	+	+
06/11/2017	B	Cabbage	*Klebsiella pneumoniae*	999	+	-	+	+
05/12/2017	C	Spinach	*Klebsiella pneumoniae*	974	+	-	+	+
05/02/2018	E	Tomatoes	*Enterobacter cloacae*	542	+	-	-	-
12/03/2018	A	Lettuce	*Enterobacter cloacae*	601	+	-	-	-
16/04/2018	E	Lettuce	*Enterobacter asburiae*	558	+	+	-	+

Table 59 The *Enterobacteriaceae* results for the antibiotic susceptibility testing (EUCAST, 2017b)

Site	Produce	*Enterobacteriaceae*	Nr	Ampi-cillin	Cefo-xitin	Chloram-phenicol	Cloxacillin	Ciprofloxacin	Gentamycin	Imipenem	Tetracyc-line	TS
B	Cabbage	*K. pneumoniae*	294	R	R	R	R	R	R	S	R	R
B	Cabbage	*K. pneumoniae*	160	R	S	S	R	R	R	S	R	R
B	Cabbage	*K. pneumoniae*	999	R	S	S	R	R	R	S	R	R
C	Spinach	*K. pneumoniae*	974	R	S	S	R	R	S	S	R	R
B	Lettuce	*E. coli*	105	R	S	R	R	S	S	S	S	S
E	Lettuce	*E. cloacae*	012	S	R	S	R	S	S	S	S	S
E	Tomatoes	*E. cloacae*	542	R	R	S	R	S	S	S	S	S
A	Lettuce	*E. cloacae*	601	R	R	R	R	S	S	S	S	R
B	Lettuce	*E. asburiae*	155	R	R	R	R	S	S	R	S	S
B	Green Beans	*E. asburiae*	853	R	R	R	R	R	R	R	S	R
D	Lettuce	*E. asburiae*	625	R	R	R	R	R	S	R	S	R
D	Spinach	*E. asburiae*	396	R	R	R	R	R	S	R	S	R
E	Lettuce	*E. asburiae*	558	R	R	R	R	R	S	S	R	R

R = Resistant, S = Sus/ceptible, TS = Trimethoprim-sulfamethoxazole

The ESBL-producers were mostly limited to cabbage sold at Site B (Mitchells plain) (n=3) during repetition 1 (data not included in this report). Two of the strains (160 and 999) have identical antibiotic resistance profiles, although it is not known whether they are clones. No ESBL-producers were isolated from samples from site D in Rylands although two MDR strains of *E. asburiae* with the same antibiotic resistance profiles was isolated on the same sampling day, but from two different produce types (data not included in this report). The possible reasons for this is only speculative at best, since a lot of information was not available (including where the products were sourced from, how produce is transported to, and handled at the vendor). Of particular concern are the *K. pneumoniae* strain 294, which was confirmed as an ESBL producer, testing positive for all three ESBL-genes (Table 58), and was found to be resistant to seven different classes of antibiotics (Table 59).

Conclusions

The microbial analyses mostly reflected the unregulated nature of produce trade at informal vendors. The sporadic ups and downs in microbial numbers linked to individual samples, as well as in the occurrence and distribution of *E. coli* specifically, highlights the inconsistencies in microbial quality of fresh produce sold in this manner. Limited information about produce sourcing, transport and subsequent handling illustrates the general lack of traceability that can also severely limit product recalls, if this is ever required. The absence of pathogens (except for one instance of STEC) is only a small comfort if one considers the occurrence of MDR strains at all sites. In this study, no MDR organisms were isolated from the same sample that tested positive for STEC. Mobile resistance elements that can potentially be transferred to pathogens (if and when they occur) can, however, pose a real threat to food safety in this sector.

4.4 References

ABADIAS M, USALL J, ANGUERA M, SOLSONA C and VIÑAS I (2008). Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments. International Journal of Food Microbiology **123**(1-2), 121-129.

AIJUKA M, CHARIMBA G, HUGO CJ and BUYS EM (2015). Characterization of bacterial pathogens in rural and urban irrigation water. Journal of Water and Health **13**, 103-117.

AL-KHAROUSI ZS, GUIZANI N, AL-SADI AM, AL-BULUSHI IM and SHAHAROONA B (2016). Hiding in Fresh Fruits and Vegetables : Opportunistic Pathogens May Cross Geographical Barriers. International Journal of Microbiology **2016**, 1-14.

ALANAZI MQ, ALQAHTANI FY and ALEANIZY FS (2018). 'An evaluation of *E. coli* in urinary tract infection in emergency department at KAMC in Riyadh, Saudi Arabia: Retrospective study', *Annals of* Clinical Microbiology and Antimicrobials. BioMed Central **17**(1), 1-7.

BAKER CA, RUBINELLI PM, PARK SH, CARBONERO F and RICKE SC (2016). Shiga toxin-producing *Escherichia coli* in food: Incidence, ecology, and detection strategies. Food Control **59**, 407-419.

BALOYI T (2021). Comparative microbiological safety study of fresh produce sold and consumed in informal markets, Gauteng. Pretoria. (Published Masters thesis). University of Pretoria, South Africa.

BAYLIS C, UYTTENDAELE M, JOOSTEN H, DAVIES A and HEINZ HJ (2011). The Enterobacteriaceae and their significance to the food industry. In ILSI Europe Report Series (https://ilsi.eu/wp-content/uploads/sites/3/2016/06/EP-Enterobacteriaceae.pdf)

BEHARIELAL T, THAMAGA-CHITJA J and SCHMIDT S (2018). Pre-and post-harvest practices of smallholder farmers in rural KwaZulu-Natal, South Africa: Microbiological quality and potential market access implications. Food Control **92**, 53-62.

BEN SAID L, KLIBI N, DZIRI R, BORGO F, BOUDABOUS A, BEN SLAMA K and TORRES C (2016). Prevalence, antimicrobial resistance and genetic lineages of Enterococcus spp. from vegetable food, soil and irrigation water in farm environments in Tunisia. Journal of the Science of Food and Agriculture **96**(5), 1627-1633.

BENCARDINO D, VITALI LA and PETRELLI, D (2018). Microbiological evaluation of ready-to-eat iceberg lettuce during shelf-life and effectiveness of household washing methods. Italian Journal of Food Safety **7**(1), 6913.

BEN SAID L, JOUINI A, KLIBI N, DZIRI R, ALONSO CA, BOUDABOUS A, BEN SLAMA K and TORRES C (2015). Detection of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in vegetables, soil and water of the farm environment in Tunisia. International Journal of Food Microbiology **203**, 86-92.

BENTI G, KEBEDE A and MENKIR, S (2014). Assessment of bacteriological contaminants of some vegetables irrigated with river water in selected farms around Adama town, Ethiopia. Journal of Microbiology and Antimicrobials **6**(2):37-42.

BEZANSON GS, MACINNIS R, POTTER G and HUGHES T (2008). Presence and potential for horizontal transfer of antibiotic resistance in oxidase-positive bacteria populating raw salad vegetables. International Journal of Food Microbiology **127**, 37-42.

BHATIA M, THAKUR A, RAT B, MISHRA B and DOGRA V (2015). International Journal of Medical and Health Sciences **4**(3), 382-384.

BHOWMIK SK (2005). Street vendors in Asia: a review. Economic and Political weekly, May 28 - June 4, 2256-2264.

BHUTANI N, MURALEEDHARAN C, TALREJA D, RANA SW, WALIA S, KUMAR A and WALIA SK (2015). Occurrence of multidrug resistant extended spectrum β-lactamase-producing bacteria on iceberg lettuce retailed for human consumption. Biomedical Research International **2015**, 547547.

BLAAK H, VAN HOEK AHAM, VEENMAN C, DOCTERS VAN LEEUWEN AE and LYNCH G (2014). Extended spectrum Beta-lactamase- and constitutively AmpC-producing Enterobacteriaceae on fresh produce and in the agricultural environment. International Journal of Food Microbiology **8**(16), 168-169.

BORGOGNA TR, BORGOGNA, J-L, MIELKE JA, BROWN CJ, TOP, EM and BOTTS RT (2016). High diversity of CTX-M extended-spectrum b-lactamases in municipal wastewater and urban wetlands. Microbial Drug Resistance **22**, 312-320.

BOURN D and PRESCOTT J (2002). A comparison of the nutritional value, sensory qualities, and food safety of organically and conventionally produced foods. Critical Reviews in Food Science and Nutrition **42** (1), 1-34.

BOUSQUET A, VAN DER MEE-MARQUET N, DUBOST C, BIGAILLON C, LARRÉCHÉ S, BUGIER S, SURCOUF C, MÉRAT S BLANCHARD H and MÉRENS A (2017). Outbreak of CTX-M-15-producing *Enterobacter cloacae* associated with therapeutic beds and syphons in an intensive care unit. American Journal of Infection Control **45**, 1160-1164.

BRINK A, FELDMAN C, DUSE A, GOPALAN D, GROLMAN D, MER M, NAICKER S, PAGET G, PEROVIC O and RICHARDS G (2006). Guideline for the management of nosocomial infections in South Africa. South African Medical Journal **96**, 642-652.

BRYAN A, YOUNGSTER I and MCADAM AJ (2015). Shiga Toxin-Producing *Escherichia coli*. Clinics in Laboratory Medicine **35**, 247-272.

CALLEJÓN RM, RODRÍGUEZ-NARANJO MI, UBEDA C, HORNEDO-ORTEGA R, GARCIA-PARRILLA MC and TRONCOSO AM (2015). Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathogens and Disease **12**(1), 32-38.

CAMPOS J, MOURÃO J, PESTANA N, PEIXE L, NOVAIS C and ANTUNES P (2013). Microbiological quality of ready-to-eat salads: An underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. International Journal of Food Microbiology **166**, 464-470.

CANTÓN R, GONZÁLEZ-ALBA JM, and GALÁN JC (2012). CTX-M enzymes: origin and diffusion. Frontier in Microbiololgy **3,** 110.

CARDAMONE C, ALEO A, MAMMINA C, OLIVERI G, DI NOTO AM (2015). Assessment of the microbiological quality of fresh produce on sale in Sicily, Italy: preliminary results. Journal of Biological Research (Thessalonike, Greece) **22**(1), 3 (doi:10.1186/s40709-015-026-3).

CENTRE FOR FOOD SAFETY (CFS) IN HONG KONG (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). Food and Environmental Hygiene Department (revised).

CHEN F, ZHANG W, SCHWARZ S, ZHU Y, LI R, HUA X and LIU S (2020). Genetic characterization of an MDR/virulence genomic element carrying two T6SS gene clusters in a clinical *Klebsiella pneumoniae* isolate of swine origin. Journal of Antimicrobial Chemotherapy **74**(6),1539-1544.

CLERMONT O, OLIER M, HOEDE C, DIANCOURT L, BRISSE S, KEROUDEAN M, GLODT J, PICARD B, OSWALD E and DENAMUR E (2011). Animal and human pathogenic *Escherichia coli* strains share common genetic backgrounds. Infection Genetics and Evolution **11**(3), 654-662.

CLINICAL LABORATORY STANDARD INSTITUTE [CLSI] (2016). Performance Standards for Antimicrobial Susceptibility testing. M100S, 26th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

CLINICAL LABORATORY STANDARD INSTITUTE [CLSI] (2018). Performance Standards for Antimicrobial Susceptibility Testing. 28th ed. CLSI Supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute.

CORZO-ARIYAMA HA, GARCÍA-HEREDIA A, HEREDIA N, GARCÍA S, LEÓN J, JAYKUS LA and SOLÍS-SOTO L (2019). Phylogroups, pathotypes, biofilm formation and antimicrobial resistance of *Escherichia coli* isolates in farms and packing facilities of tomato, jalapeño pepper and cantaloupe from Northern Mexico. International Journal of Food Microbiology **290**, 96-104.

DE BRUIN W, OTTO D and KORSTEN L (2016). Microbiological status and food safety compliance of commercial basil production systems. Journal of Food Protection **79**, 43-50.

DECOL LT, CASARIN LS, HESSEL CT, BATISTA ACF, ALLENDE A and TONDO EC (2017). Microbial quality of irrigation water used in leafy green production in Southern Brazil and its relationship with produce safety'. Food Microbiology **65**,105-113.

DENIS N, ZHANG H, LEROUX A, TRUDEL R and BIETLOT H (2016). Prevalence and trends of bacterial contamination in fresh fruits and vegetables sold at retail in Canada. Food Control **67**, 225-234

DEPARTMENT OF WATER AFFAIRS (DWAF) (1996). South African Water Quality Guidelines. Volume 4. Agricultural Use: Irrigation. Second Edition. Edited by S. Holmes, CSIR Environmental Services. Pretoria: Department of Water Affairs and Forestry.

DESK N (2019). Alfalfa sprouts linked to *Salmonella* infections in Australia | Food Safety News. Retrieved September 23, 2020, from https://www.foodsafetynews.com/2018/07/alfalfa-sprouts-linked-to-salmonella-infections-in-australia/.

DOYLE MP, LONERAGAN GH, SCOTT HM and SINGER RS (2013). Antimicrobial resistance: Challenges and perspectives. Comprehensive Reviews in Food Science and Food Safety **12**, 234-248.

DU PLESSIS EM, DUVENAGE F and KORSTEN L (2015). Determining the potential link between irrigation water quality and the microbiological quality of onions by phenotypic and genotypic characterisation of *Escherichia coli* isolates. Journal of Food Protection **78**(4), 643-651.

DU PLESSIS EM, GOVENDER S, PILLA, B, and KORSTEN L (2017). Exploratory study into the microbiological quality of spinach and cabbage purchased from street vendors and retailers in Johannesburg, South Africa. Journal of Food Protection **80**(10), 1726-1733.

DUVENAGE S and KORSTEN L (2017). Assessment of foodborne pathogen presence in the peach supply chain and its potential risk to the end consumer. Food Control **78**, 374-382.

EHLERS MM, VELDSMAN C, MAKGOTLHO EP, DOVE MG, HOOSEN AA and KOCK MM (2009). Detection of blaSHV, blaTEM and blaCTX-M antibiotic resistance genes in randomly selected bacterial pathogens from the Steve Biko Academic Hospital. FEMS Immunology Medical Microbiology **56**,191-196.

ESPIGOL AMD, DEL CARMEN DR, ESGUERRA EB, LUALHATI RAO and ALCANTARA GAP (2018). Microbiological quality of selected organically-grown fruits and vegetables in Luzon, Philippines. International Food Research Journal **25** (6), 2337-2344.

EUROPEAN FOOD SAFETY COMMISSION (EFSA) (2007). Commission regulation on microbiological criteria for foodstuffs. Commission Regulation (EC) No 1441/2007 of 5 December 2007. Official journal of the European Union, **322**, 12-29.

EUCAST (2017a). EUCAST guidelines for detection of resistance mechanisms and specific resistance of clinical and/or epidemiological importance. [Internet document]. URL http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_170711.pdf. Assessed date 18/05/2017.

EUCAST (2017b). EUCAST Disk Diffusion Method for Antimicrobial Susceptibility Testing. [Internet document]. URL http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/Version_5/Manual_v_6.0_EUCAST_Disk_Test_final.pdf. Assessed date 18/05/2017.

EUCAST (2018). European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. [Internet document]. URL http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf. Assessed date 18/05/2017.

FALOMIR MP, RICO H and GOZALBO D (2013). *Enterobacter* and *Klebsiella* species isolated from fresh vegetables marketed in Valencia (Spain) and their clinically relevant resistances to chemotherapeutic agents. Foodborne Pathogens and Disease 1**0** (12), 1002-7.

FAIR RJ and TOR Y (2014). Perspectives in medicinal chemistry antibiotics and bacterial resistance in the 21st Century. Perspectives in Medical Chemistry **6**, 25.

FOOD SAFETY AUTHORITY IRELAND (FSAI) (2016). Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 4) https://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=16771.

FOOD SAFETY STANDARDS NEW ZEALAND (FSANZ) (2001). Guidelines for the microbiological examination of ready-to-eat foods. Retrieved from http://foodstandards.gov.au.

FRESH PRODUCE SAFETY CENTRE AUSTRALIA AND NEW Zealand (FPSC A-NZ) (2019). Guidelines for Fresh Produce Food Safety. https://fpsc-anz.com/wp-content/uploads/2019/06/Guidelines-for-Fresh-Produce-Food-Safety-2019_WEB.pdf

FREITAG C, MICHAEL GB, JUN L, KADLEC K, WANG Y, HASSEL M and SCHWARZ S (2018). Occurrence and characterisation of ESBL- encoding plasmids among *Escherichia coli* isolates from fresh vegetables. Veterinary Microbiology **219**, 63-69.

GEKENIDIS, M. T, QI W, HUMMERJOHANN J, ZBINDEN R, WALSH F and DRISSNER D (2018). Antibiotic-resistant indicator bacteria in irrigation water: High prevalence of extended-spectrum ß-lactamase (ESBL)-producing *Escherichia coli*. PLoS One **13** (11), e020785.

GEMMELL ME and SCHMIDT S (2012). 'Microbiological assessment of river water used for the irrigation of fresh produce in a sub-urban community in Sobantu, South Africa', Food Research International **47**(2), 300-305.

GRAÇA A, ESTEVES E, NUNES C, ABADIAS M and QUINTAS C (2017). Microbiological quality and safety of minimally processed fruits in the marketplace of southern Portugal. Food Control **73**, 775-783.

GRACE D, DIPEOLU M, and ALONSO S (2019). Improving food safety in the informal sector: nine years later. Infection Ecology and Epidemiology **9**(1), 1579613.

HEALTH PROTECTION AGENCY (2009). Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. London.

HEALTH CANADA (2002). Health products and food Branch methods for the microbiological analysis of foods: Determination of coliforms, faecal coliforms and *Escherichia coli* in foods (MFHPB-19). Health Canada. Ontario. https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/methods-microbiological-analysis-foods-compendium-analytical-methods.html, Accessed 20-1-2021.

HERMAN K, HALL A and GOULD L (2015). Outbreaks attributed to fresh leafy vegetables, United States, 1973-2012. Epidemiology and Infection **143**(14), 3011-3021.

HOLVOET K, SAMPERS I, SEYNNAEVE M and UYTTENDAELE M (2014). Relationships among hygiene indicators and enteric pathogens in irrigation water, soil and lettuce and the impact of climatic conditions on contamination in the lettuce primary production. International Journal of Food Microbiology **171**, 21-31.

HÖLZEL CS, TETENS JL and SCHWAIGER K (2018). Unravelling the role of vegetables in spreading antimicrobial-resistant bacteria: a need for quantitative risk assessment. Foodborne Pathogens and Disease **15**, 671-688.

IJABADENIYI OA, DEBUSHO LK, VAN DER LINDE M and BUYS E M (2011). Irrigation water as a potential preharvest source of bacterial contamination of vegetables. Journal of Food Safety **31**, 452-461.

JANS C, SARNO E, COLLINEAU L, MEILE L, STÄRK KDC and STEPHAN R (2018). Consumer exposure to antimicrobial resistant bacteria from food at Swiss retail level. Frontiers in Microbiology **9**, 362.

JENA J, DEBATA NK, SAHOO RK, GAUR M and SUBUDHI E (2018). Molecular characterization of extended spectrum β-lactamase-producing *Enterobacteriaceae* strains isolated from a tertiary care hospital. Microbial Pathogenesis **115**, 112-116,

JONGMAN M and KORSTEN L (2016a). Assessment of irrigation water quality and microbiological safety of leafy greens in different production systems. Journal of Food Safety **37**, 1-12.

JUNG Y, JANG H AND MATTHEWS KR (2014). Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. Microbial Biotechnology **7**, 517-527

KHARAT AA, KHARAT KR, CHAUDHARI SG, KADAM DG, and KHARAT AS (2017). Co-existence of multiple β-lactamase traits among clinical isolates of *Escherichia c*oli from rural part of Maharashtra, India. African Journal of Microbiology Research **11**, 278-286.

KHARI FIM, KARUNAKARAN R, ROSLI R, and TAY ST (2016). Genotypic and phenotypic detection of AmpC b-lactamases in *Enterobacter* spp. Isolated from a teaching hospital in Malaysia. PLoS One **11**, 1-12.

KILONZO-NTHENGE A, LIU S, HASHEM F, MILLNER P and GITHUA S (2018). Prevalence of Enterobacteriaceae on fresh produce and food safety practices in small-acreage farms in Tennessee, USA. Journal of Consumer Protection and Food Safety **13,** 279-287.

KIM HS, CHON JW, KIM YJ, KIM DH, KIM M and SEO KH (2015). Prevalence and characterization of extended-spectrum-β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in ready-to-eat vegetables. International Journal of Food Microbiology **207**, 83-86.

KIM YJ, PARK KH, PARK DA, PARK J, BANG BW, LEE SS, LEE EJ, LEE HJ, HONG SK and KIM YR (2019). Guideline for the antibiotic use in acute gastroenteritis, Infection and Chemotherapy **51**(2), 217-243.

KORIR RC, PARVEEN S, HASHEM F and BOWERS J (2016). Microbiological quality of fresh produce obtained from retail stores on the Eastern Shore of Maryland, United States of America. Food Microbiology **56**, 29-34.

LAUBSCHER A (2019). Determination of microbiological safety of selected fresh produce of informal retailers at point-of-sale. Master Thesis, University of Stellenbosch, South Africa.

LI D, YU T, ZHANG Y, YANG M, LI Z, LIU M and QI R (2010). Antibiotic resistance characteristics of environmental bacteria from an oxytetracycline production wastewater treatment plant and the receiving river. Applied and Environmental Microbiology **76**, 3444-3451.

LIU C, HOFSTRA N and FRANZ E (2013). Impacts of climate change on the microbial safety of pre- harvest leafy green vegetables as indicated by *Escherichia coli* O157 and *Salmonella* spp. International Journal of Food Microbiology **163**, 119-128.

LODE HM (2008). Rational antibiotic therapy and the position of ampicillin/sulbactam. International Journal of Antimicrobial Agents **32**, 10-28.

LOWE C, WILLEY B, O'SHAUGHNESSY A, LEE W, LUM M, PIKE K, LAROCQUE C, DEDIER H, DALES L, MOORE C and MCGEER A (2012). Outbreak of extended-spectrum β-lactamase-producing *Klebsiella oxytoca* infections associated with contaminated handwashing sinks. Emerging Infectious Diseases **18**, 1242-1247.

MA L, LI A, YIN XL, AND ZHANG T (2017). The Prevalence of integrons as the carrier of antibiotic resistance genes in natural and man-made environments. Environmetal Science and Technology **51**, 5721-5728.

MAGWEDERE K, RAUFF D, DE KLERK G, KEDDY KH, and DZIVA F (2015). Incidence of nontyphoidal Salmonella in food-producing animals, animal feed, and the associated environment in South Africa, 2012-2014. Clinical Infectious Diseases **61**(4), 283-289.

MARSHALL BM, OCHIENG DJ and LEVY SB (2009). Commensals: Underappreciated reservoir of antibiotic resistance. Microbes **4**(5), 231-238.

MARUTLULLE NK (2017). Causes of informal settlements in Ekurhuleni Metropolitan Municipality: An exploration. Africa's Public Service Deliveeery Performance Review **5**(1), 11.

MASONGANYE M (2010). Street trading in Tshwane Metropolitan Municipality: realities and challenges. Urban Landmark. [Online]. Available from http://www.urbanlandmark.org.za/downloads/report_street_trading_jan2010. pdf,

MERLINI VV, PENA FL, CUNHA DT, OLIVEIRA JM, ROSTAGNO MA AND ANTUNES AEC (2018). Microbiological quality of organic and conventional leafy vegetables. Hindawi Journal of Food Quality Volume 2018, Article ID 4908316, 7 pages.

MESA RJ, BLANC V, BLANCH AR, CORTÉS P, GONZÁLEZ JJ, LAVILLA S, MIRÓ E, MUNIESA M, SACO M, TÓRTOLA MT, MIRELIS B, COLL P, LLAGOSTERA M, PRATS G and NAVARRO F (2006). Extended-spectrum β-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). Journal of Antimicrobial Chemotherapy **58**(1), 211-5.

MOISSENET D, SALAUZE B, CLERMONT O, BINGEN E, ARLET G, DENAMUR E, MÉRENS A, MITANCHEZ D and VU-THIEN H (2010). Meningitis caused by *Escherichia coli*-producing TEM-52 extended-spectrum β-lactamase in an extensive outbreak in a neonatal ward: Epidemiological investigation and characterization of the strain. Jornal of Clinical Microbiology **48**(7), 2459-2463.

MTHEMBU TP, ZISHIRI OT and EL ZOWALATY ME (2019). Detection and molecular identification of Salmonella virulence genes in livestock production systems in South Africa. Pathogens **8**(3), 124.

MURRAY K, WU F, SHI J, XUE SJ and WARRINER K (2018). Challenges in the microbiological food safety of fresh produce: Limitations of post-harvest washing and the need for alternative interventions. Food Quality and Safety **1**(4), 289-301.

NATIONAL DEPARTMENT OF HEALTH (NDOH) (2000). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. Department Of Health Directorate: Food Control, South Africa.

NATIONAL INSTITUTE OF COMMUNICABLE Disease (NICD) (2017). Foodborne disease outbreaks reported to the NICD. Communicable Diseases Communiqué **16**, 9-10.

NDE CW and LOGUE CM (2008). Characterization of antimicrobial susceptibility and virulence genes of Salmonella serovars collected at a commercial turkey processing plant. Journal of Applied Microbiology **104**(1), 215-223.

NEW SOUTH WALES (NSW) FOOD AUTHORITY (2009). Microbiological quality guide for ready-to-eat foods: A guide to interpreting microbiological results. http://www.foodauthority.nsw.gov.au. (Accessed 15 March 2020).

NGUZ K, SHINDANO J and SAMAPUNDO S (2005). Microbiological evaluation of fresh-cut organic vegetables produced in Zambia **16**, 623-628.

NJAGE PMK and BUYS EM (2014). Pathogenic and commensal *Escherichia coli* from irrigation water show potential in transmission of extended spectrum and AmpC β-lactamases determinants to isolates from lettuce. Microbial Biotechnology **8**, 462-473.

OJER-USOZ E, GONZÁLEZ D, GARCÍA-JALÓN I AND VITAS AI (2014). High dissemination of extended-spectrum β-lactamase-producing Enterobacteriaceae ineffluents from wastewater treatment plants. Water Research **56**, 37-47.

ORTEGA-PAREDESA D, BARBAB P, MENA-LÓPEZC S, ESPINELA N and ZURITAA BJ (2018). *Escherichia coli* hyperepidemic clone ST410-A harboring *bla*CTX-M-15 isolated from fresh vegetables in a municipal market in Quito-Ecuador. International Journal of Food Microbiology **280**, 41-45.

PAHL DM, TELIAS A, NEWELL M, OTTESEN AR and WALSH C (2013). Comparing source of agricultural contact and the presence of faecal indicator organisms on the surface of Juliet grape tomatoes. Journal of Food Protection **6**, 928-1108.

PETERSEN LM, and CHARMAN AJE (2018). The scope and scale of the informal food economy of South African urban residential townships: Results of a small-area micro-enterprise census. Development Southern Africa, **35**(1), 1-23.

PIRZAMAN NA and MOJTAHEDI A (2019). Investigation of antibiotic resistance and the presence of integron genes among ESBL-producing *Klebsiella* isolates. Meta Gene **19**, 37-41.

PITOUT JD and LAUPLAND KB (2008). Extended-spectrum β-lactamase-producing *Enterobacteriacea*e: an emerging public-health concern. *The Lancet Infectious Diseases*, **8**, 159-166.

REULAND EA, AL NAIEMI N, RAADSEN, S.A, SAVELKOUL PHM, KLUYTMANS JAJW and VANDENBROUCKE-GRAULS CMJE (2014). Prevalence of ESBL-producing Enterobacteriaceae in raw vegetables. European Journal of Clinical Microbiology and Infectious Diseases, **33**, 1843-1846.

RICHTER L, DU PLESSIS EM, DUVENAGE S and KORSTEN L (2019). Occurrence, identification, and antimicrobial resistance profiles of extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae from fresh vegetables retailed in Gauteng Province, South Africa. Foodborne Pathogens Disease **16**, 421-427.

RICHTER L, DU PLESSIS EM, DUVENAGE S and KORSTEN L (2020). Occurrence, Phenotypic and Molecular Characterization of extended-spectrum and ampc-β-lactamase-producing enterobacteriaceae isolated from selected commercial spinach supply chains in South Africa. Frontiers in Microbiology **11**, 638.

SARIFFUDDIN S, WAHYONO H and BROTOSUNARYO B (2017). Street Vendors Hypergrowth: Consequence of Uncontrolled Urbanization in Semarang City. Komunitas: International Journal of Indonesian Society and Culture **9**(1), 81-91.

SHAHID M, SINGH A, SOBIA F, RASHID M, MALIK A, SHUKLA I and KHAN HM (2011). *Bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, and *bla*$_{\text{SHV}}$ in *Enterobacteriaceae* from North-Indian tertiary hospital: High occurrence of combination genes. Asian Pacific Journal of Tropical Medicine **4**, 101-105.

STATISTICS SOUTH AFRICA (STATSSA) (2016). Statistics South Africa on GHS Series VIII: Water and Sanitation, 2002-2016 report. https://www.gov.za/speeches/statistics-south-africa-ghs-series-viii-water-and-sanitation-2002-2016-report-23-nov-2016. Accessed 04 May 2020.

SKINNER C (2008). Street trade in Africa: A review. KwaZulu-Natal, South Africa: School of Development Studies, University of KwaZulu-Natal.

STATSSA (2019). [Online]. Available from: http://www.statssa.gov.za/?p=12121Accessed: May 19 2019.

TAU NP, SMITH AM, SOOKA A, and KEDDY KH (2012). Molecular characterization of extended-spectrum b-lactamase-producing Shi-gella isolates from humans in South Africa, 2003-2009. Journal of Medical Microbiology **61**,162-164.

VAN DYK BN, DE BRUIN W, DU PLESSIS EM and KORSTEN L (2016). Microbiological food safety status of commercially produced tomatoes from production to marketing. Journal of Food Protection **79**, 392-406.

VAN HOEK AHAM, VEENMAN C, VAN Overbeek WM, LYNCH G, DE RODA HUSMAN AM AND BLAAK H (2015). Prevalence and characterization of ESBL- and AmpC-producing Enterobacteriaceae on retail vegetables. International Journal of Food Microbiology **204**, 1-8.

VASAIKAR S, OBI L, MOROBE I and BISI-JOHNSON M (2017). Molecular characteristics and antibiotic resistance profiles of *Klebsiella* isolates in Mthatha, Eastern Cape province, South Africa. International Journal of Microbiology **2017**, 8486742.

VERMA P, SAHARAN V V, NIMESH S and SINGH AP (2018). Phenotypic and virulence traits of *Escherichia coli* and Salmonella strains isolated from vegetables and fruits from India. Journal of Applied Microbiology **125**(1, 270-281.

VESEY D, CHAPMAN, P FORDE, B ROBERTS, L BERGH, H, JENNISON A, BEATSON S and HARRIS P (2018). Managing an outbreak of extended spectrum beta-lactamase (ESBL)-producing *Klebsiella oxytoca* in a special care nursery (SCN). Infection, Disease and Health, **23**, S6.

VITAL PG, ZARA ES, PARAOAN CEM, DIMASUPIL MAZ, ABELLO JJM, SANTOS ITG and RIVERA WL (2018). Antibiotic resistance and extended-spectrum beta-lactamase production of *Escherichia coli* Isolated from Irrigation Waters in Selected Urban Farms in Metro Manila, Philippines. Water **10**(5), 548.

WELDHAGEN GF (2004). Integrons and β-lactamases – A novel perspective on resistance. International Journal of Antimicrobial Agents **23**, 556-562.

WORLD HEALTH ORGANISATION (WHO) (1989). Health guidelines for use of wastewater in agriculture and aquaculture. Technical Report series no 778 Switzerland, Geneva. WHO Press.

WORLD HEALTH ORGANIZATION (2008). Microbiological hazards in fresh fruits and vegetables. Microbiological Risk assessment series (https://www.who.int/foodsafety/publications/micro/MRA_FruitVeges.pdf).

XU J and HE F (2019). Genomic analysis of two bacterial strains co-isolated from a urinary tract infection: NDM-1-producing *Enterobacter cloacae* accompanied by extended-spectrum β-lactamase-producing *Escherichia coli*. Journal of Global Antimicrobial Resistance **17**,198-200.

YE Q, WU Q, ZHANG S, ZHANG J, YANG G, WANG H and WANG J (2017). Antibiotic-resistant extended spectrum ß-Lactamase- and plasmid-mediated AmpC-Producing Enterobacteriaceae isolated from retail food products and the Pearl River in Guangzhou, China. Frontiers in Microbiology, **8**, 1-12.

ZEKAR FM, GRANIER SA, MARAULT M, YAICI L, GASSILLOUD B, MANCEAU C, TOUATI A and MILLEMANN Y (2017). From Farms to Markets: Gram-Negative bacteria resistant to third-generation cephalosporins in fruits and vegetables in a region of North Africa. Frontiers in Microbiology **8**, 1569.

ZHANG H, ZHOU Y, GUO S and CHANG W (2015). Multidrug resistance found in extended-spectrum 562 ß-lactamase-producing Enterobacteriaceae from rural water reservoirs in Guantao, China. Frontiers in Microbiology **6**, 267.

ZURFLUH K, NUESCH-INDERBINEN M, MORACH M, BERNER AZ, HACHLER H and STEPHAN R (2015). Extended-spectrum-β-lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. Applied Environmental Microbiology **81**, 3115-3120.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

WRC Report 1875/1-2/15 *Investigation into the link between water quality and microbiological safety of selected fruit and vegetables from farming to processing stages*" focused on analysing river- and dam water quality in the context of assessing any link with the fresh produce up to, at- and after-harvest, as well as at the point of sale. A clear link was established between contaminants isolated from the irrigation water and the associated fresh produce. The current solicited project K5/2706//4 focused on determining the microbial quality, safety and prevalence of multidrug resistant pathogenic bacteria in fresh, minimally processed and ready-to-eat (RTE) vegetable basket components from the farming, through processing (washing, cutting, packaging) to the retail level in both the formal and informal sector.

The most important findings of this study are summarised in this Chapter. Results of a scoping study of the microbiological quality, occurrence and characteristics of antimicrobial resistance in potential human pathogenic bacteria from fresh vegetables (lettuce, spinach, tomatoes, green beans and cucumbers) at the point-of-sale (formal and informal retailers) in the Tshwane Metropole was initially conducted**.** Microbial analysis demonstrated that the *E. coli* counts on the fresh vegetables (lettuce, spinach, tomatoes, green beans and cucumbers) ranged from 0 up to 5 log CFU/g (high counts mostly at informal vendors and fresh produce markets). The average coliform counts of fresh vegetables were above the acceptable limit of 2.3 log CFU/g as stipulated in the Department of Health (NDOH, 2000) guidelines which are currently under revision. One of the most significant findings was that the hygiene indicator bacteria counts were mostly not significantly different between formal and informal markets, with exceptions noted on occasion.

Fresh produce (lettuce, cabbage, spinach, tomatoes, green beans and green peppers) was sampled at five selected informal vendors in the Cape Town Metropole. The general hygiene counts for all sites were well above the advised coliform limits according to the previous Department of Health

guidelines. No *Salmonella* or *L. monocytogenes* was detected in any of the fresh produce, while E. *coli* was sporadically detected.

Hazard identification and characterisation was also performed on commercially produced leafy green vegetables from five selected supply chains in Gauteng Province and North West Province supplying retailers in Tshwane Metropole. Microbial analysis showed that the *E. coli* levels on fresh produce from the farm, through processing at a retail ranged from zero to counts above the acceptable limits according to the Department of Health (NDOH) guidelines for ready to eat fresh fruit and vegetables, currently under revision (*E. coli* 0 CFU/g) (NDOH, 2000). Similarly the coliform counts exceeded the maximum limit of 2.3 log CFU/g allowed in the previous national guidelines. In addition the Enterobacteriaceae counts of the leafy green vegetables were similar to the coliform counts and counts of up to 6 log CFU/g were obtained.

In addition, microbial analysis of broccoli coleslaw (broccoli stems, carrots and cabbage) and lettuce samples collected at different processing points at a packhouse in Philippi, Western Cape South Africa and from retailers were performed. Processing steps such as washing in a chlorine (150-200 ppm) and peeling did lower the microbial counts on the vegetables significantly. However, an increase in microbial counts to levels significantly higher than on the treated samples was observed in shredded samples and bagged mix coleslaw samples and were higher in samples from the retailer. The coliform and Enterobacteriaceae counts were similar to *E. coli* results obtained for the selected fresh produce in the Tshwane Metropole and *E. coli* was detected intermittently. No *Salmonella* or Shiga-toxin-producing *E. coli* (STEC) were detected. Similar to results of WRC project K5/2875//4 *E. coli*, *Salmonella* and *Listeria monocytogenes* were isolated from fresh produce at certain stages along the supply chain**,** *but not throughout the chain in both the Tshwane and Cape Town Metropoles.*

The Enterobacteriacea, *E. coli* and coliform counts of the African leafy green vegetables (chinensis, chamolia, rape) and spinach sampled from the small-scale farms and associated formal and informal vendors supplied were similar to results obtained for fresh produce from informal retailers and formal retail shops. However, *Listeria* spp. was detected in irrigation water and 4.93% of the fresh produce sampled were contaminated with *Salmonella* spp. which included

Salmonella IIIb 42 or II 42 subspecies, *Salmonella* Enteritidis and *Salmonella* II 42:z29 or *Salmonella* Djama, *Salmonella* Havana and *Salmonella* Typhimurium.

The *E. coli* levels in river water used on commercial fresh produce production farms did not often exceed the maximum limit of <1000 *E. coli*/ 100 ml limit for safe irrigation water (DWAF, 1996). *E. coli* numbers were generally lower in water from holding dams and the irrigation pivot points, with some exceptions noted due to bird droppings. *However, where river water contaminated with E. coli was directly applied via overhead irrigation, E. coli was enumerated from the irrigation pivot point, washwater and from the spinach samples throughout the supply chain from farm to retail as well as from contact surfaces.*

Phenotypic (antimicrobial) and genotypic (virulence gene prevalence, DNA fingerprinting) analysis of *E. coli* isolates clearly showed a link between the irrigation water source/s (river, dam, irrigation pivot point) and fresh produce isolates in the Tshwane and Cape Town Metropoles from selected informal vendors, commercial supply chains (from the farm, through processing and at the point-of-sale), small-scale farmers and at the point-of-sale.

Antibiotic resistance in irrigation water, fresh produce and the environment

Plants can absorb antibiotics from antibiotic contaminated water or manure amended soil, which contributes to the presence of antibiotic resistant microorganisms on fresh produce (Azanu *et al*., 2016). According to EFSA (2018), *E. coli* isolates displaying levels of >70% resistance to antibiotics tested are regarded as extremely high (EFSA, 2018). High antimicrobial resistance levels observed in clinically important ESBL/AmpC-producing Enterobacteriaceae included *E. coli, Klebsiella pneumoniae & Enterobacter* spp. High prevalence of ESBL & AmpC genetic determinants posed a concern, since there is a risk of transfer to commensal bacteria.

5.2 Recommendations for future research

The results from this study confirmed the need for scientific knowledge-based microbiological quality and safety guidelines for determining the microbiological quality and safety of the entire,

minimally processed and ready-to-eat (RTE) fresh produce from formal and informal supply chains, including small-scale farms.

Similar to the results from this study, many other studies have enumerated coliforms ranging from approximately 2 log CFU/g up to 6 log CFU/g from a variety of fresh produce samples globally. The national guideline for fresh produce, currently under revision, provides guidance values only for ready-to-eat stage of vegetables and fruit (NDOH, 2000). Internationally, no consensus exist regarding the microbiological standards that apply to ready-to-eat minimally processed vegetables (Health Protection Agency, 2009; [Food Safety Authority of Ireland (FSAI), 2016]; Fresh Produce Safety Centre Australia & New Zealand [FPSC A-NZ], 2019). A number of countries did not include coliform counts in the microbiological guidelines for RTE fresh produce and included Hong Kong [Centre for Food Safety (CFS), 2014], Australia [New South Wales (NSW) Food Authority, 2009] and Canada (Health Canada, 2002). The Canadian guidelines indicated that high coliforms are expected on leafy greens (Health Canada, 2002). Furthermore, the European microbiological criteria for Enterobacteriaceae on leafy greens states that levels higher than >4 log CFU/g were unsatisfactory [Health Protection Agency (HPA), 2009]. The fact that coliform and Enterobacteriaeae counts are naturally high and part of the biome should be considered during the revision of the national guidelines and align with other international guidelines. The guidelines for *E. coli* count limits for fresh vegetables also differ for each country including Hong Kong (20-100 CFU/g), United Kingdom (20-100 CFU/g) and Australia (3-100 CFU/g). A higher percentage of fresh produce analysed in this study would have been acceptable according to the international criteria which are more realistic.

In South Africa the Department of Water Affairs and Forestry (DWAF), stipulated the microbiological guidelines and other physical parameters for irrigation water (DWAF, 1996). In our previous WRC project report 1875/1-2/15 we concluded that revision of current microbiological guidelines for irrigation water and fresh produce based on scientific data (including actual natural microbial levels and pathogen presence/absence) is required. Results from the current project confirmed the need for the development of South African fit-for-purpose guidelines for irrigation water for both the formal and informal sector taking the unique challenges faced by the two sectors into consideration. However, the use of indicator organisms alone is not

enough to indicate the microbiological safety status in irrigation water and processing water. The presence of foodborne pathogens such as Shiga toxin-producing *E. coli* (i.e. *E. coli* O157:H7), *Salmonella* spp. and *Listeria monocytogenes* should also be monitored.

Surveillance of antimicrobial resistance (AR) in agroecosystems

In order to inform future risk analysis, develop and implement mitigation strategies/guidelines/policy changes scientific data on the prevalence and dissemination of antimicrobial resistance in the water-plant-food-human health nexus is needed. The threat of antimicrobial resistance in water and the subsequent irrigated crops is real and should be further investigated to determine the potential risk associated with the presence of antimicrobial resistant bacteria (ARBs) and antimicrobial resistance genes (ARGs).

Research focus areas/activities include:

- *It is important to include Plant Health as part of a holistic One Health* approach to develop and implement mitigation strategies.
- Establishment of a One Health Ethics Committee is needed to enhance the ethical clearance process.
- Establishing an integrated surveillance system should be established to create a knowledge base with regards to emerging challenges including multidrug resistant bacteria (MRB).
- Guidelines for irrigation water need to be expanded to include not only hygiene indicator microorganisms (coliforms, *E. coli*) that have traditionally been used, but other members of for example the Enterobacteriaceae family, i.e. *Salmonella* spp. and *E. coli* O157:H7 should also be considered.
- The occurrence and characterisation of chemical pollutants should also be investigated, since resistance to chemicals exists in the agricultural environments. In addition, chemical disinfectants need longer contact times, higher doses and have several disadvantages such as persistent use and use for aspects beyond its registered use, i.e. plant disease control.
- There is a national need to map out the potential contributors to the growing AMR problems, i.e. sewage plants, the mining sector, animal husbandry, etc.

- Establishing and maintaining a national central database of antimicrobial resistance surveillance data is imperative to characterize the potential risk with a view to develop and implement risk mitigation strategies.
- Testing methodologies to test resistance should be replicated across laboratories/ research groups in order for the results to be comparable.
- Investigate treatment options to ensure safe water and fresh produce. All disinfection treatments are influenced by the water quality, i.e. organic pollutants, etc.
- Risk assessment of results obtained to date should be done to determine the risk associated with potential human pathogenic bacteria isolated from the water-plant-food-public health interphase.
- Link isolates/info between different sectors/stakeholders. Results from antimicrobial resistance studies (ARBs, ARGs, whole genome sequencing of isolates, resistomes in water, fresh produce) should be linked with reference labs.
- Establishment of core training including food safety training, training on practices and improved Hygiene.
- Education is key to educate the public, farmers, retailers (formal and informal), municipalities, advisory boards and policy makers.
- Develop an action plan with the relevant role player, i.e. Veterinary Sciences, Plant Sciences, other academic institutions, NICD, DAFF, GDARD and NDOH.
- Food safety is an integral part of food security. The question was raised on how AMR issues can be addressed without compromising food security in the country.
- Community of practise and policy makers should function in a more integrated manner.
- *One issue we need to raise with policy makers, if we are planning to transform the economy of this country to a knowledge-based one, is that they should support research and development activities. The model of research-intensive support programmes in China and the USA could be excellent examples.*
- Scientists should focus on engaging with government with a view to influence policy. There is a definite change and a big drive to convert the information we have in the scientific field to influence policy.
- In order to make an impact on policy, working with government at all three levels, national, provincial and local and trying to push the agenda on improved governance.

- Summarise the existing information in a simplified way to communicate this with municipalities/government. The summary document can be used to engage with relevant stakeholders at municipality level to show the status of rivers and why people are getting sick with a view to developing and implementing mitigation strategies.
- Governmental departments, academia and policy makers should work together closely to develop and implement measures to mitigate the risk associated with AR.

5.3 References

AZANU D, MORTEY C, DARKO G, WEISSER JJ, STYRISHAVE B, and ABAIDOO RC (2016). Uptake of antibiotics from irrigation water by plants. Chemosphere **157**, 107e114.

CENTRE FOR FOOD SAFETY (CFS) IN HONG KONG (2014). Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items). Food and Environmental Hygiene Department (revised).

DEPARTMENT OF WATER AFFAIRS (DWAF) (1996). South African Water Quality Guidelines. Volume 4. Agricultural Use: Irrigation. Second Edition. Edited by S. Holmes, CSIR Environmental Services. Pretoria: Department of Water Affairs and Forestry.

DU PLESSIS M, ANNANDALE J, BENADE N, VAN DER LAAN M, JOOSTE S, DU PREEZ C, BARNARD J, RODDA N, DABROWSKI J, GENTHE B and NELL P (2017). Risk-Based, Site-Specific, Irrigation Water Quality Guidelines; Description of Decision Support System; WRC Report No TT 727/17; Water Research Commission: Pretoria, South Africa.

EFSA (European Food Safety Authority) (2018). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. EFSA Journal, **16**(2), 5182.

FOOD SAFETY AUTHORITY IRELAND (FSAI) (2016). Guidelines for the Interpretation of Results of Microbiological Testing of Ready-to-Eat Foods Placed on the Market (Revision 4) https://www.fsai.ie/WorkArea/DownloadAsset.aspx?id=16771.

FOOD SAFETY STANDARDS NEW ZEALAND (FSANZ) (2001). Guidelines for the microbiological examination of ready-to-eat foods. Retrieved from http://foodstandards.gov.au.

FRESH PRODUCE SAFETY CENTRE AUSTRALIA AND NEW ZEALAND (FPSC A-NZ) (2019). Guidelines for Fresh Produce Food Safety. https://fpsc-anz.com/wp-content/uploads/2019/06/Guidelines-for-Fresh-Produce-Food-Safety-2019_WEB.pdf

HEALTH PROTECTION AGENCY (HPA) (2009). Guidelines for Assessing the Microbiological Safety of Ready-to-Eat Foods Placed on the Market. London.

HEALTH CANADA (2002). Health products and food Branch methods for the microbiological analysis of foods: Determination of coliforms, faecal coliforms and *Escherichia coli* in foods (MFHPB-19). Health Canada. Ontario. https://www.canada.ca/en/health-canada/services/food-nutrition/research-programs-analytical-methods/analytical-methods/compendium-methods/methods-microbiological-analysis-foods-compendium-analytical-methods.html, Accessed 20-1-2021.

NATIONAL DEPARTMENT OF HEALTH (NDOH) (2000). Guidelines for environmental health officers on the interpretation of microbiological analysis data of food. South African Department of Health Directorate: Food Control. Available at: www.NDOH.co.za. Accessed July 10th, 2019

APPENDIX I

POST-GRADUATE TRAINING OF STUDENTS AND HUMAN CAPACITY BUILDING (THESES AND DISSERTATIONS AWARDED AND IN PROGRESS)

In total six female post graduate students of which two were white and four black were trained during the course of WRC project K5/2706/4 from 2017-2021 (Table 1). This included 4 MSc and two PhD students which were all South African citizens. Three Masters students have completed their studies, while the other three candidates will complete their studies during 2021.

Table 1: Students 2017 to 2021

	Student name and surname	Gender	Race	Degree	Progress	University	Country of origin	Student e-mail address
1	Muneiwa Ratshilingano	Female	Black	MSc Microbiology	In progress-Complete 2021	UP*	South Africa	tshidino86@yahoo.com
2	Loandi Richter	Female	White	MSc Plant Pathology	In progress-Complete 2021	UP*	South Africa	loandi.richter@yahoo.com
3	Degracious Kgoale	Female	Black	PhD Biotechnology	In progress-Complete 2021	UP*	South Africa	dm.kgoale@gmail.com
4	Tintswalo Baloyi	Female	Black	MSc Plant Pathology	**Completed 2021**	UP*	South Africa	u13131975@tuks.co.za
5	Anika Laubscher	Female	White	MSc Food Science	**Completed 2018**	US**	South Africa	17792819@sun.ac.za
6	Efaishe TA Kavela	Female	Black	MSc Food Science	**Completed 2019**	US**	South Africa	efaishe.kav@gmail.com

UP* University of Pretoria; US** Stellenbosch University

APPENDIX II

OVERVIEW OF KNOWLEDGE DISSEMINATION THROUGH CONFERENCE PAPERS PRESENTED, SCIENTIFIC AND POPULAR ARTICLES

Overview of knowledge dissemination

Publications in peer reviewed scientific journals for the duration of the project period from 2017/18, 2018/19, 2019/20 and 2020/21 were summarised in Table1. In total four papers have been published, one accepted and two are under review. In addition, one popular paper has been published. A total of 33 webinar, conference and workshop presentations were summarised in Table 2. Media engagement activities were summarised in Table 3.

Table 1 List of scientific publications

Publications in Scientific Journals				
	Authors and Titles	**Journal**	**Publication status**	**Attachment**
1	**Richter L, Du Plessis EM, Duvenage S and Korsten L (2021)** High prevalence of multidrug resistant *Escherichia coli* isolated from fresh vegetables sold by selected formal and informal traders in the most densely populated Province of South Africa.	Journal of Food Science **86(1)**, 161-168. https://DOI:10.1111/1750-3841.15534.	Published	
2	**Tintswalo Baloyi, Stacey Duvenage, Erika Du Plessis, Germán Villamizar-Rodríguez & Lise Korsten (2021)** Multidrug resistant *Escherichiacoli* from fresh produce sold by street vendors in South African informal settlements.	International Journal of Environmental Health Research https://doi.org/10.1080/09603123.2021.1896681	Published	
3	**Muneiwa T. Ratshilingano , Erika M. du Plessis, Stacey Duvenage and Lise Korsten** (2021) Multidrug resistance and molecular characterisation of generic and extended-spectrum β-lactamase-producing *Escherichia coli* isolated from selected commercially produced lettuce and spinach supply chains	Journal of Food Protection	Submitted March 2021	
4	**Richter L, Du Plessis EM, Duvenage S and Korsten L (2020)** Occurrence, Phenotypic and molecular Characterization of Extended-Spectrum- and AmpC- β-Lactamase-Producing Enterobacteriaceae Isolated from Selected Commercial Spinach Supply Chains in South Africa	Frontiers in Microbiology **11**, 638. https://doi.org/10.3389/fmicb.2020.00638.	Published	

Table 1 cont.

5	**Iwu CD, Du Plessis, EM, Korsten L and Okoh AI. (2020)** Prevalence and antibiogram imprints of *E. coli* O157:H7 and its Shiga toxigenic strains in irrigation water and agricultural soil: A potential threat to public health.	International Journal of Environmental Health research https://doi.org/10.1080/09603123.2021.1896681	Published	
6	**Iwu CD, Du Plessis, EM, Nontongana N, Korsten L and Okoh AI (2020)** Antibiogram signatures of some Enterobacteria recovered from irrigation water and agricultural soil in two District Municipalities of South Africa.	Microorganisms **8**(8), 1206. Published online doi: 10.3390/microorganisms8081206. (Journal impact factor 2019 (Incites): 4.167)	Published	
7	**Richter L, Du Plessis E M, Duvenage S and Korsten L (2019)** Occurrence, identification, and antimicrobial resistance profiles of Extended-Spectrum and AmpC β-Lactamase-producing Enterobacteriaceae from Fresh Vegetables Retailed in Gauteng Province, South Africa.	Foodborne Pathogens and Disease, **16(6)** 421-427. https://doi:10.1089/fpd.2018.2558.	Published	5
8	**Dlangalala M, du Plessis EM, Duvenage S and Korsten L (2020)** A review on antibiotic resistance, global mitigation strategies and detection of β-lactamase resistance genes.	South African Journal of Science (Journal impact Factor 2019 (Incites): 0.910). Submitted 28-9-2020.	Resubmit April 2021	
	Popular paper/s			
9	**Duvenage S, Du Plessis EM and Korsten L** Are concerns of informally traded fresh produce justified?	Food and Beverage Reporter October 2020	Published	

Table 2 List of presentations at webinars, conferences and workshops

	2020	
	Webinars	
1	Du Plessis EM, Duvenage S and Korsten L	WRC virtual symposium: World antimicrobial awareness week (WAAW) November 2020 Antimicrobial resistance in the water-plant-food public health nexus: A reason for concern?
2	Richer L, Du Plessis EM, Duvenage S and Korsten L	Multidrug resistant extended-spectrum β-lactamase-producing Enterobacteriaceae in spinach production from farm to retail.
3	Ratshilingano M, Du Plessis EM, Duvenage S and Korsten L	Multidrug resistance and molecular characteristics of generic and extended-spectrum β- lactamase-producing *Escherichia coli* sold from selected commercially produced lettuce and spinach supply chain.
4	Kgoale DM, Duvenage S, Du Plessis EM and Korsten L	Multidrug resistant *Salmonella enterica* identified from small-scale supply chains in North West, South Africa.
5	Du Plessis EM, Duvenage S and Korsten L	University of Nottingham Sutton Bonington AMR Webinar Series" 4 November 2020 Antimicrobial resistance in the water-plant-food public health nexus: A reason for concern? (webinar presentation).
6	Korsten L and Du Plessis EM	WRC Dialogue 24 April 2020 on "Susceptibility of food security and safety (urban and rural areas) due to coronavirus (COVID-19) (webinar presentation).

Table 2 cont.

	2019	
	IAFP European Symposium on Food Safety 24-26 April 2019 Nantes France	
7	**Korsten L**	Presentation "Clarity through Chaos: International Perspectives on Food Safety after Recent High-Profile Foodborne Outbreaks (presentation)
8	**Duvenage S**, Du Plessis EM, Kgoale DM, Ratshilingano TM, Baloyi T, Richter L and Korsten L	Formal and informal spinach safety from farm to fork: a South African case study (poster)
	International Association of Food Protection's Annual Meeting, Louisville, USA, 21-24 July 2019	
9	Kgoale DM, Duvenage S, **Du Plessis EM** and Korsten L	Microbial safety status of Rape produced and sold from small-scale farming in South Africa (Poster).
10	Dlangalala M, Villamizar-Rodríguez G, Du **Plessis EM** and Korsten L	Prevalence of Extended Spectrum β-lactamase-producing genes, a South African cucumber agroecosystem case study (Poster).
	Workshop 20 June 2019 @ UP	
11	Sigge G	Reducing microbial contamination in irrigation water: some perspectives and challenges in food safety.
11	Du Plessis EM, Duvenage S, Kgoale DM, Richter L, Dlangalala M, Ratshilingano M, Baloyi T and Korsten L	Contribution of fresh vegetables & the production environment to antimicrobial resistance: A reason for concern?
13	Duvenage S, Du Plessis EM, Kgoale DM, Ratshilingano M, Baloyi T, Richter L and Korsten L	Formal and informal spinach safety from farm to fork: a South African case study.
14	Kgoale DM	Microbial safety status of rape produced and sold from small-scale farming in South Africa
14	Richter L	Prevalence and characterisation of antimicrobial resistant Enterobacteriaceae in fresh vegetables from farm to retail
15	Ratshilingano M	Prevalence and dissemination of antimicrobial resistance in the water-soil-fresh produce interphase
16	Discussion session: workshop participants	Academia and representatives from DAFF/WRC/NDOH/GDARD. How do we inform risk analysis, develop and implementation mitigation strategies/ guidelines/policy changes based on scientific data of the prevalence and dissemination of AR in the environment
	2018	
17	Du Plessis EM	Microbiological quality and safety of our fresh produce: A reason for concern? AEC Amersham Listeriosis Workshop, Listeria – The Way Forward", 18 April 2018. AEC Amersham, 6 Indianapolis Street, Kyalami Business Park, Kyalami 16
18	Du Plessis EM	Multidrug resistant extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae in agroecosystems and fresh vegetable supply chains: an emerging health threat in South Africa? AEC Amersham Listeriosis Workshop, Listeria – The Way Forward", 19 April 2018. AEC Amersham facility in Cape Town, South Africa.
19	Richter L, Du Plessis EM, Duvenage S and Korsten, L	Mikrobiologiese kwaliteit en veiligheid van vars produkte: Rede tot kommer? Poster presentation at SAAWK Studente simposium, Pretoria, 26 October 2018.
	2nd International Conference for Food Safety and Security, Pretoria, 15-17 October 2018	
20	Du Plessis EM, Duvenage S, Villamizar-Rodriguez G and Korsten L	Multidrug resistant extended-spectrum and AmpC β-lactamase-producing Enterobacteriaceae in agroecosystems and fresh vegetable supply chains: an emerging health threat in South Africa? (oral presentation)
21	Baloyi T, Duvenage S , Du Plessis, EM and Korsten L	Microbiological quality and safety of fresh produce sold in informal street vending green grocers in Atteridgeville, South Africa (Best student oral prize)
22	Msimango T, Duvenage S, Du Plessis EM and Korsten L	Food safety assessment of fresh produce served and grown at schools in the Tshwane West District of the Gauteng Province, South Africa.

Table 2cont.

23	Richter L, Du Plessis EM, Duvenage S and Korsten L	Microbiological quality, safety and prevalence of antimicrobial resistance in isolates from commonly consumed fresh vegetables sold at two farmer's markets in Gauteng, South Africa.
24	Ratshilingano M, Du Plessis EM, Duvenage S and Korsten L	Microbiological quality and prevalence of antimicrobial resistance of isolates from minimally processed fresh vegetable from commercial farms to retail markets in Gauteng, South Africa (Best student poster prize received).
25	Kgoale DM, Duvenage S, Du Plessis EM and Korsten L	Microbial quality and prevalence of *Escherichia coli*, *Salmonella* spp. and ESBL-producing Enterobacteriaceae on *Brassica rapa* L. subsp. Chinensis from small-scale farming to retailers (Poster)
28	Duvenage S and Korsten, L	Bacterial biomes and the potential foodborne pathogens on spinach and tomatoes sold from street-trading green grocers, mobile trolleys and retailers in Gauteng, South Africa (Poster)
29	Duvenage S, Gcanga T, Du Plessis EM and Korsten L	Microbiological quality and safety of coleslaw and related fresh vegetables sold at retailers (Presentation)
		2017
		Food Safety Collaboration Workshop 25 April 2017, Fabi auditorium, Fabi @UP
30	Korsten L	Water-energy-food nexus to address Food Safety for Food Security? (Presentation)
31	Richter L	Prevalence and characterisation of antimicrobial resistant Enterobactericeae in fresh vegetables from farm to retail. University of Pretoria Prof. L Korsten, Dr EM Du Plessis and Dr S Duvenage (Presentation)
32	Ratshilingano M	Assessing microbial quality and tracking of antimicrobial resistance in the water-fresh produce interface. University of Pretoria Prof L Korsten, Dr E. Du Plessis and Dr S Duvenage (Presentation)
33	Kgoale DM	A comparative microbial analysis of fresh produce produced, prepared and sold formally and informally (Presentation).

Table 3 Summary of media engagement activities

Year	Media engagement
2019	Radio interview Alex FM Ms Manana Dlangalala. 30/09/2019
2019	Radio interview on Valley FM Ms Manana Dlangalala. 26/09/2019
2019	Nutritious but risky, published in Cape Argus. Dr S. Duvenage. 24/08 /2019
2019	Radio interview on Pretoria FM Dr S. Duvenage. 15/08/2019.
2019	Radio interview on Capricorn FM Dr S Duvenage 18/06/2019.
2018	TV Interview Business Report. SA hosts global conference on food safety, security. https://www.iol.co.za/business-report/economy/sa-hosts-global-conference-on-food-safety-security-17478920
2018	2nd International Conference for Food Safety and Security, radio interviews on SA FM, Radio Islam (http://www.peararchive2.co.za/SynopsisClip/2018-10-24/1549010.mp3?utm_source=MASTER+CoE-FS&utm_campaign=76b5a4c196-EMAIL_CAMPAIGN_2018_10_29_02_02&utm_ medium=email&utm_term=0_8830373c10-76b5a4c196-111816713).
2018	News Report one NCA (https://foodsecurity.us11.listmanage.com/track/click?u=51cd31d3e2aa1119f8c5d4faf&id=030b76ea7f&e=8a0df66168).
2017	Interview Radio FM - Korsten, L and E du Plessis. Characterising and tracking antimicrobial resistance in the water-plant-food-public health interface.

APPENDIX III

STUDENT THESES AND DISSERTATIONS

1. **Ms Anika Laubscher** completed her MSC in Food Science at Stellenbosch University
Supervisor: Prof G.O. Sigge and Co-supervisor: Dr C Lamprecht

Determination of the microbiological safety of selected fresh produce of informal retailers at point-of-sale

Abstract

The global consumption of fresh produce has increased as consumers have become more health conscious. With the rise of fresh produce consumption, fresh produce related foodborne outbreaks also increased globally. Recent outbreaks have included the *E. coli* O157:H7*, Listeria monocytogenes* and *Salmonella* spp. infections caused by contaminated fresh produce in 2018, 2016 and 2015, respectively. To minimise the risk for foodborne outbreaks in fresh produce it is important to know the current microbiological safety status of fresh produce in South Africa. Limited information is available about the microbiological safety of fresh produce sold at informal markets. Fresh produce is often consumed raw and therefore the microbiological risk is higher. A group of environmental bacteria, the Extended Spectrum β-Lactamases (ESBL)-producing Enterobacteriaceae, are also of concern because of their ability to counteract the effect of antibiotics and spread to the environment and fresh produce.

The aim of this study was to determine the microbiological safety of fresh produce sold at the informal market in the Cape Town Metropolitan area, South Africa, by enumerating hygiene indicator systems such as coliforms, *E. coli* and Enterobacteriaceae. Indicator systems, however, do not give an indication of the presence of specific pathogens. The presence of produce-related pathogens such as Salmonella, *Listeria monocytogenes* and Shiga Toxin-producing *E. coli* (STEC) were also investigated. Also included in this study was the detection of Extended Spectrum β-Lactamase (ESBL)-producing bacteria and their antibiotic resistance profiles.

Five informal vendors were selected to represent the informal market in the Cape Town metropolitan area. Each site was visited three times and at each site, two different products were selected for sampling (five replicates of each product). The fresh produce tested in this study included lettuce, cabbage, spinach, tomatoes, green beans and green peppers.

The general hygiene counts for all sites were well over the advised coliform limits according to the Department of Health. No *Salmonella* spp. or *Listeria monocytogenes* was detected in any of the fresh produce. The presence of *E. coli* occurred in sporadic cases indicating evidence of poor handling practices at the informal vendors. The prevalence of ESBL-producing Enterobacteriaceae was relatively low with 4% of the fresh produce sampled that tested positive for ESBL-producing Enterobacteriaceae. Multiplex polymerase chain reaction (PCR) was used to confirm the presence of the most prevalent ESBL genes in an isolate namely bla $_{TEM}$, bla $_{CTX-M}$ and bla $_{SHV}$. Out of the seven phenotypically confirmed ESBL-producing Enterobacteriaceae, five isolates were confirmed as containing at least one of the ESBL genes of interest. All ESBL-producing Enterobacteriaceae were multidrug resistant as well, being resistant to at least Ampicillin, Cloxacillin and/or Cefoxitin, Tetracycline, Ciprofloxacin and Trimethoprim-sulfamethoxazole. Taking all the evidence into consideration, it is clear that post-harvest handling of fresh produce can be improved. In this study, the presence of ESBL-producing Enterobacteriaceae on fresh produce has been confirmed in samples sold at informal markets in the Cape Town metropolitan area. It is therefore recommended that the prevalence of these organisms is further monitored in the future.

2. **Ms Efaishe Kavela** completed her MSC in Food Science at Stellenbosch University

Supervisor: Prof G.O. Sigge and Co-supervisor: Dr C Lamprecht

Microbial evaluation of selected produce pre- and post-packhouse and at the formal retail point-of-sale

Abstract

Fresh produce consumption is important to humans as it provides important nutrients and other compounds that promote good health. However, consumption of contaminated produce can be detrimental to human health. Outbreaks linked to fresh produce consumption have been reported globally, with Enterobacteriaceae members such as *Escherichia coli* and Salmonella being the most frequently implicated bacteria. Fresh produce isolates carrying the extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae has been reported. These organisms can resist the action of penicillin and the broad-spectrum cephalosporins, and they are also resistant to other antimicrobials. This is such a concern because fresh produce is eaten raw and these organisms are

not inactivated before consumption. To be able to control the spread of contaminations and antimicrobial resistance along the fresh produce production chain, it is essential to know the microbiological quality of fresh produce at different stages of production.

The aim of this study was to determine the changes in the microbiological quality of fresh produce pre- and post-packhouse processing and at the formal point-of-sale, in order to identify potential contamination points along the supply chain. Different fresh produce types: broccoli coleslaw (broccoli stems, carrots and cabbage) and lettuce samples were collected at different processing points in a packhouse situated in Philippi, Western Cape, South Africa. Some packhouse samples (mixed coleslaw bags and lettuce pre-packs) were also collected from retail outlets. All samples were tested for microbial indicators (Enterobacteriaceae, coliforms and *E. coli*), *Salmonella* and Shiga-toxin-producing *E. coli* (STEC). Produce samples were also screened for ESBL-producing Enterobacteriaceae.

The untreated/unprocessed samples had high microbial counts which were then reduced to significantly lower levels after peeling and washing in a chlorine (150-200 ppm) solution. An increase in microbial counts to levels significantly higher than on the treated samples was observed in shredded samples and bagged mix coleslaw samples. Mixed coleslaw bags sampled from the retailer two days after packaging also had significantly higher microbial levels than mixed coleslaw from the same batch sampled at the packhouse directly after packaging. Lettuce samples have indicated a gradual decrease on microbial levels throughout, and the lowest reduction was detected on pillow-packs samples. Throughout the study, no *Salmonella* or STEC were detected. Fifty isolates were identified as Enterobacteriaceae with MALDI-TOF, of which 22% were confirmed as ESBL producers according to the EUCAST method (2017b). All 50 Enterobacteriaceae were also subjected to genotypic confirmation, and seven of them were carrying the ESBL genes: $bla_{CTX\text{-}M}$ and bla_{TEM}. *Enterobacter cloacae* and *Klebsiella oxytoca* isolates were found carrying $bla_{CTX\text{-}M}$ and bla_{TEM}, and a single bla_{TEM} was found on an *E. coli* isolate. All 50 Enterobacteriaceae were also tested for resistance against ampicillin, gentamicin tetracycline, ciprofloxacin, and chloramphenicol. Five of the 50 tested isolates were found to be multidrug resistant. Fresh produce is eaten raw without thermal treatment to deactivate these organisms carrying ESBL genes. Through ingesting of this produce the ESBL genes could be transferred to the intestinal microorganisms and will confer resistance to important antimicrobials. This study investigated the microbiological quality of fresh produce sold in the Western Cape and

has also identified shredding and packaging as potential contamination points. Given favourable conditions, microorganisms may grow on stored fresh produce over time.

3. **Ms Garce Baloyi**, MSc in Plant Pathology, Department of Plant and Soil Sciences, University of Pretoria

Supervisor: Prof Lise Korsten; Co-supervisors: Dr Stacey Duvenage and Dr Erika du Plessis

Comparative microbiological safety study of fresh produce sold and consumed in informal markets, Gauteng

Abstract

Poor food safety handling practices, personal hygiene and environmental conditions as well as lack of adequate infrastructure and sanitation systems have been identified as important means for contact-contamination of fresh produce with foodborne pathogenic bacteria. During further unfavourable conditions such as temperature abuse, foodborne pathogens can proliferate on fresh produce causing disease outbreaks. Globally, people living in informal settlements often lack basic infrastructure (potable water and fridges) as well as support services such as basic sanitation and waste removal systems. Given the added burden of poverty and lack of supportive regulatory systems, these people are often exposed to unhygienic conditions to sell their food. The aim of this study was therefore to investigate the microbiological quality and safety of fresh produce purchased from street vendors and then stored in homes until consumption in informal settlements. A secondary aim was to assess the food safety knowledge, attitudes and practices among households in these informal settlements.

Microbiological analyses included the enumeration of quality indicators such as coliforms, *Enterobacteriaceae* and *Escherichia coli*, and detection of selected foodborne pathogens. The data from 735 samples showed coliform counts exceeded previously used criteria of "safe" food (2 log CFU/g) for ready-to-eat fresh produce. Bacterial contaminants detected included *E. coli* (n=99, 13.47%*), Salmonella* spp. (n=six, 0.82%), *Listeria monocytogenes* (n=one, 0.14%) and extended spectrum β-lactamase (ESBL) -producing *Enterobacteriaceae* (n=83, 11.29%). The later included *Enterobacter* spp., *Rahnella* spp., *Proteus* spp., *Serratia* spp., *Citrobacter* spp., *Hafnia alvei and Klebsiella pneumoniae.* The *E. coli* isolated from the study showed substantial antimicrobial

resistance with more than 80% of the isolates having a multiple antibiotic resistance index higher than 0.2.

Even though, consumers assessed in the study had knowledge about food safety of fresh produce, most experienced a lack of resources to implement these practices. The presence of bacteria such as *L. monocytogenes*, *Enterobacteriaceae, E. coli*, *Salmonella* spp. and high counts of quality indicators demonstrate possible faecal contamination of fresh produce. The high levels of contamination can be attributed to a lack of resources to maintain the microbiological quality and safety of fresh produce for people living in informal settlements. The high antimicrobial resistance observed in *E. coli* isolates presents a potential health risk for the community living under these unhygienic conditions. Further, people in these communities represent a higher proportion of people that has higher levels of compromised immune systems such as HIV/AIDS, pregnant women, the elderly and people who are taking immune suppressants. This study clearly showed that people need to be educated about proper hygiene practices and healthier environmental conditions which will reduce the risks associated with exposure to foodborne pathogens. Improved food safety knowledge, attitudes and practices will allow consumers to make better choices and change behaviour to create a more hygienic environment. This can contribute to improved food safety, health and well-being.

4. **Ms Muneiwa Ratshilingano,** MSc Biotechnology in the Plant and Soil Sciences Department, University of Pretoria

Supervisor: Prof Lise Korsten; Co-supervisors: Dr Erika Du Plessis; Dr Stacey Duvenage

Determining the microbiological safety of commercially produced lettuce and spinach

Abstract

The increased consumption of minimally processed fresh produce (spinach and lettuce) has been linked to increased foodborne outbreaks worldwide. Ready to eat (RTE) leafy vegetables in South Africa are produced in formal controlled supply chain with reasonably effective food safety assurance systems. The microbiological quality of leafy greens was investigated from commercial farms to retailers. A total of 136 samples were collected comprising of spinach (n=68), lettuce (n=68) from two commercial farms (in the field followed through to the packhouses and associated major retail outlets) in Gauteng Province and North west Province, South Africa. Total coliforms,

Escherichia coli, Enterobacteriaceae counts were determined for all the collected samples. Presumptive Extended Spectrum β-Lactamase-producing Enterobacteriaceae (ESBL), *Salmonella* spp. and *Listeria monocytogenes* were isolated following selective enrichment and plating on appropriate chromogenic media. Isolate identities were determined using MALDI-TOF analysis. Total coliform counts of lettuce samples ranged between log 2.26 and 4.61 CFU/g for supply chain scenario 4, while the counts ranged between 3, 28 log CFU/g and 4,66 log CFU/g in supply chain scenario 5. The total coliform counts of spinach samples ranged between 2,89log CFU/g and 4,64 log CFU/g in supply chain 4, while the counts ranged between 2,65 log CFU/g and 4,71 log CFU/g in supply chain 5. *Salmonella* spp. and *Listeria monocytogenes* were not isolated from any of the supply chain sampling points. S*erratia fonticola* isolates were prevalent in spinach samples from the field, packhouse and retail samples in the spinach and lettuce supply chain scenario 3. Presumptive ESBL/AmpC-producing Enterobacteriaceae included *E. coli*, *Citrobacter freundii*, *Klebsiella pneumoniae* and *Serratia fonticola.*

5. **Ms Laondi Richter** PhD in Biotechnology in the Plant and Soil Sciences Department, University of Pretoria

Supervisor: Prof Lise Korsten; Co-supervisors: Dr Erika Du Plessis; Dr Stacey Duvenage

Prevalence and characterisation of antimicrobial resistant Enterobacteriaceae in fresh vegetables from farm to retail

Abstract

Contaminated fresh produce has increasingly been implicated in foodborne disease outbreaks. The associated irrigation water has been recognised as an important source of contamination of potential pathogenic antimicrobial resistant bacteria in fresh produce production. Moreover, the increasing occurrence of multidrug-resistant extended-spectrum β-lactamase- (ESBL) and/or AmpC β-lactamase-producing Enterobacteriaceae represent risks related to environmental integrity and food safety. However, surveillance in South African markets and fresh produce production systems are limited.

This study aimed to determine the microbiological quality, prevalence of foodborne pathogens (*Escherichia coli*, *Salmonella* spp., and *Listeria monocytogenes*) and prevalence of multidrug resistant ESBL/AmpC-producing Enterobacteriaceae, including phenotypic and

genotypic characterisation in fresh produce at the point of sale from formal retailers and informal street traders, trolley vendors and farmers' markets, as well as two commercial spinach production systems on farm, through processing and up to retail, in Gauteng, the most densely populated province of South Africa. Further, to genotypically characterise selected ESBL/AmpC- producing Enterobacteriaceae isolates with whole genome sequence analysis.

A total of 833 samples were analysed. This included 545 vegetable samples (spinach, tomatoes, lettuce, cucumber and green beans) purchased from formal and informal retailers in Gauteng. Furthermore, 288 samples were collected from two commercial spinach production scenarios with different irrigation water (river and borehole) sources. This included spinach samples at harvest, during processing and from the associated retailers, irrigation water at the source, storage dams, irrigation pivot point in the field and water used during processing, as well as soil at harvest and contact surfaces including crates, floors and cutting surfaces throughout the respective production systems.

Coliforms, *E. coli* and Enterobacteriaceae enumerated from fresh produce at the point of sale were mostly not significantly different between formal and informal markets, with exceptions noted on occasion. In the spinach production systems, where river water was directly used as overhead irrigation, *E. coli* was enumerated from spinach at harvest, during processing as well as from the ready-to-eat retail samples. Following selective enrichment and plating onto chromogenic media, potential pathogens were identified using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) analysis. In total, 17,5% (n=146) of the samples harboured *E. coli*, which included 81 samples from the point of sale and 65 samples from the spinach production systems. Except for one *stx2* positive *E. coli* isolate from river irrigation water, no virulence genes (*lt*, *st*, *bfpA*, *eagg*, *eaeA*, *stx1*, *stx2*, *ipaH*) were detected in any of the *E. coli* isolates (n=147) following PCR and sequencing. S*almonella* spp. isolates (n=11) were only recovered from river water samples, whilst no *Listeria* spp. were isolated from any of the samples. Cluster analysis of enterobacterial repetitive intergenic consensus PCR DNA fingerprints from the *E. coli* isolated from the commercial supply chains showed in each production system, *E. coli* isolated from spinach that clustered together with *E. coli* from irrigation water at a 70% cut-off value.

Phenotypic antimicrobial resistance profiles (Kirby-Bauer disk diffusion) revealed multidrug resistance in 38,8% of the generic *E. coli* isolates from the entire study. Overall, 16,4%

(137/833) of the samples were found to be contaminated with ESBL/AmpC-producing Enterobacteriaceae which included 95/544 vegetable samples at the point of sale and 42/288 samples throughout spinach production. Dominant species included *E. coli*, *Enterobacter cloacae*, *Enterobacter asburiae* and *K. pneumoniae* from vegetables at the point of sale and *Serratia fonticola*, *E. coli* and *K. pneumoniae* from the spinach supply chains. In total, 96.8% (121/125) of the ESBL/AmpC-producing Enterobacteriaceae isolates were multidrug resistant. Genotypic characterisation (PCR of ESBL/AmpC resistance genes) revealed domination of the CTX-M group 9 ESBL type in isolates from vegetables at the point of sale, whilst the CTX-M group 1 ESBL type were the most prevalent in the spinach supply chains. Selected ESBL/AmpC-producing *E. coli*, *K. pneumoniae*, *Salmonella* spp., and *S. fonticola* isolates (n=19) from the spinach supply chains were subjected to whole genome sequencing. The presence of integron In191 were revealed in six strains, with the plasmid-borne trimethoprim resistance gene, *dfrA14*, in the cassette array. In three *K. pneumoniae* strains, $bla_{\text{CTX-M-15}}$ was related to IncFII_pKP91 and/or IncFIB(K)_1_Kpn3 plasmids. Other resistance genes including $bla_{\text{CTX-M-14}}$, *sul2*, and *qnrB1* related to insertion sequences were also identified in *E. coli*, *K. pneumoniae* and *S. fonticola* strains. All multidrug resistant ESBL/AmpC-producing isolates showed relevant similarity to human pathogens with the probability of being a human pathogen ranging between (0,852-0,931) for *E. coli*, (0,796-0,899) for *K. pneumoniae*, (0,635-0,721) for *S. fonticola* and the *Salmonella enterica* strain had a probability of 0,939 for being a human pathogen.

Main findings from this study include the highlighted need for continued surveillance of multidrug resistant foodborne pathogens in fresh produce retailed formally and informally. Furthermore, the necessity of using clean and safe irrigation water with the need for standardised microbiological safety parameters for irrigation water of ready-to-eat fresh vegetables, as a link between *E. coli* from irrigation water and spinach at different points of the respective production systems were shown. For the first time, the presence of multidrug resistant ESBL/AmpC-producing Enterobacteriaceae in raw vegetables sold at selected retailers in Gauteng Province, SA were also reported, highlighting potential consumer health risks upon consumption of raw, unwashed fresh produce. The results further add to the global knowledge base regarding the prevalence and characteristics of ESBL/AmpC-producing Enterobacteriaceae in fresh vegetables and the agricultural environment, required for future risk analysis, and emphasises the need for strategies to minimise the environmental spread of multidrug resistant strains.

6. **Ms Degracious Kgoale,** PhD Biotechnology in the Plant and Soil Sciences Department, University of Pretoria

Supervisor: Prof Lise Korsten; Co-supervisors: Dr Stacey Duvenage and Dr Erika du Plessis

Bacterial profiles and foodborne pathogens in leafy greens from informal supply chains

Abstract

This study aims to evaluate the microbial quality and prevalence of foodborne pathogens on morogo cultivated from small-scale farms as the informal supply chains in Gauteng, Mpumalanga and the North-West Provinces of South Africa. We also aim to evaluate the food safety status of morogo sold in the formal and informal markets, by following the morogo from production through to the point of sale. The study investigated eight six small-scale farms and their associated formal and informal retailers supplied, visited once in two production seasons. Irrigation water, soil and cultivated morogo (farm and point of sales samples) were investigated in order to determine the main source of contamination. This information will be used to inform mitigation and risk assessment studies to improve food safety. Overall, fifteen sampling trips have been completed and 454 samples have been evaluated. All cultures generated from this project form part of the CoE-FS Virtual Microbial Database and characterization of these isolates will contribute towards a better knowledge of pathogen prevalence and persistence. This information will further be used to determine the South African genomic signal, antimicrobial resistance gene presence and virulence of foodborne pathogens as part of ongoing isolate characterisation.

SUPPLEMENTARY INFORMATION

Questionnaire A

DEPARTMENT OF PLANT AND SOIL SCIENCE

SOUTH AFRICAN CONSUMERS' FOOD SAFETY KNOWLEDGE, ATTITUDES AND PRACTICES, FRESH VEGETABLES CONSUMED IN TSHWANE AND CAPE TOWN METROPOLES

Please follow the instructions for each question very carefully. Your responses will be treated confidentially and you will remain anonymous as your identity can not be retrieved or disclosed in any way.

Section A *What do you know about Food Safety?* ***Please indicate your agreement with the following statements and mark only the most relevant answer with an X in the relevant column.***	**Respondent number:**				**Office use**	

1. Please answer the following statements by indicating True, False or Uncertain.	**True**	**False**	**Uncertain**		
1.1. Foodborne illnesses are diseases that are passed on to people by harmful bacteria that are present in contaminated food.	1	2	3	Q1.1	
1.2. Cross contamination is the passing of harmful substances or bacteria to food from food or from dirty equipment, utensils or hands.	1	2	3	Q1.2	
1.3. The temperature danger zone at which harmful bacteria will flourish is 0-15˚C.	1	2	3	Q1.3	
1.4. Cross contamination is the main cause of food poisoning.	1	2	3	Q1.4	
1.5. Harmful bacteria multiply quickly at room temperature.	1	2	3	Q1.5	
1.6. Cutting boards and cooking utensils should be washed thoroughly with hot soapy water.	1	2	3	V1.6	
1.7. *Salmonella* is a harmful bacteria which causes food poisoning.	1	2	3	Q1.7	
1.8. Nausea and vomiting are common symptoms when you have food poisoning.	1	2	3	V1.8	

2. In your opinion, how knowledgeable are you about food safety? *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Strongly disagree" and ''5" would indicate "Strongly agree".*	**Strongly disagree**	**Disagree**	**Neutral**	**Agree**	**Strongly agree**		
2.1. Compared to other people, I know more about food safety.	1	2	3	4	5	Q2.1	
2.2. People who know me consider me to be a food safety expert.	1	2	3	4	5	Q2.2	
2.3. Compared to other people, I know more about how to assess the safety of food.	1	2	3	4	5	Q2.3	
2.4. I am knowledgeable about the causes of food poisoning.	1	2	3	4	5	Q2.4	
2.5. I am knowledgeable about how to store perishable foods (spoil easily) correctly to prevent food poisoning.	1	2	3	4	5	Q2.5	
2.6. I am knowledgeable about how to clean work surfaces correctly to prevent bacterial growth.	1	2	3	4	5	Q2.6	

Section B *What are your thoughts about Food Safety?* ***Answer <u>every question</u> and mark only the most relevant answer with an X in the relevant column.***						**Office use**	
3. How do you feel about the following: *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Strongly disagree" and ''5" would indicate "Strongly agree".*	**STRONGLY DISAGREE**	**DISAGREE**	**NEUTRAL**	**AGREE**	**STRONGLY AGREE**		
3.1. Good personal hygiene of those that work with food can prevent foodborne illness.	1	2	3	4	5	Q3.1	
3.2. It is the responsibility of all food handlers to ensure that food is safe to eat.	1	2	3	4	5	Q3.2	
3.3. I am willing to change my food handling behaviours when I know they are incorrect.	1	2	3	4	5	Q3.3	
3.4. I am willing to learn more about food safety.	1	2	3	4	5	Q3.4	
3.5. It is more important to have tasty food than safe food.	1	2	3	4	5	Q3.5	
3.6. I select a place to buy food from based on its reputation for cleanliness.	1	2	3	4	5	Q3.6	
3.7. Washing my hands before touching food reduces the risk of spreading harmful bacteria.	1	2	3	4	5	Q3.7	
3.8. I can reduce the risk of foodborne illness by thoroughly washing areas where foods are prepared beforehand.	1	2	3	4	5	Q3.8	
3.9. Food products purchased from a trustworthy retailer are always safe.	1	2	3	4	5	Q3.9	
3.10. Branded food products are always safe.	1	2	3	4	5	Q3.10	
3.11. Ready-made foods purchased from retailers are prepared in a clean environment.	1	2	3	4	5	Q3.11	
3.12. Ready-made foods purchased from retailers cannot make me sick.	1	2	3	4	5	Q3.12	
3.13. The ''best before date'' is an indication of whether food is safe to eat or not.	1	2	3	4	5	Q3.13	
3.14. The quality of food products in general keeps improving in South Africa.	1	2	3	4	5	Q3.14	
3.15. In recent years, my confidence in fresh produce food safety has increased.	1	2	3	4	5	Q3.15	
3.16. I am concerned about the safety of food products in general.	1	2	3	4	5	Q3.16	
Food products in general have never been as safe as they are nowadays.	1	2	3	4	5	Q3.17	
4. How confident are you about the safety of food originating from the following sources? *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Not confident at all" and ''5" would indicate "Highly confident".*	**NOT CONFIDENT AT ALL**	**NOT VERY CONFIDENT**	**UNCERTAIN**	**FAIRLY CONFIDENT**	**HIGHLY CONFIDENT**		
4.1. Fast food outlets	1	2	3	4	5	Q4.1	
4.2. Restaurants	1	2	3	4	5	Q4.2	

4.3. Farm stalls	1	2	3	4	5	Q4.3	
4.4. Imported	1	2	3	4	5	Q4.4	
4.5. Retailers	1	2	3	4	5	Q4.5	
4.6. Food markets	1	2	3	4	5	Q4.6	
4.7. Street food vendors	1	2	3	4	5	Q4.7	
5. How concerned are you about possible food contamination originating from: *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Not concerned at all" and ''5" would indicate "Highly concerned".*	**NOT CONCERNED AT ALL**	**NOT VERY CONCERNED**	**UNCERTAIN**	**FAIRLY CONCERNED**	**HIGHLY CONCERNED**		
5.1. Insect and rodent droppings	1	2	3	4	5	Q5.1	
5.2. Pesticides	1	2	3	4	5	Q5.2	
5.3. Mercury	1	2	3	4	5	Q5.3	
5.4. Lead	1	2	3	4	5	Q5.4	
5.5. Irradiation	1	2	3	4	5	Q5.5	
5.6. Bacteria	1	2	3	4	5	Q5.6	
5.7. Human excretion	1	2	3	4	5	Q5.7	
5.8. Dirty dishcloths	1	2	3	4	5	Q5.8	
6. How confident are you about the safety of fresh produce in general purchased from the following retailers? *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Not confident at all" and ''5" would indicate "Highly confident".*	**NOT CONFIDENT AT ALL**	**NOT VERY CONFIDENT**	**UNCERTAIN**	**FAIRLY CONFIDENT**	**HIGHLY CONFIDENT**	Q6	
6.1. Checkers	1	2	3	4	5	Q6.1	
6.2. Food Lovers Market	1	2	3	4	5	Q6.2	
6.3. Pick n Pay	1	2	3	4	5	Q6.3	
6.4. Shoprite	1	2	3	4	5	Q6.4	
6.5. Spar	1	2	3	4	5	Q6.5	
6.6. Woolworths	1	2	3	4	5	Q6.6	
7. How confident are you about the safety of the following food items? *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Not confident at all" and ''5" would indicate "Highly confident".*	**NOT CONFIDENT AT ALL**	**NOT VERY CONFIDENT**	**UNCERTAIN**	**FAIRLY CONFIDENT**	**HIGHLY CONFIDENT**		
7.1. Fresh Whole Vegetables	1	2	3	4	5	Q7.1	
7.2 Washed prepackaged vegetables						Q7.2	
7.3 Ready-prepared mixed salads, i.e. Greek salad, Coleslaw						Q7.3	

Section C *What do you do regarding food safety?* ***Please answer <u>every question</u> and mark only the most relevant answer with an X in the relevant column.***						**Office use**	
8. How often do you perform the following food handling practices: *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Never" and ''5" would indicate "Always".*	**NEVER**	**RARELY**	**SOMETIMES**	**FREQUENTLY**	**ALWAYS**		
AT HOME:							
8.1. Store perishable foods bought from the retailer immediately in the refrigerator.	1	2	3	4	5	Q8.1	
8.2. Use perishable foods after the expiration date.	1	2	3	4	5	Q8.2	
8.3. Use non-perishable foods after the expiration date.	1	2	3	4	5	Q8.3	
8.5. Keep fruits and vegetables separate from raw meat in the refrigerator.	1	2	3	4	5	Q8.5	
8.7. Check the temperature of refrigerators/freezers regularly to prevent food from going off.	1	2	3	4	5	Q8.7	
8.8. Wash fresh fruits and vegetables even if the skin is not eaten.	1	2	3	4	5	Q8.8	
8.9. Wash fresh fruits and vegetables before cooking them.						Q8.9	
8.10. Wash fresh fruits and vegetables before eating them.						Q8.10	
8.11. Wash hands with soap and water after handling raw foods.	1	2	3	4	5	Q8.11	
8.12. Clean food preparation areas in the kitchen with hot soapy water.	1	2	3	4	5	Q8.12	
8.13. Wash cutting boards/utensils before preparing raw foods.	1	2	3	4	5	Q8.13	
8.14. Use the same work surface for raw and cooked meat.	1	2	3	4	5	Q8.14	
8.15. Consult the label for storage conditions of food items.	1	2	3	4	5	Q8.15	
8.16. Store raw food items separate from cooked food items.	1	2	3	4	5	Q8.16	
AT THE RETAILER:	1	2	3	4	5		
8.17. Examine food packaging to check for damages before purchasing.	1	2	3	4	5	Q8.17	
8.18. Consult the expiry date of food products to decide if it is safe to purchase.	1	2	3	4	5	Q8.18	
8.19. Purchase only branded food products thinking they are the safest.	1	2	3	4	5	Q8.19	
8.20. Leave perishable foods in the car for more than an hour after purchasing it at the retailer.	1	2	3	4	5	Q8.20	
8.21. Choose a clean trolley/basket for shopping.	1	2	3	4	5	Q8.21	
8.22. Place fresh vegetables you won't peel or cook before eating directly into the trolley/basket.	1	2	3	4	5	Q8.22	
8.23. Re-use the same plastic shopping bags for food purchases.	1	2	3	4	5	Q8.23	
8.24. Use insulated cooler bags to carry perishable foods home in from the store.	1	2	3	4	5	Q8.24	
8.25. Shop for non-perishable foods first and leave the perishable foods for last.	1	2	3	4	5	Q8.25	

9. Who is your retailer of choice for safe food purchases?	_____ (Drop down options)		
	9.1. Checkers	Q9.1	
	9.2. Food Lovers Market	Q9.2	
	9.3. Pick n Pay	Q9.3	
	9.4. Shoprite	Q9.4	
	9.5. Spar	Q9.5	
	9.6. Woolworths	Q9.6	

10. In your opinion, who is responsible for making sure that food is safe to eat? *Please answer the following statemens using a rating of 1 to 5, where ''1" would indicate "Definitely" and ''5" would indicate "Definitely not".*	**DEFINITELY**	**TO SOME EXTENT**	**UNCERTAIN**	**TO LESSOR EXTENT**	**DEFINITELY NOT**		
10.1. Government	1	2	3	4	5	Q10.1	
10.2. Farmers	1	2	3	4	5	Q10.2	
10.3. Manufacturers/producers	1	2	3	4	5	Q10.3	
10.4. Retailers (e.g. Checkers, Food Lovers Market, Pick n Pay, Shoprite, Spar, Woolworths)	1	2	3	4	5	Q10.4	
10.5. Department of Health	1	2	3	4	5	Q10.5	
10.6. Food Safety inspectors	1	2	3	4	5	Q10.6	
10.7. Myself	1	2	3	4	5	Q10.7	

Section D **Vegetables and Fruit Types Consumed** ***Please answer <u>every question</u> and mark only the most relevant answer with an X in the relevant column.***						**Office use**	
11. Which vegetables and fruit from the list below do you typically buy; do you eat it raw, cooked or both? ***Please answer <u>every question</u> and mark only the most relevant answer with an X in the relevant column.***	**Eat**	**Don't eat**	**raw**	**cooked**	**Both raw & cooked**		
11.1 Cabbage	1	2	3	4	5	Q11.1	
11.2 Carrot	1	2	3	4	5	Q11.2	
11.3 Green beans	1	2	3	4	5	Q11.3	
11.4 Spinach	1	2	3	4	5	Q11.4	
11.5 Morogo/African leafy vegetables	1	2	3	4	5	Q11.5	
11.6 Lettuce	1	2	3	4	5	Q11.6	
11.7 Kale	1	2	3	4	5	Q11.7	
11.8Cucumber	1	2	3	4	5	Q11.8	
11.9 Sweet corn	1	2	3	4	5	Q11.9	
11.10 Peas	1	2	3	4	5	Q11.10	
11.11 Onions	1	2	3	4	5	Q11.11	
11.12 Butternut	1	2	3	4	5	Q11.12	
11.13 Beetroot	1	2	3	4	5	Q11.13	
11.14 Pumpkin	1	2	3	4	5	Q11.14	

11.15 Sweet potato	1	2	3	4	5	Q11.15	
11.16 Potatoes	1	2	3	4	5	Q11.16	
11.17 Cauliflower	1	2	3	4	5	Q11.17	
11.18 Broccoli	1	2	3	4	5	Q11.18	
11.19 Green pepper	1	2	3	4	5	Q11.19	
11.20 Tomatoes	1	2	3	4	5	Q11.20	

Section E *PLEASE TELL US MORE ABOUT YOURSELF* ***Answer every question and mark only the most relevant answer with an X.***									**Office use**	
12. What is your gender?	1	Male	2	Female					Q12	
13. What is your age at your last birthday?	☐☐ Years								Q13	
14. What is your highest level of education?	1	Lower than Grade 12	2	Grade 12	3	Degree/Diploma	4	Post-graduate Qualification	Q14	
15. What is the name of the area where you live in South Africa?	_____								Q15	
16. Who mainly purchases groceries in your household?	_____								Q16	
17. Approximately how much money do you spend on food <u>per month</u>?	R_____								Q17	

Thank you for your participation and valuable contribution to this research.
Your information will remain anonymous throughout the research process.

Questionnaire B

DEPARTMENT OF PLANT AND SOIL SCIENCES

SOUTH AFRICAN CONSUMERS BUYING FRESH PRODUCE FROM INFORMAL TRADING GREEN GROCERS/STREET VENDORS:
FOOD SAFETY KNOWLEDGE, ATTITUDES AND PRACTICES, VEGETABLES CONSUMED

Please follow the instructions for each question very carefully. Your responses will be treated confidentially and you will remain anonymous as your identity can not be retrieved or disclosed in any way.

Section A: Personal Information										**Respondent number:**	
1. What is your gender	1	Male	2	Female						Q1	
2. What is your age at your last birthday?									Years	Q2	
3. What is your highest level of education?	1	Grade 7	2	Lower than grade12	3	Grade 12	4	Degree/ diploma	5 Post-graduate qualification	Q3	
4. Which country do you come from, if SA specify province.										Q4	
5. Where do you reside? (Please name city/town or township)										Q5	
6. What is your business monthly income?	R									Q6	
7. Do you have another source of income? If yes, please specify										Q7	
8. Who mainly handle/s the fresh produce at the market stall? a. Only you b. Someone else, please specify										Q8	

Section B: Fresh produce sourcing and handling			
Please answer the following questions by selecting one of the listed options.	Answer	Office Use	
1. What do you typically do before handling fresh produce? 1. I don't do anything before handling fresh produce. 2. I wash my hands with water to remove dirt. 3. I wash my hands with water and soap to remove dirt. 4. I wash my hands with water and soap to prevent the spread of bacteria.		Q9	
2. Where do you mostly source the fresh produce (spinach, cabbage, tomatoes, carrots, etc.) that you sell? 1. Fresh Produce Market (Tshwane/Johannesburg) 2. Pick n Pay 3. Spar 4. Shoprite 5. Checkers 6. Farms 7. Other		Q10	
10.1 If other, please specify		Q10.1	
1. What is most important when you decide where to buy fresh produce? 1. Price 2. Quality 3. Convenience 4. Cleanliness 5. Distance 6. Other		Q11	

11.1 If other, please specify		Q11.1	

Section C Fresh Vegetables Consumed							
12. Which vegetables and fruit from the list below do you typically buy? If yes circle 1, if no circle 2, Do you eat it raw, cooked or both? If eaten raw circle 3, cooked circle 4, raw and circle 5	Eat	Don't eat	Raw	Cooked	Raw &Cooked		
12.1 Cabbage	1	2	3	4	5	Q12.1	
12.2 Carrot	1	2	3	4	5	Q12.2	
12.3 Green beans	1	2	3	4	5	Q12.3	
12.4 Spinach	1	2	3	4	5	Q12.4	
12.5 Morogo/African leafy vegetables	1	2	3	4	5	Q12.5	
12.6 Lettuce	1	2	3	4	5	Q12.6	
12.7 Kale	1	2	3	4	5	Q12.7	
12.8 Cucumber	1	2	3	4	5	Q12.8	
12.9 Sweet corn	1	2	3	4	5	Q12.9	
12.10 Peas	1	2	3	4	5	Q12.10	
12.11 Onions	1	2	3	4	5	Q12.11	
12.12 Butternut	1	2	3	4	5	Q12.12	
12.13 Beetroot	1	2	3	4	5	Q12.13	
12.14 Pumpkin	1	2	3	4	5	Q12.14	
12.15 Sweet potato	1	2	3	4	5	Q12.15	
12.16 Potatoes	1	2	3	4	5	Q12.16	
12.17 Cauliflower	1	2	3	4	5	Q12.17	
12.18 Broccoli	1	2	3	4	5	Q12.18	
12.19 Green pepper	1	2	3	4	5	Q12.19	
12.20 Tomato	1	2	3	4	5	Q12.20	

Section D: What do you know about Food Safety?					
Please answer the following statements by indicating True, False or Uncertain	**True**	**False**	**Uncertain**		
13. Fresh produce can be contaminated with bacteria/germs that cause disease.		2	3	Q13	
3. Cutting boards and knives should be washed thoroughly with hot water.	1	2	3	Q13	
4. Vomiting, diarrhoea are symptoms of food poisoning.	1	2	3	Q13	

How do you feel about the following: *Please answer the following statement using a rating of 1 to 5, where 1 would indicate strongly disagree and 5 would indicate strongly agree*	Strongly disagree	Disagree	Neutral	Agree	Strongly agree		

14. .Good personal hygiene of those that work with food can prevent foodborne illness.	1	2	3	4	5	Q14	
15. It is the responsibility of all food handlers to ensure that fresh is fresh and clean.	1	2	3	4	5	Q15	
16. Quality is more important than price.	1	2	3	4	5	Q16	
17. Washing my hands before touching food is necessary	1	2	3	4	5	Q17	
18. I think it is unsafe to leave fresh produce outside the fridge overnight.	1	2	3	4	5	Q18	
19. Do you think fresh produce sold at retailers are better quality/safer than at green grocers or markets.	1	2	3	4	5	Q19	

Section E: OBSERVATION/S			
1. GPS coordinates		Q20	

Thank you for your participation and valuable contribution to this research. Your information will remain anonymous throughout the research

Questionnaire C

SOUTH AFRICAN STREET TRADING GREEN GROCERS AND FARMERS MARKET/S FOOD SAFETY KNOWLEDGE, ATTITUDES AND PRACTICES, VEGETABLES SOLD BY INTERVIEWED QUESTIONNAIRE

Section A: Personal Information										**Respondent number:**	
1. What is your gender	1	Male	2	Female						Q1	
2. What is your age at your last birthday?									Years	Q2	
3. What is your highest level of education?	1	Grade 7	2	Lower than grade12	3	Grade 12	4	Degree/ diploma	5 Post-graduate qualification	Q3	
4. Which country do you come from, if SA specify province.										Q4	
5. Where do you reside? (Please name city/town or township)										Q5	
6. What is your business monthly income?	R									Q6	
7. Do you have another source of income? If yes, please specify										Q7	
8. Who mainly handle/s the fresh produce at the market stall? a. Only you b. Someone else, please specify										Q8	

Section B: Fresh produce sourcing and handling				
Please answer the following questions by selecting one of the listed options.	Answer		Office Use	
9. What do you typically do before handling fresh produce? 1. I don't do anything before handling fresh produce. 2. I wash my hands with water to remove dirt. 3. I wash my hands with water and soap to remove dirt. 4. I wash my hands with water and soap to prevent the spread of bacteria.			Q9	
10. Where do you mostly source the fresh produce (spinach, cabbage, tomatoes, carrots, etc.) that you sell? 1. Fresh Produce Market (Tshwane/Johannesburg) 2. Pick n Pay 3. Spar 4. Shoprite 5. Checkers 6. Farm 7. Other			Q10	
10.1 If other, please specify			Q10.1	
11. What is most important when you decide where to buy fresh produce? 1. Price 2. Quality 3. Convenience, 4. Cleanliness 5. Distance 6. Other			Q11	
11.1 If other, please specify			Q11.1	

12. How do you transport fresh produce to the market stall? 1. In a vehicle? 2. In a taxi? 3. Other			Q 12	
12.1 If other, please specify			Q12.1	
13.Do you wash your fresh produce 1. Yes 2. No			Q13	
14. If you wash your fresh produce, what is the washwater source? 1. Tap water 2. Borehole 3. Other			Q14	
14.1If other, please specify.			Q14.1	
15.What do you wash it in 1. Bucket 2. Washbasin @ home 3. Other			Q15	
15.1 If other, please specify.			Q15.1	
16. For how many days to you buy fresh produce to sell? 1. One day 2. Two days 3. Three days 4. Four days 5. Five days 6. Six days 7. A week 8. Other			Q16	
16.1 If other, please specify			Q16.1	
17. If you buy bulk, where do you store excess fresh produce? 1. In a fridge 2. At home in a box 3. At room temperature in the store 4. At room temperature in a storeroom 5. Other			Q17	
17.1 If other, please specify.			Q17.1	
18. For how long do you keep the fresh produce? 1. One day 2. Two days 3.Three days 4. Four days 5. Five days 6. Six days 7. A week 8. Until it's quality does not allow it to be sold			Q18	
19. How many days a week are you selling fresh produce? 2. Every day (7 days a week) 3. Monday to Saturday (6 days a week) 4. None			Q19	
19.1 If none of the above are accurate, please specify.			Q19.1	
20 Is there a bathroom that you can use near your market stall? 1. Yes 2. No			Q20	

21 Do you have water to wash your hands? 1. Yes 2. No			Q21	
22 What is the water source you use for hand washing 1. Tap water 2. Borehole 3. Other			Q22	
23 Who looks after your market stall when you at the bathroom? 1. A neighbouring vendor 2. An assistant 3. No one				
24 Do you come to sell fresh produce when you have diarrhoea? 1. Yes 2. No				

Section C **Fresh Vegetables sold**							
25. Which vegetables and fruit from the list below do you typically buy to sell: If yes circle 1, if no circle 2, Do you eat it raw, cooked or both? If eaten raw circle 3, cooked circle 4, raw and circle 5	Eat	Don't eat	Raw	Cooked	Raw &Cooked		
25.1 Cabbage	1	2	3	4	5	Q25.1	
25.2 Carrot	1	2	3	4	5	Q25.2	
25.3 Green beans	1	2	3	4	5	Q25.3	
25.4 Spinach	1	2	3	4	5	Q25.4	
25.5 Morogo/African leafy vegetables	1	2	3	4	5	Q25.5	
25.6 Lettuce	1	2	3	4	5	Q25.6	
25.7 Kale	1	2	3	4	5	Q25.7	
25.8 Cucumber	1	2	3	4	5	Q25.8	
25.9 Sweet corn	1	2	3	4	5	Q25.9	
25.10 Peas	1	2	3	4	5	Q25.10	
25.11 Onions	1	2	3	4	5	Q25.11	
25.12 Butternut	1	2	3	4	5	Q25.12	
25.13 Beetroot	1	2	3	4	5	Q25.13	
25.14 Pumpkin	1	2	3	4	5	Q25.14	
25.15 Sweet potato	1	2	3	4	5	Q25.15	
25.16 Potatoes	1	2	3	4	5	Q25.16	
25.17 Cauliflower	1	2	3	4	5	Q25.17	
25.18 Broccoli	1	2	3	4	5	Q25.18	
25.19 Green pepper	1	2	3	4	5	Q25.19	
25.20 Tomato	1	2	3	4	5	Q25.20	

Section D: What do you know about Food Safety?					
Please answer the following statements by indicating True, False or Uncertain	**True**	**False**	**Uncertain**		
26. Fresh produce can be contaminated with bacteria/germs that cause disease.	1	2	3	Q26	

27. Cutting boards and knives should be washed thoroughly with hot water.	1	2	3	Q27	
28. Vomiting, diarrhoea are symptoms of food poisoning.	1	2	3	Q28	

Section E: What are your thoughts about Food Safety?							
How do you feel about the following: *Please answer the following statement using a rating of 1 to 5, where 1 would indicate strongly disagree and 5 would indicate strongly agree*	Strongly disagree	Disagree	Neutral	Agree	Strongly agree		
29. Good personal hygiene of those that work with food can prevent foodborne illness.	1	2	3	4	5	Q29	
30. It is the responsibility of all food handlers to ensure that fresh is fresh and clean.	1	2	3	4	5	Q30	
31. Quality is more important than price.	1	2	3	4	5	Q31	
32. Washing my hands before touching food is necessary	1	2	3	4	5	Q32	
33. I think it is unsafe to leave fresh produce outside the fridge overnight.	1	2	3	4	5	Q33	
34. Do you think fresh produce sold at retailers are better quality/safer than at green grocers or markets.	1	2	3	4	5	Q34	

Does contamination of fresh produce from the following concerns you? *Please answer the following statements using a rating of 1 to 5, where 1 would indicate "not concerned at all" and 5 "would indicate Highly concerned"*	Not concerned at al	Not very concerned	Uncertain	Fairly concerned	Highly concerned		
35. Insects and rodent dropping	1	2	3	4	5	Q35	
36. Pesticides	1	2	3	4	5	Q36	
37. Bacteria/germ	1	2	3	4	5	Q37	
38. Human excretion	1	2	3	4	5	Q38	
39. Dumping sites	1	2	3	4	5	Q39	

Historic events **Please circle the correct answer**	Not concerned at al	Highly concerned		
40. Do you have a problem with rubbish next to your markets? 1. Yes 2. No	1	5	Q40	
41. Do you have problems of sewage overflowing? 1. Yes 2. No	1	5	Q41	
42. Do you have chicken farms/cages close to your market stall? 1. Yes 2. No	1	5	Q42	
43. Do you have cattle farms/cops close to your market stall? 1. Yes 2. No	1	5	Q43	

Section E: OBSERVATION/S			
1. GPS coordinates		Q45	

Thank you for your participation and valuable contribution to this research.
Your information will remain anonymous throughout the research.