Assessing Faecal-Pathogen Contamination and Exposure to Risk in Community Ablution Blocks (CABs): A Case Study of Informal Settlements within eThekwini Municipality, South Africa

Final Report to the Water Research Commission

Report to the
Water Research Commission

by

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WRC Report No. 2896/1/21
ISBN 978-0-6392-0318-8

February 2022

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EXECUTIVE SUMMARY

The goal of sanitation coverage is to enable and improve human health by offering protection against faecal-oral exposure. Insufficient knowledge of hygiene levels within *Community Ablution Blocks* (CABs) coupled with individual and community behavioural determinants could potentially increase the risk for sanitation-related disease transmission. The risk for diarrhoeal disease transmission from shared sanitation is well known. This risk is thought to be primarily posed by the contamination of contact surfaces within these shared facilities with potentially pathogenic microorganisms.

This project used a combination of qualitative and quantitative methods to determine the potential of the CABs within informal settlements in the eThekweni Municipality (KwaZulu-Natal) to contribute to diarrhoeal disease transmission through contamination with potentially pathogenic microorganisms. Metagenomics was used to characterise the microbial communities on key contact surfaces within CABs in two informal settlements. This was followed by an in-depth study to determine the concentration of *Escherichia coli* (*E. coli*) on these surfaces, serving as input data for the *Quantitative Microbial Risks Assessment* (QMRA). The QMRA approach was used to determine the probable risks of diarrhoea due to contact with these surfaces. In addition to the microbial studies, the general hygiene and safety conditions around the CABs were also determined. Challenges faced by caretakers of these CABs in the daily cleaning and maintenance of these facilities was also assessed. This report details the results of the study including findings and recommendations on how risks associated with the use of CABs can be monitored.

The common bacterial phyla identified on these surfaces were Actinobacteria, Firmicutes, Proteobacteria and Bacteriodetes. Actinobacteria were the most abundant, up to about 90% bacteria on the cistern handles. The relative abundance of the bacterial phyla mentioned above differed across contact surfaces, which could be due to differences in source of contamination on these contact surfaces. Focusing on the Enterobacteriaceae, due to their role in diarrhoea, we identified *Enterobacter cloacea, Escherichia. coli, Klebsiella oxytoca, Klebsiella pneumoniae, Raoultella ornithinolytica* and *Salmonella enterica* as the most common species. *Enterobacter cloacae* (85% of bacterial species) and *E. coli* were the most prevalent of these species (23% of bacterial species). Additionally, we detected uncommon potential human pathogens such as *Cedeceae neteri, Enterobacter hormaechei, Klebsiella michiganensis, Pluralibacter gergoviae, Kosakonia cowanii* and *Raoultella ornithinolyitca.* Another significant finding was the detection of *Mycobacterium* spp on the different contact surfaces. A total of 18 species were detected accounting for both male and female toilets in the two study sites. *M. tuberculosis*, *M. ulcerans* and *M. leprae* were the most abundant with relative abundance of up 6% for *M. tuberculosis.*

The highest concentration of *E. coli* was found on cistern handles (6.01 Log10 CFU/cm^2), floor surfaces in front of toilets (6.23 Log10 CFU/cm^2) and tap handles (6.25 Log10 CFU/cm^2), all within female toilets. This shows that contact with these surfaces poses the highest risks of infection which was corroborated by the QMRA results. For instance, for one-time exposure,

at least two people (2) out of 100 users of the CABs may be infected when they touch surfaces such as the cistern handle, external door latch and tap handle in both the shower and wash basin. However, these risks increase when people touch these surfaces multiple times over the course of the day or year. The poor hygiene conditions observed in the CABs which explains the contamination levels measured could be due to the challenges faced by the caretakers. Survey findings indicated that these caretakers received some level of training from the Municipality and are provided with the necessary materials and personal protective equipment to carry out their duties. However, they expressed the need for refresher training programmes. The CAB risk assessments and audit showed that despite reports of daily cleaning of the CABs, these facilities had faecal contamination on internal contact surfaces. In addition, the presence of overgrown bush and vegetation around the CABs and the lack of external lighting at night may pose serious safety concerns and could account for the open defaecation observed around the CABs. We also observed that despite CABs being accessible, toilet and sewer blockages with newspapers, sanitary pads cement bags and plastics was a major issue that led to non-functional toilets and sewer overflows. Notably, the audit also revealed that there are no refuse bins within or near CABs.

Based on the results of this project, we can conclude that contact surfaces in shared sanitation facilities such as the CABs investigated here harbour potentially pathogenic bacteria. Therefore, there is a critical need for risk reduction interventions such as effective cleaning, focusing on frequently touched surfaces, and implementing personal hygiene practices, such as hand washing to reduce oral transfer of pathogens.

ACKNOWLEDGEMENTS

Special thanks to Mr V. Govender (UKZN School of Built Environment) for assistance with Figures 2 and 3. To our beloved Prof C.A. Buckley, thank you for years of encouragement, advice, friendship, mentorship, support, supervision and wisdom, and certainly your vast networking which has contributed immense value to the project.

The Project Team would also like to thank the Reference Group for their contribution:

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1. BACKGROUND

Research has shown that shared sanitation can play a critical role in achieving sanitation coverage (Pickering et al., 2015; Garn et al., 2017). For most people living in densely populated urban areas, such as informal settlements, the alternative to open defaecation is the use of shared sanitation (Busquet, 2015) resulting in a global increase in the use of shared sanitation. However, despite the increase in the use of shared sanitation, there is divided opinion on its appropriateness. While some researchers contend that shared or public toilets (as opposed to individual household toilets) are the best option for densely populated urban slums to deal with space constraints (Schouten and Mathenge, 2010; Katukiza et al., 2012), others believe that shared sanitation may be a contributory factor to increased infections (Fenn et al., 2012; Patil et al., 2014; Heijnen et al., 2014; Pickering et al., 2015). The argument against shared sanitation is mainly from the viewpoint that the use of these facilities may play a role in predisposing users to increased risk of diarrhoea, soil-transmitted helminths, and trachoma (Guerrant et al., 2013) due to contamination of contact surfaces with faeces.

Faecal matter contains a variety of potential pathogens and normal microbiota of the gut. Exposure to these pathogens could, therefore, result in diarrhoeal infections and other diseases. The primary aim of sanitation is, therefore, to limit exposure to these pathogens present in faecal matter (Stenström et al., 2011). During outbreaks of diseases, such as diarrhoea, pathogen contamination of key contact surfaces within sanitation facilities could be increased, due to the shedding of large numbers of microorganisms (Kay et al., 2006). The conditions in this environment are ideal for microorganism survival and proliferation, thereby increasing faecal-oral exposure (Kagan et al., 2002; Kay et al., 2006). Survival of microorganisms on surfaces such as plastics and metals has been reported extensively (Barker and Bloomfield, 2000; Neely and Maley, 2001; Curtis et al., 2003; Alsallaiy et al., 2016). It is therefore likely that the surfaces in the sanitation facilities may harbour a wide range of microorganisms, some of which may be pathogens.

Viruses and bacteria have been found on bathroom surfaces, from airborne dissemination during toilet flushing (Barker and Bloomfield, 2000; Gerhardts et al., 2012). The concentration of microorganisms on contact surfaces within shared sanitation facilities could be higher compared to single-household or private facilities. However, despite the preventive role of sanitation in relation to disease transmission, several studies have reported that domestic bathrooms, showers and toilets serve as reservoirs for pathogens (Curtis et al., 2003; Kay et al., 2006; Gerhardts et al., 2012). Additionally, the role of shared sanitation in the spread of diarrhoeal disease has been reported extensively (Rah et al., 2015; Baker et al., 2016; Crocker and Bartram, 2016; Ramlal et al., 2019). Disease transmission in sanitation facilities could occur either through toilets-to-hands-to-mouth contact or from contaminated hands to surfaces (Barker and Bloomfield, 2000; Curtis et al., 2003).

In most settings where shared sanitation is practiced the responsibility of cleaning is shared by the users or volunteers (Kwiringira, 2017). This has been reported to result in apathy towards the hygienic maintenance of these facilities (Tumwebaze and Mosler, 2015; Kwiringira, 2017).

Therefore, the employment of caretakers, living in the communities where shared sanitation facilities are located, like the *Community Ablution Blocks* (CABs) in the informal settlements of the eThekwini Municipality has been recommended as an alternative.

To ascertain the role of shared sanitation facilities in the transmission of diseases, especially diarrhoea there is the need to determine the microbial community on key contact surfaces. This could be achieved through a variety of methods. The most common approach used to determine bacterial contamination of contact surfaces is the culturing of swabs using specific media (DeVita et al., 2007; Atnafie, et al., 2017; Keeratipibul et al., 2017), or molecular techniques such as the *Polymerase Chain Reaction* (PCR) (Martinon et al., 2012; Selvaganapathi et al., 2018; Amin et al., 2018). However, these methods are targeted or specific to organisms of interest. For instance, culturing requires the selection of media that contains the nutrient requirement for the growth of the bacteria. In PCR methods, primers need to be selected that target specific regions of the target bacteria. These methods, therefore, have the potential to introduce a bias. The advent of advanced molecular techniques such as metagenomics has addressed this issue of bias to a great extent.

Metagenomics allows for the complete profiling of all bacteria without the need to be specific. A few studies have reported on the metagenomic profile of microorganisms on contact surfaces within sanitation facilities (Flores et al., 2011; Mukherjee et al., 2014; Hsu et al., 2016). However, none of these studies focused on shared sanitation facilities. This report, therefore, presents information on the common microbial communities on key contact surfaces within the CABs. Additionally, it also includes a probabilistic assessment of the risks of diarrhoeal infections associated with the use of these facilities using the *Quantitative Microbial Risk Assessment* (QMRA) approach. This approach has been used extensively to determine the health risks associated with wastewater reuse, drinking water and food consumption. However, its application for the determination of health risks associated with the use of shared sanitation facilities is limited.

The main aim of this project was to determine whether the use of these facilities could contribute to diarrhoea infections among the populations as a result of contact-surface contamination. One of the key objectives of the project involved assessing the potential contamination of contact surfaces within CABs in informal settlements in the eThekwini Municipality through pathogen characterisation and quantification. The project was structured around the deliverables listed below.

1.1 Project Deliverables

The project was divided into five (5) deliverables presented in the table below:

Deliverable	**Description**
Detailed experimental plan for the study	Details of how swab sampling will be conducted. Selection of control and experimental study sites. Describing and detailing the methods that will be applied in the study.
Metagenomics profiling of microorganisms on contact surfaces within CABs	Identification and characterisation of microorganisms within the *Community Ablution Blocks* (CABs) (shared sanitation). The methodology and results presented in this report start from this deliverable.
Quantification of diarrhoea-associated microorganisms on contact surfaces	This is the quantification (using *quantitative Polymerase Chain Reactio*n - qPCR) of the common pathogens related to diarrhoeal infection chosen based on deliverable 2. This also presents the temporal variation in the concentration of these pathogens.
Probabilistic risks of diarrhoeal infections for users of CABs	*Quantitative microbial risk assessment* (QMRA) for diarrhoeal infection based on the concentration of pathogens determined with qPCR and user behavioural information. This was expanded to include an audit of the physical structure of the CABs to ascertain the health and safety risks. We also interviewed the caretakers to determine the challenges they face in their daily activities.
Final report	Final report: This reports on the results of the bacterial profiles on the contact surfaces, the concentration of indicator bacteria on these surfaces and the associated risks of infections. This also includes proposed interventions to reduce the risks estimated for users of the CABs.

2. METHODOLOGY

2.1 Study area

Two peri-urban informal settlements in the eThekwini Municipality were chosen for this study, locally known as the Kennedy Road (Settlement A) and Foreman Road (Settlement B) informal settlements (**Figure 1**). Selection for the project was based on the following criteria: population density, spatial distribution of CABs within each settlement and environmental factors such as elevation and slope. The spatial distribution of CABs in the two settlements are dissimilar, in that those located within Settlement A are settled along its boundaries whilst those at Settlement B are interspersed within the settlement; these differences allowed for comparative analyses.

This study was approved by the Biomedical Research Ethics Committee, University of KwaZulu-Natal (reference number BE 339/19). Gatekeeper permission was granted by the eThekwini Municipality to conduct the study in the informal settlements selected.

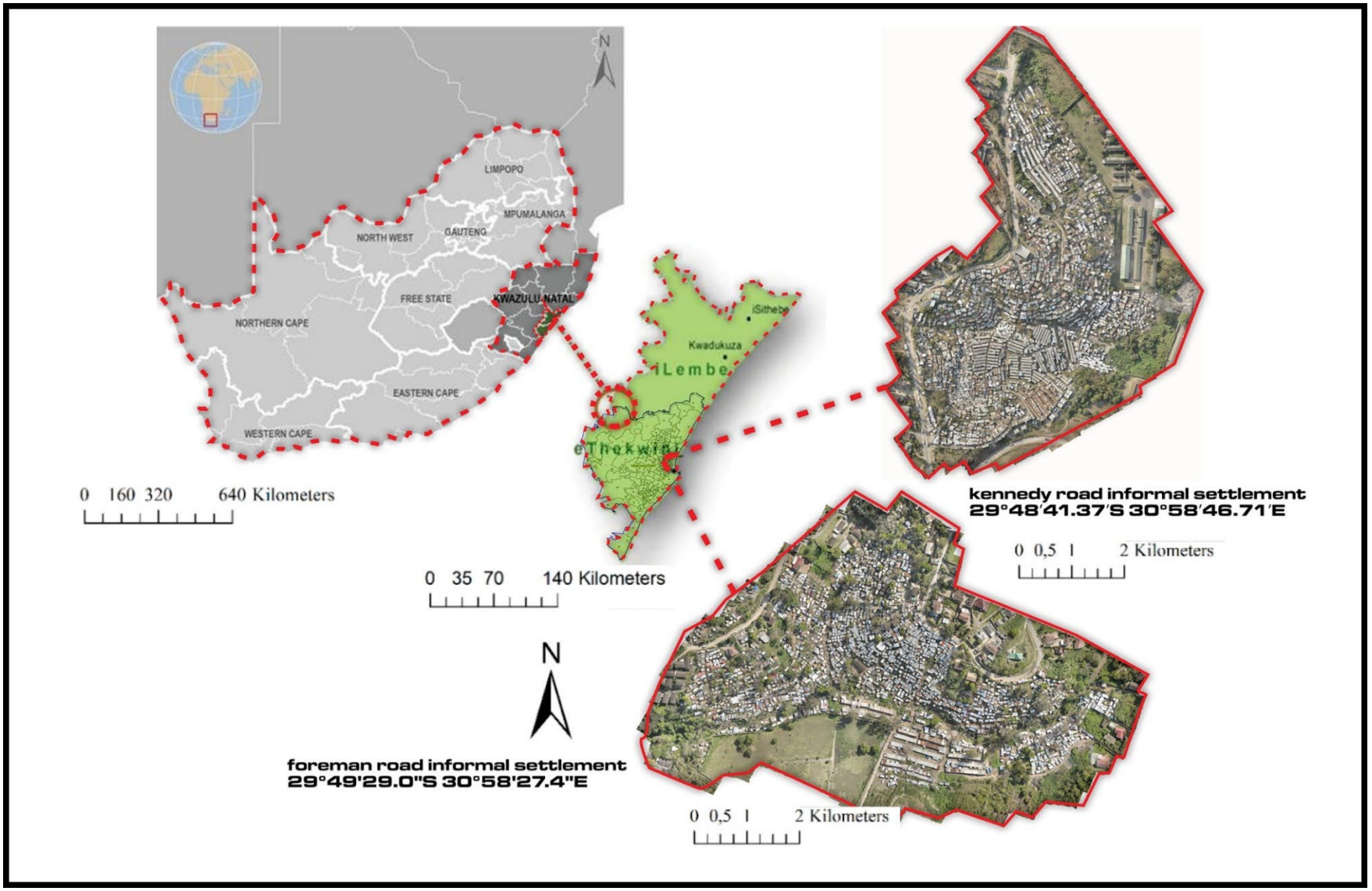

Figure 1. Location of Kennedy and Foreman Road informal settlements in Durban, eThekwini Municipality, KwaZulu-Natal.

2.2 Metagenomics profiling of microorganisms on contact surfaces within CABs

2.2.1 Swab Sampling for metagenomics analysis

The key contact surfaces sampled for the metagenomics profiling included the internal door latch of cubicle door, toilet cistern handle, toilet seat, and tap in wash hand basin (**Figure 2**). Samples were taken with the FLOQSwabs™ swabs (Seegene Inc., USA), following the protocol proposed by Park et al. (2017). The kit ensures stability of the nucleic acid during sample storage and transportation at ambient temperature without changing the composition of the samples. An initial study was conducted to determine the suitability of either a wet or a dry swab for this kind of study. In this study, swab samples were initially taken with dry swabs and followed up with wet swabs, this was done once for the initial study. Wet swabs were prepared by soaking the swabs with *Phosphate-Buffered Saline* (PBS). The diversity and abundance of the different microbes picked up by these two sampling approaches were compared.

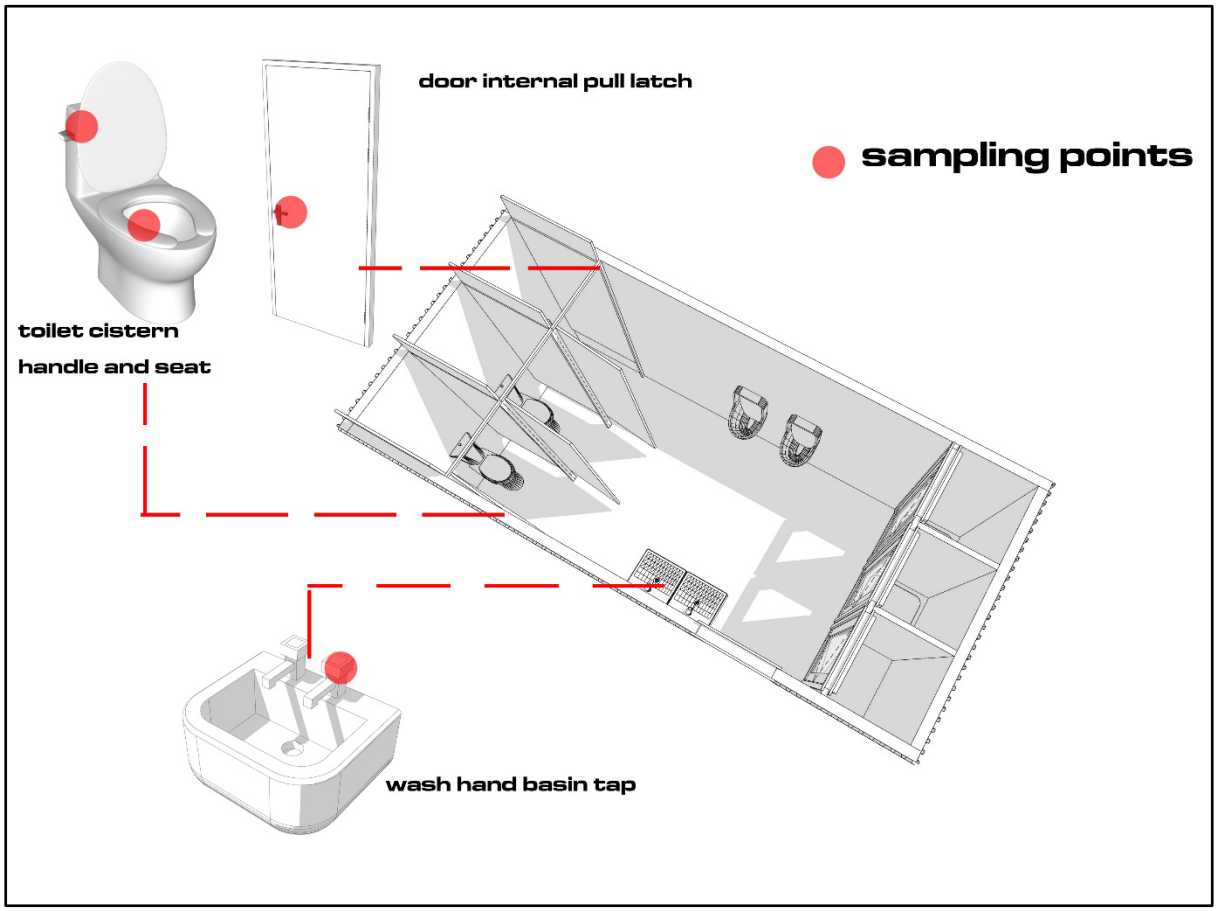

Figure 2. Key contact points/surfaces swabbed for the metagenomic profiling.

2.2.2 Sample preparation and metagenomic profiling of microorganisms

Sample processing and the metagenomic profiling was carried out by Inqaba Biotechnical Industries (Pty) Ltd (Pretoria, South Africa). Briefly, *Deoxyribonucleic Acid* (DNA) was extracted using Zymo Research's ZymoBIOMICS DNA Kit. The quantity and quality of extracted DNA were assessed prior to profiling. The *genomic Deoxyribonucleic Acid* (gDNA)

samples were then fragmented using an enzyme-based approach (part of NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina workflow). The resulting fragments were purified (size selected, using AMPure XP Beads), end-repaired and an Illumina specific adapter sequence was ligated to all fragments. Following quantification, the samples were individually indexed, and a second size selection step was performed, using AMPure XP Beads. The libraries were quality controlled on a DNA chip (Agilent's TapeStation) and then sequenced on Illumina's NextSeq platform, using a NextSeq (300 cycle) kit according to the manufacturer's protocol. For each sample, 1.5 GB of data was produced (2 x 150 bp paired-end reads).

2.2.3 Bioinformatic analysis

Taxonomic classification of the bacterial, archaeal, viral, and eukaryotic community in the sampled surfaces was performed using Kraken2 (Wood et al., 2019). Data obtained from Kraken was converted to a phyloseq object and analysed using the R package Phyloseq (McMurdie et al., 2019). Phyloseq functions were used to calculate and plot relative abundance for each of the identified taxonomic units. Read counts for abundance estimation were normalized for each sample using median sequencing depth (McMurdie et al., 2019).

2.3 Quantification of diarrhoea-associated microorganisms on contact surfaces

2.3.1 Swab sampling for *E. coli* concentration

E. coli was selected as an indicator of the faecal contamination on the contact surfaces based on the bacterial profile and previous studies which showed it to be a reliable indicator. The number of sites, CABs and contact surfaces selected in this study was based on financial and time constraints associated with the sampling methods and analyses used but care was taken that replication levels are in line with recent studies in the field (see **Table S1** in **Appendix I**) (Mpotane et al., 2013; Bohnert et al., 2016). The contact surfaces selected for this aspect of the study included the following: cistern handle, toilet seat, floor surface in front of the toilet, internal pull latch of cubicle door, external cubicle door handle, tap handle in shower cubicle, internal common floor surface and tap in wash hand basin **(Figure 3)**. These contact surfaces were chosen based on a recommendation made in previous studies (Mpotane et al., 2013; Bohnert et al., 2016). One cubicle each in the male and female facilities was sampled in each of the selected CABs. Figure 3 lists the eight specific sampling points that were swabbed within each CAB in this study.

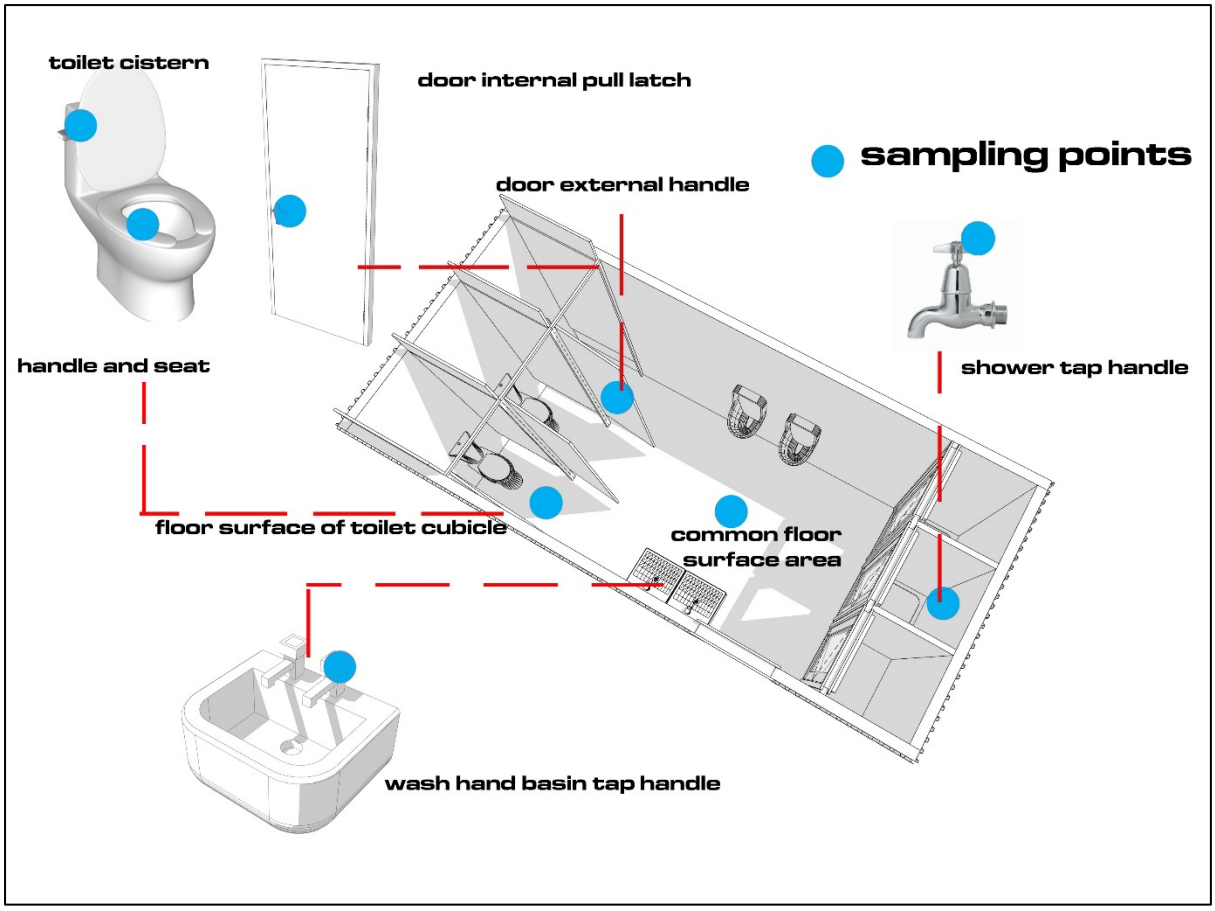

Figure 3. Key contact surface areas swabbed to determine *E.coli* concentration within internal surfaces of CABs.

2.3.2 Enumeration of *E. coli*

Aliquots of 0.1 ml were spread-plated on labelled agar plates (mFC -faecal coliform counts, Chromocult - *E. coli* counts and Nutrient agar - colony counts) after a series of 10^{-4} to 10^{-10} dilutions per sampling point on a CAB. The plate lids were left slightly open after spread-plating for 2-3 minutes to allow for sample absorption, this was followed by packaging and incubation at 37°C for 24 hours. To determine the number of *coliform forming units* (CFU) per millilitre (ml) of sample (CFU/ml), plates with 30-300 colonies were used. This information enabled the determination of the presumptive count of colony-forming units per swab area (CFU/cm^2).

$$\textbf{CFU/cm}^2 = \textbf{n} \times \textbf{dilution factor (y/z)}$$

Where:

n: Number of colony counts

y: ml of sample solution

z: Swab area (cm^2)

The surface area swabbed for each surface is presented in **Table S1 (Appendix I).**

2.3.3 Isolation of *E. coli* isolates

Ten single colonies per sampling site from Chromocult agar plates were selected based on morphological characteristics and colour. The *E. coli* colonies were shiny, mucoid, and dark blue to violet in colour. Colonies that met the criteria were inoculated by streaking into nutrient agar to obtain a pure culture of *E. coli.* The *E. coli* isolates were grown overnight on nutrient agar at a temperature of 37°C.

2.3.4 Biochemical confirmation of *E. coli* isolates

E. coli isolates were confirmed using a traditional procedure that is referred to as the IMVic test (Lupindu, 2017). The IMVic test is made up of four tests, which are the Indole, Methyl red, Voges-Proskauer (VP), and Citrate utilization test. The Indole test is used to check bacteria for the ability to produce indole from tryptophan with the use of tryptophanase. The indole reacts with the aldehyde in the Kovac's reagent and exhibits a red or a pink ring at the top of the tube. The peptone water that contained tryptophan was inoculated with the *E. coli* isolate. The mixture was incubated at 37°C for 24 hours. A few drops of Kovac's reagent was then be added to the mixture, and the formation of a red or a pink coloured ring at the top indicated a positive reaction. *E. coli* are indole-positive bacteria.

The methyl red test determines the ability of bacteria to generate acid from glucose fermentation. Methyl red retains the red colour at a pH less than or equal to 4.4. The isolates were inoculated into glucose phosphate (*Methyl Red* (MR) and *Voges-Proskauer* (VP) - MRVP) broth, consisting of glucose and phosphate buffer. They were then incubated at 37°C for 48 h. About 3 to 5 drops of MR reagent was added to the tube, and a red colour indicated a positive reaction. Yellow discoloration indicated a MR-negative reaction.

Voges-Proskauer tests for presence of acetoin in the bacteria-harbouring media. Diacetyl, in the presence of alpha-naphthol, reacts with guanidine to produce red colour. The VP test was performed by inoculating the *E. coli* isolates into glucose phosphate (MRVP) broth in a tube and this was incubated for 72 hours at 37°C. 15 drops of alpha-naphthol were then added to the test broth, accompanied by shaking. 5 drops of 40% potassium hydroxide (KOH) were added to the broth and shaken well, and the tube was allowed to stand for 15 minutes before showing a red discolouration. If the isolate stood for over an hour with no change, it was classified as VP-negative. The citrate utilisation test identified the ability of *E. coli* isolates to utilise citrate as their sole source of carbon and energy. Citrate agar media consists of a pH indicator referred to as bromothymol blue and this agar switches from green to blue under alkaline pH conditions. A loopful of *E. coli* isolate was streaked on a citrate agar slant without stabbing the butt and this was incubated with a loosened cap at 37°C for 24 hours. The development of a blue colour indicated a positive reaction, whereas the persistence of a green colour signaled a negative result.

2.3.5 Confirmation of *E. coli* isolates using polymerase chain reaction (PCR)

Each *E. coli* isolate was confirmed using PCR according to the methods of Abid and AL-zuwainy (2014). DNA was extracted by boiling (Peng et al., 2013). A single colony of the presumptive positive culture was suspended on a test tube consisting of 1 ml of distilled water, and the contents were boiled in a water bath for 10 minutes. The contents were transferred into an Eppendorf tube and then centrifuged for 5 minutes at 1,000 rpm. 3 µl of DNA was used as a template for PCR.

The *uidA* was used as the marker gene for the confirmation of *E. coli* isolates, and its amplification was carried out with the following reaction mixture. The total reaction volume was 25 µl, consisting of 12.5 µl green master mix (Go Taq DNA polymerase which is provided in 2x Green tag reaction buffer pH 8.5, 400 µm dATP, 400 µm d GTP, 400 µm dCTP, 400 µm dTTP and 3 µM $MgCl_2$), 2.5 µl of the forward primer and 2.5 µl of the reverse primer, 3 µl of DNA template and 4.5 µl of distilled water. The PCR-amplified fragments (10 µl) were separated using 2% (w/v) agarose gel electrophoresis and viewed under UV light after staining with ethidium bromide. The primer sequences used for the PCR are presented in **Table S2** (**Appendix I**).

2.4 Probabilistic risks of diarrhoeal infections for users of CABs

2.4.1 Microbial Infection Risk Assessment

The QMRA (Haas et al., 2014) approach was used for the microbial health risk assessment. According to Haas et al. (2014), the QMRA approach involves a sequence of interrelated steps: a) hazard identification; b) exposure assessment; c) dose-response assessment and d) risk characterisation. This approach has been used widely in assessing the health risk associated with exposure to different pathogens (WHO, 2006).

(a) Hazard Identification

The hazard of choice for this study was pathogenic *E. coli. E. coli* isolated from environmental samples could include environmental strains of non-pathogenic sources, and the mean *E. coli* counts would therefore be relatively higher than the pathogenic strains. Therefore, the risk of infection was calculated assuming that 8% of average *E. coli* counts are pathogenic (Howard et al., 2006; George et al., 2013; Machdar et al., 2013). The pathogenic *E. coli* concentrations were then used as doses that were incorporated into the QMRA at the dose-response modelling stage to ascertain the risks.

(b) Exposure Assessment

The exposure assessment was based on surveys (household and caretaker) and CAB observation checklist. The exposure scenario used in the health risk assessments was contact with contaminated surfaces within the CABs. The frequency of exposure to these contaminated

surfaces was determined based on the frequency of use of the CABs as provided by the respondents during this study. The risk assessment framework is presented in **Figure 4.**

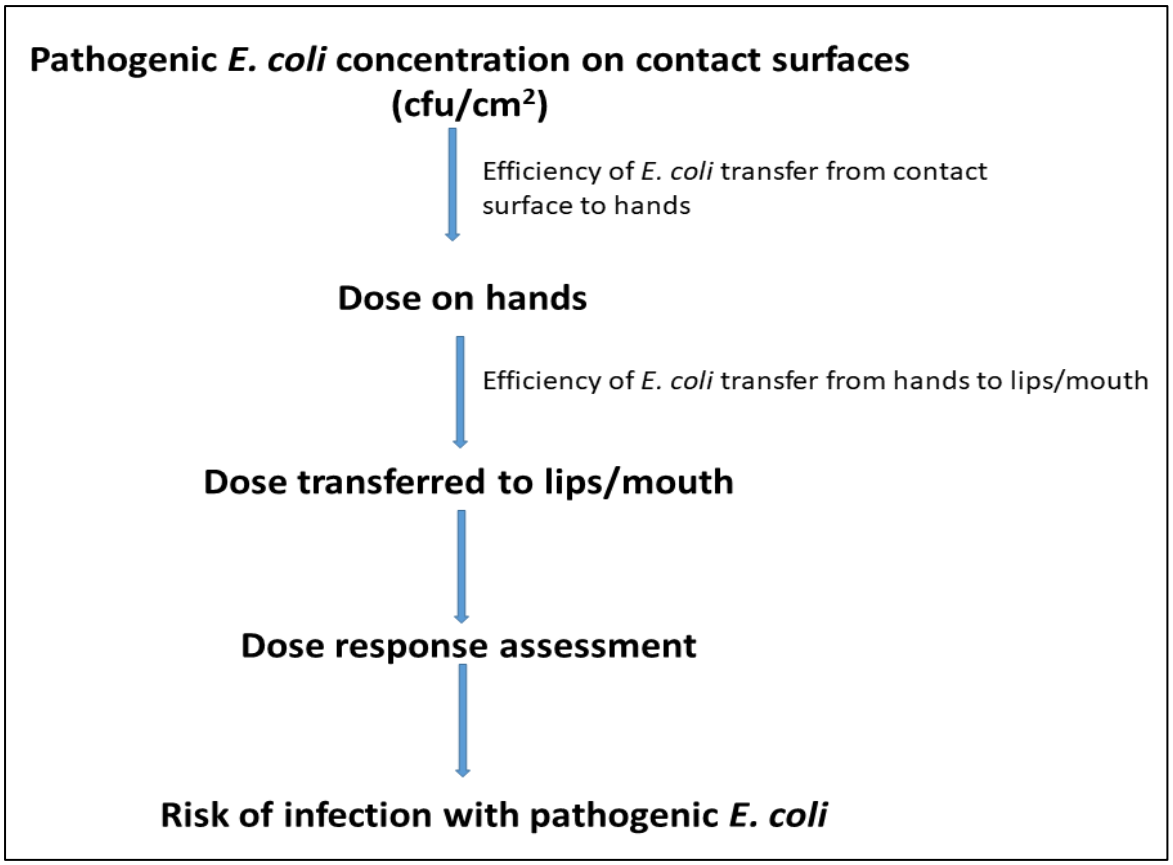

Figure 4. Scenario for assessing the exposure and possible risks associated with contamination of the contact surfaces (Adapted from Ryan et al., 2014).

(c) Dose Response Assessment

Several dose-response models have been developed for the estimation of risk posed by exposure to pathogenic *E. coli*. In this study, the beta-poisson dose-response model was used (Haas et al., 2014). The beta-poisson model is defined by the following equation:

$$p(d) = 1 - \left(1 + \left(\frac{d}{N_{50}}\right)\left(2^{\frac{1}{\alpha}} - 1\right)\right)^{-\alpha}$$

With $p(d)$ being the risk of infection, and 'd' the total concentration of pathogenic *E. coli* ingested (Haas et al., 2014).

(d) Risk Characterisation

In the risk characterisation, all the outcomes of the hazard identification, exposure assessment and dose-response assessment were combined to characterise the infections risks for exposed individuals. The risk of infection [P1(A)] associated with multiple exposures will be determined using the following formula:

$$P1(A) = 1 - \left(1 - P1(d)\right)^{n}$$

Where P1 (d) is the risk of infection from a single exposure to a dose *d* of the pathogen; and *n* being the number of days of exposure to the single dose *d* (Sakaji and Funamizu, 1998). The "*n*" was taken from the user frequency surveys conducted during this project. This gives information on the number of times the users could potentially be exposed to the pathogen.

2.5 Semi-quantitative assessment of risks associated with exposure to hazards around the CABs

A CAB observation checklist was developed to evaluate the physical condition, structural state and hygiene status quo of the CABs **(Appendix II).** The purpose of these observations was to understand and examine potential disease transmission routes in the area immediately around the CABs. For this observational assessment, three CABs in the Foreman Road settlement were selected. These CABs were selected from different locations within the settlement, one located close to a road, the second in the middle, and the third on the southern slope of the settlement. In the Kennedy Road study area five CABs were selected. These ablution blocks are located along the northern, western and eastern peripheries of the settlement. The CAB selection for this assessment was as follows: one at the northern periphery, two from the western side, and two alongside the eastern boundary, due to a high density of the CABs in this cardinal zone. The selection of a higher number of CABs in the Kennedy Road informal settlement was due to the higher number of CABs and households in this area. The inhabitants of these settlements use CABs based on proximity and availability; therefore, they are likely to use more than one CAB.

The tool instrument used for the semi-quantitative CAB risk assessment focused on the physical conditions of the areas surrounding the CABs and an assessment of the internal structure and state of the CABs. A risk score was developed for each of these different sections. To generate the risk score, each individual parameter assessed was scored "0" if it was not present around the CABs or did not pose any risk or "1" if it was present or is a potential risk.

2.6 Challenges faced by caretakers

This component of the study was conducted in the two chosen informal settlements. Data was collected from designated caretakers responsible for the upkeep and maintenance of these facilities. To facilitate ease of communication, the principal investigator was accompanied by a fieldworker conversant in IsiZulu and English during face-to-face interviews with caretakers. At present 23 CABs are located and are operational within the Kennedy Road (n = 14) and Foreman Road (n = 9) settlements respectively. The caretakers involved in the maintenance and cleaning of these CABs during the day of the fieldwork (27-02-2020) were randomly selected and interviewed. The survey instrument is divided into the following three sections; (**Appendix III**):

- Section A: Socio-Economic and Demographic Profile of Caretaker
- Section B: Maintenance and Operational Duties
- Section C: Hygiene and Occupational Risk

2.7 Statistical Analysis

The Chi-square test and the Fisher exact test was used for comparison of categorical variables and the Mann–Whitney U test was used for continuous variables. These comparative statistical analyses were performed using GraphPad Prism (version 7). All the QMRA models and health risk assessment were performed with @Risk (Palisade Corporation, USA) add on to Excel (Microsoft Corporation, USA).

3. RESULTS AND DISCUSSION

3.1 Preliminary testing of dry and wet swab samples

The preliminary assessment of the suitability of dry and wet swabs for sampling of contact surfaces within the CABs provided useful insights for the full-scale sampling. The wet swabs gave higher reads per sample compared to the dry swabs, except for the door handle where the dry swabs had slightly more reads (1369) compared with the wet swab (1139). Two additional bacteria were detected on the cistern handle using the wet swab compared to the dry swab. In relation to bacterial diversity, we observed differences in the type of bacteria that dominated these contact surfaces based on the type of swab. For instance, samples from the dry swabs taken from the cubicle handle had *Corynebacterium* as the most common known bacteria (20.15%), compared with the wet swab that had *Rothia nasimurium* as the most common known bacterial species (10.11%). Additionally, the toilet seat samples taken with the dry swab was dominated by *Prevotella copri* (13.15%) whilst the wet swab samples were dominated by *Kocuria palustris* (0.98%). Consideration of the full profile indicated a higher diversity in the wet swab as compared with the dry swab.

Whether a swab is wet or dry is just one of the many factors that may affect the swabbing process. Factors such as handling by the operator and swab type (cotton, foam, viscosin, polyester, nylon) could potentially have an impact (Verdier et al., 2014). However, in our study, the same operator used the different swab types, eliminating that sampling influence. The higher diversity in the wet swabs could potentially be due to the improved recovery of bacteria on the surfaces due to the moisture content on the swabs. Lahou and Uyttendaele (2014) observed that on dry surfaces the recovery of spiked bacterial cells was reduced by up to 11%.

Therefore, at the end of the preliminary study, we made the following observations;

(i) Both wet and dry swabs could be used to sample contact surfaces within sanitation facilities.
(ii) Wet swabs provide higher diversity of bacteria on the contact surfaces.

Therefore, the main study was performed using wet swabs.

3.2 Bacterial phyla detected on key contact surfaces

A total of 36 different bacterial phyla were detected on the key contact surfaces within the selected CABs (**Figure 5**). The most abundant of these phyla were: Actinobacteria, Firmicutes, Proteobacteria and Bacteriodetes. The dominance of these four phyla of bacteria is not surprising as these are diverse and are found in different environments. Actinobacteria are distributed ubiquitously in different ecosystems (both aquatic and terrestrial) (Barka et al., 2016), therefore their occurrence in high abundance on the contact surfaces is expected. Firmicutes and Bacteriodetes are well-known microbiota found in human intestines (Mariat et al., 2009; Thomas et al., 2011); this could therefore account for their occurrence on contact surfaces within sanitation facilities. The predominance of these four bacterial phyla on contact surfaces within different facilities has been reported elsewhere. For instance, Mukherjee et al. (2014), Jeon et al. (2013) and Flores et al. (2011) all reported these four phyla as the most abundant of bacterial taxa on contact surfaces within a fitness centre, households and public restrooms respectively.

The relative abundance of the bacterial phyla based on the average count/reads obtained presented similar findings compared to the abundance data. For instance, Actinobacteria had average counts up to 12 Log_{10} for both female and male toilets with a prevalence of up to 100%. Some of the rare phyla detected, based on the average counts and prevalence, were Caldiserica, Calditrichaeota, Candidatus, Ignavibacteriae, Kiritimatiellaeota, Lentisphaerae, Fibrobacteres and Coprothermobacterota. These phyla had an average count abundance of between 0-4 Log_{10} as shown in Figure 5, indicating low prevalence on the contact surfaces.

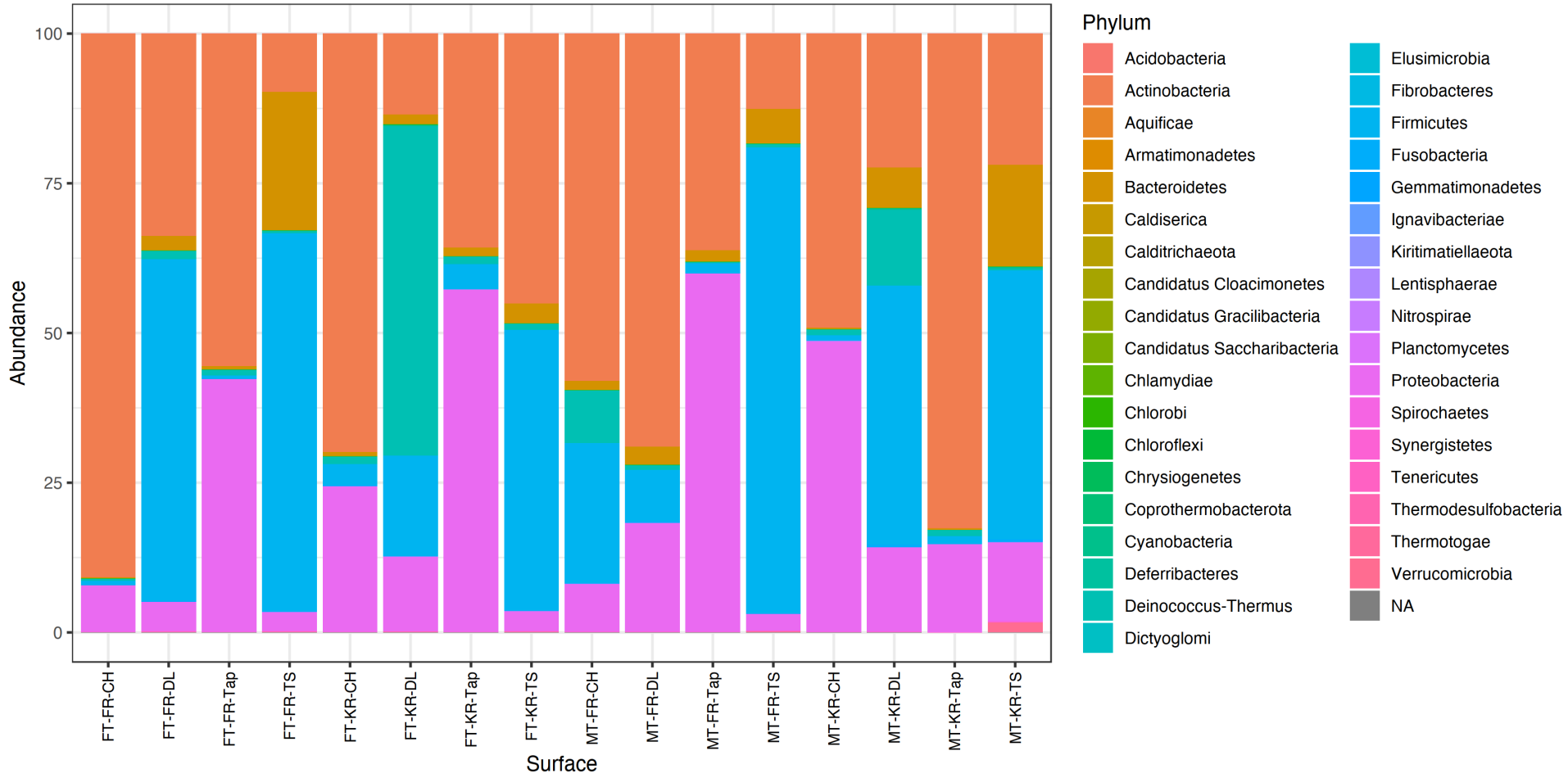

Figure 5. Metagenomic profile of abundant bacterial phyla on the contact surfaces in the community ablution blocks.

**FT= female toilets, MT= male toilets, KR= Kennedy Road informal settlement, FR= Foreman Road informal settlement*
CH= cistern handle, DL= door latch, Tap= tap in wash hand basin, TS= toilet seat

3.3 Principal component analysis of bacterial phyla diversity on contact surfaces

A *Principal Component* (PC) analysis did not show any relationship between diversity of the bacterial phyla on the contact surfaces and toilet user gender (**Figure 6; Part A**), and geographic location (**Figure 6; Part B**) of the ablution blocks. However, we observed a few outliers. For example, the female toilets showed higher levels on PC2 but low on PC1. When considering Proteobacteria diversity, we observed the same trend of results where no difference was observed in the different CABs based on gender (**Figure 6; Part C**) and location (**Figure 6; Part D**). Once again, a few outliers could be observed but did not necessarily follow any trend. Despite this outcome, we observed that the four most abundant bacterial phyla occurred predominantly on the different surfaces sampled. For instance, the tap handles predominated by Actinobacteria and the toilet seats by Firmicutes. Firmicutes are very abundant in gut microbiota (Mariat et al., 2009). This could explain their abundance on the toilet seats due to the possibility of easier contamination by faecal matter. Additionally, Hsu et al. (2016) observed that the bacterial community on contact surfaces is dependent on the type of material, which could explain the difference in abundance of the key phyla of bacteria on various surfaces.

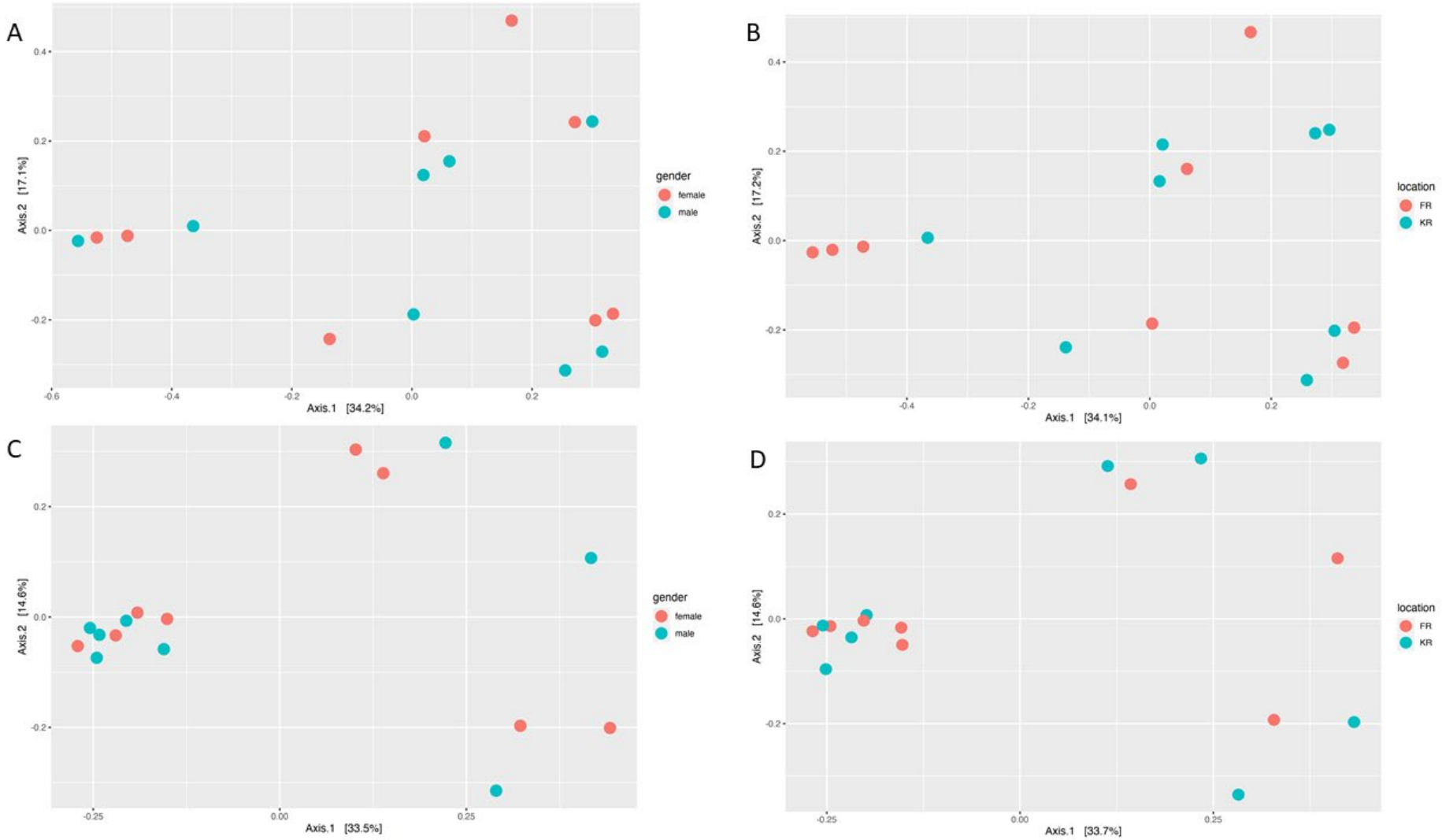

Figure 6. Principal component plots of bacterial (Part A & B) and Proteobacteria (Part C& D) diversity in the ablution blocks categorized based on gender and location.*FR: Foreman Road, KR: Kennedy Road

3.4 Profile of Enterobacteriaceae on contact surfaces

The family Enterobacteriaceae has been known to contain most of the diarrhoea-causing pathogens, we, therefore, focused on these to determine their diversity on the key contact surfaces. The results show a difference in the abundance of this family on contact surfaces within the male and female toilets, with nine and ten genera detected in the male and female toilets, respectively (**Figure 7**). The common genera were *Citrobacter, Enterobacter, Escherichia, Klebsiella, Lelliottia, Pluralibacter, Raoultella* and *Salmonella*. It is worth noting that *Cedecea* was found in the male toilets only, whilst *Kosakonia* and *Leclercia* were found in the female toilets only. Diversity of these genera on the contact surfaces was similar among the 8 contact surfaces studied, with no significant difference in their diversity ($p \geq 0.05$). However, it is worth noting the Genus *Enterobacter* mainly dominated the taps, whilst *Escherichia* was the most abundant genus on toilet seats and *Klebsiella* the most abundant on the door latch (Figure 7). The diversity of Enterobacteriaceae species on the contact surfaces was also determined. However, it must be noted that a large proportion of the genus was unclassified, making it difficult to identify the species (**Figure 8**). However, based on the classified proportion, the most common potentially pathogenic Enterobacteriaceae species detected included *Enterobacter cloacea, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Raoultella ornithinolytica* and *Salmonella enterica*. The abundance of *S. enterica*, an important enteric pathogen, was high on cistern handles for both the male toilets for both settlements. In addition to the cistern handles, the abundance of *Salmonella enterica* was high on taps and toilet seats of Foreman Road for the female toilets, with an abundance of 5% and 6%, respectively. This could also be due to the difference in materials in each of these surfaces. For instance, the toilet seat and tap comprises plastic and the door latch metal materials. The most abundant species of Enterobacteriaceae identified on the surfaces are all potentially pathogenic to humans. The abundance of enteric pathogens on these surfaces shows the potential contamination by faecal matter either through direct deposition or aerosols (toilet seat) or through contaminated hands (taps and door latch). The tap handle and door latch contamination by these potentially pathogenic enteric bacteria could be due to contaminated hands. A study by De Alwis et al. (2012) observed that door handles in male toilets were more contaminated than female toilets which they attributed to the fact that only about 50% of the males washed their hands with soap. The generation of droplets and aerosols during flushing of the toilets could be the reason for the contamination of the toilet seats (Flores et al., 2011), especially with *Escherichia*, a known gut microbiota. Potentially, the detection of these pathogens could therefore contribute towards diarrhoeal disease dissemination among the users.

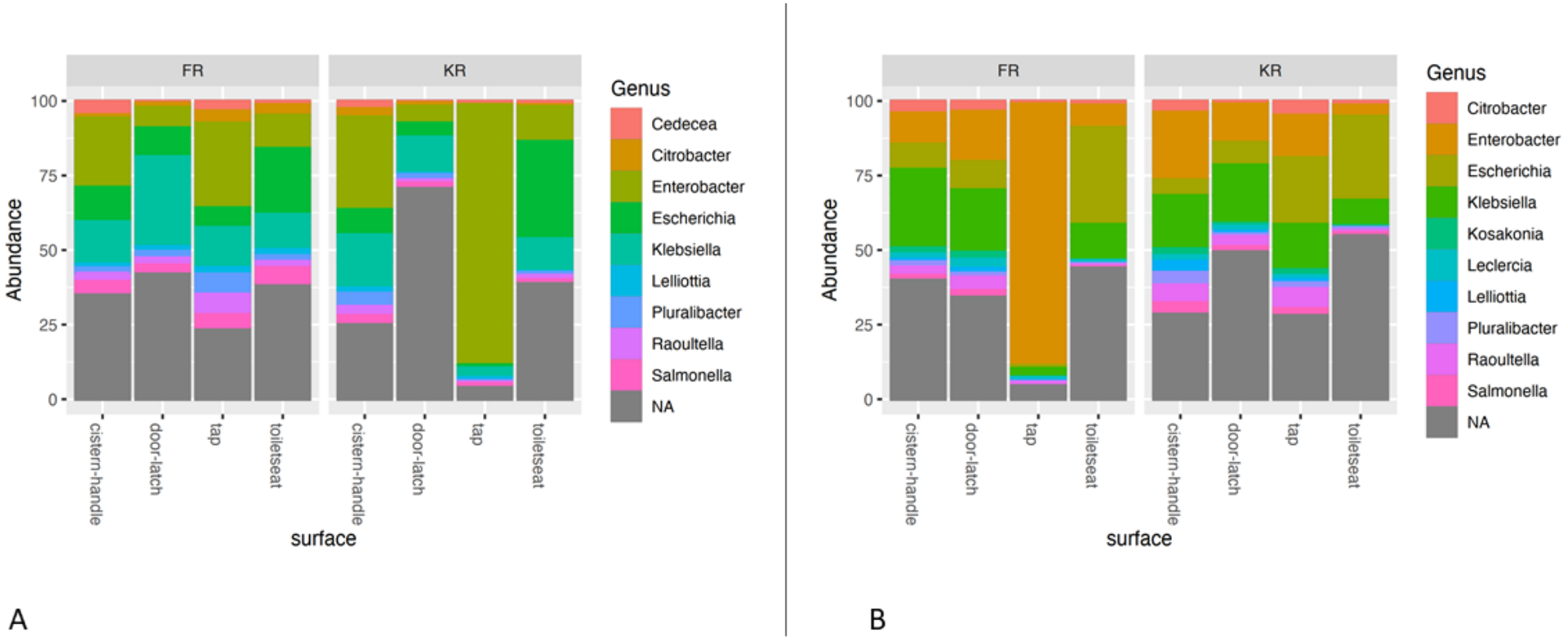

Figure 7. Abundance of Enterobacteriaceae genera on the contact surfaces: 'A' refers to Male toilets and 'B' refers to the female toilets

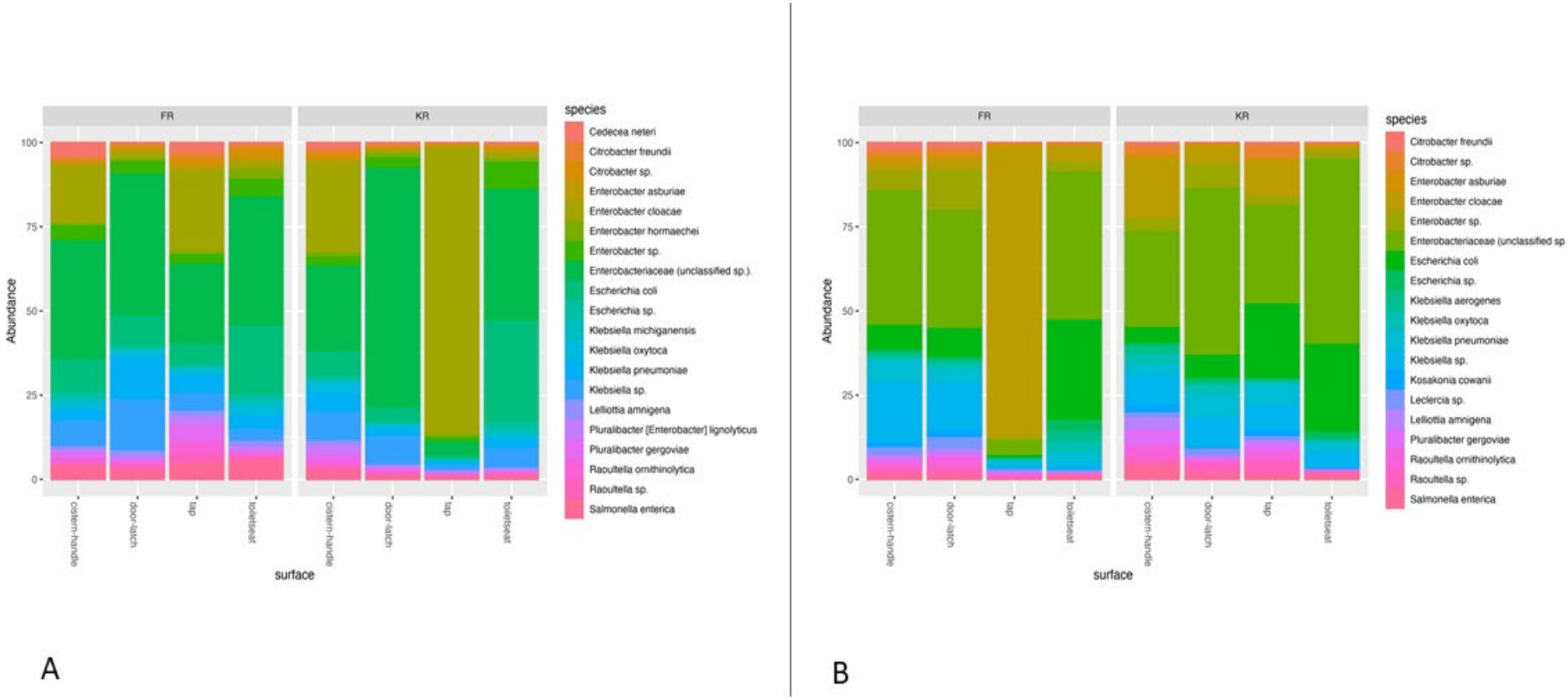

Figure 8. Abundance of Enterobacteriaceae species on the contact surfaces: 'A' refers to Male toilets and 'B' refers to the female toilets

3.5 Rare bacteria on key contact surfaces

Some rare potentially pathogenic bacterial species were detected, these include *Klebsiella michiganensis, Pluralibacter gergoviae, Kosakonia cowanii* and *Raoultella ornithinolytica.* Furthermore, non-human pathogenic species of Enterobacteriaceae detected were *Lelliottia amnigena* and *Pluralibacter* (Enterobacter) *lignolyticus*. Despite being rare in comparison to other forms of Enterobacteriaceae, there some reports that show the presence of these in South Africa (Samie et al., 2011; 2012). It is also worth mentioning that although these were found on almost all surfaces, their abundance was highest on toilet seats. This could be attributed to potential shedding in faecal matter, therefore, the presence of these rare pathogens on contact surfaces signifies infection within the populations that use the toilets. For instance, *R. ornithinolytica* is known to inhabit aquatic environments, however, in recent years it has become an emerging human pathogen (Seng et al., 2016a; 2016b). There are reports of urinary infections with *R. ornithinolytica* (Nakasone et al., 2015), which could account for the occurrence in a sanitation environment.

3.6 Presence of *Mycobacterium* spp on the contact surfaces

The study also detected members of the *Mycobacterium* spp on the different contact surfaces within the study area. A total of 18 species were detected accounting for both male and female toiles in the two study sites. An additional 13 species could not be classified by name (**Figures 9 and 10**). The well-known human pathogenic species of *Mycobacterium tuberculosis*, *Mycobacterium ulcerans,* and *Mycobacterium leprae* were detected on almost all contact surfaces, irrespective of site. *M. tuberculosis* had a similar abundance (no statistically significant difference) on all contact surfaces in the male toilets ranging from 3% to 7% (Figure 9). In the male toilets, *M. ulcerans* abundance was slightly higher on the toilet seats, compared to the other contact surfaces. In the female toilets, *M. tuberculosis* was the most abundant, which was consistent on all contact surfaces with no significant difference (Figure 10). The relative abundance of these Mycobacteria species also differed between the study locations. For instance, in Kennedy Road, the relative abundance of *M. tuberculosis*, *M. ulcerans,* and *M. leprae* were 6.4%, 0.6% and 4.5% respectively. However, in Foreman Road informal settlement, the relative abundance of these species on the contact surface was 21%, 1.3% and 1.3% respectively.

Other non-tuberculosis *Mycobacterium* spp. detected on the contact surfaces included; *M. avium, M. intracellulare, M. canettii, M. colombiense, M. dioxanotrophicus, M. haemophilum* and others as shown in Figures 9 and 10.

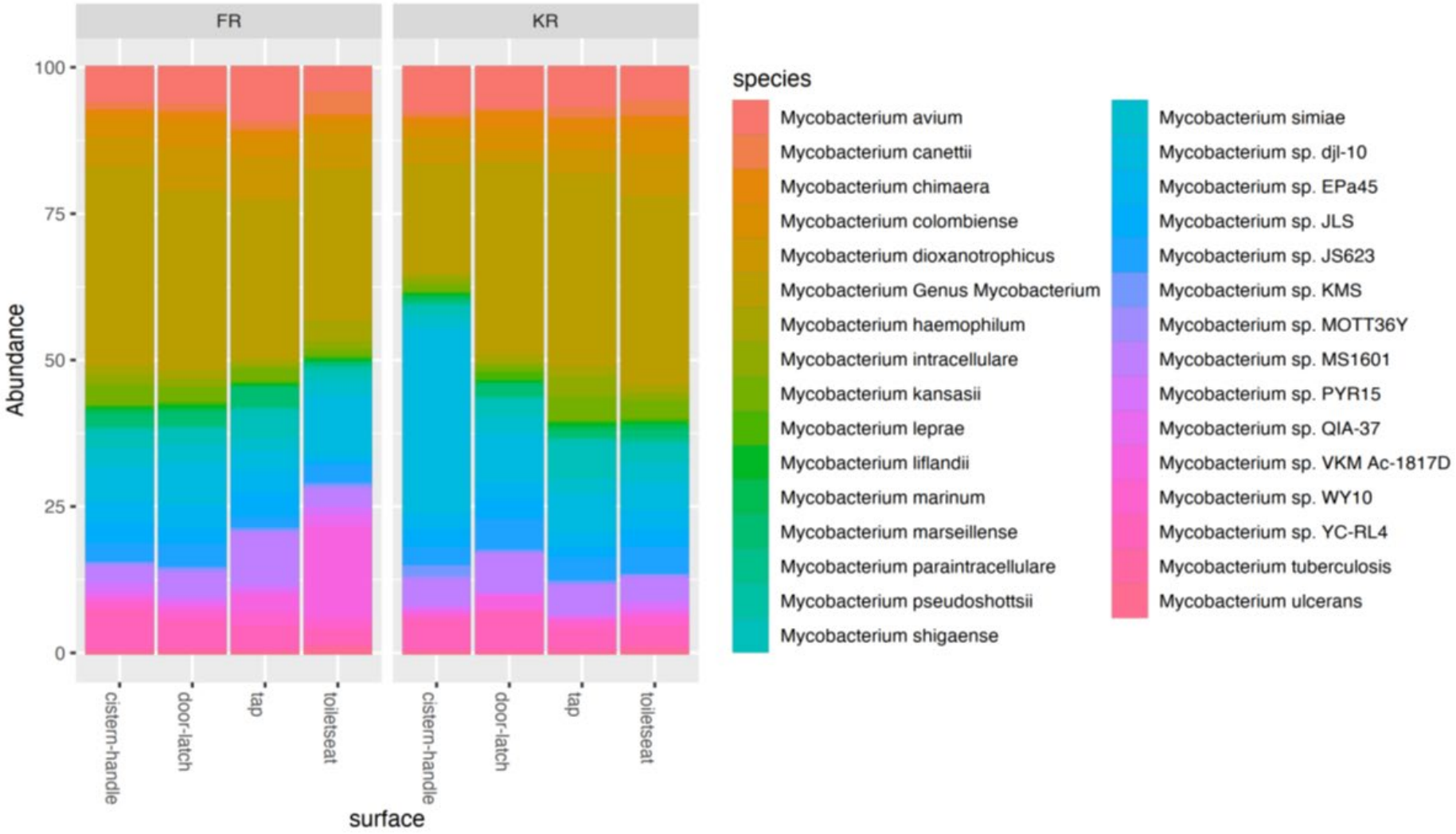

Figure 9. The abundance of *Mycobacterium* species on the contact surfaces within the male toilets.

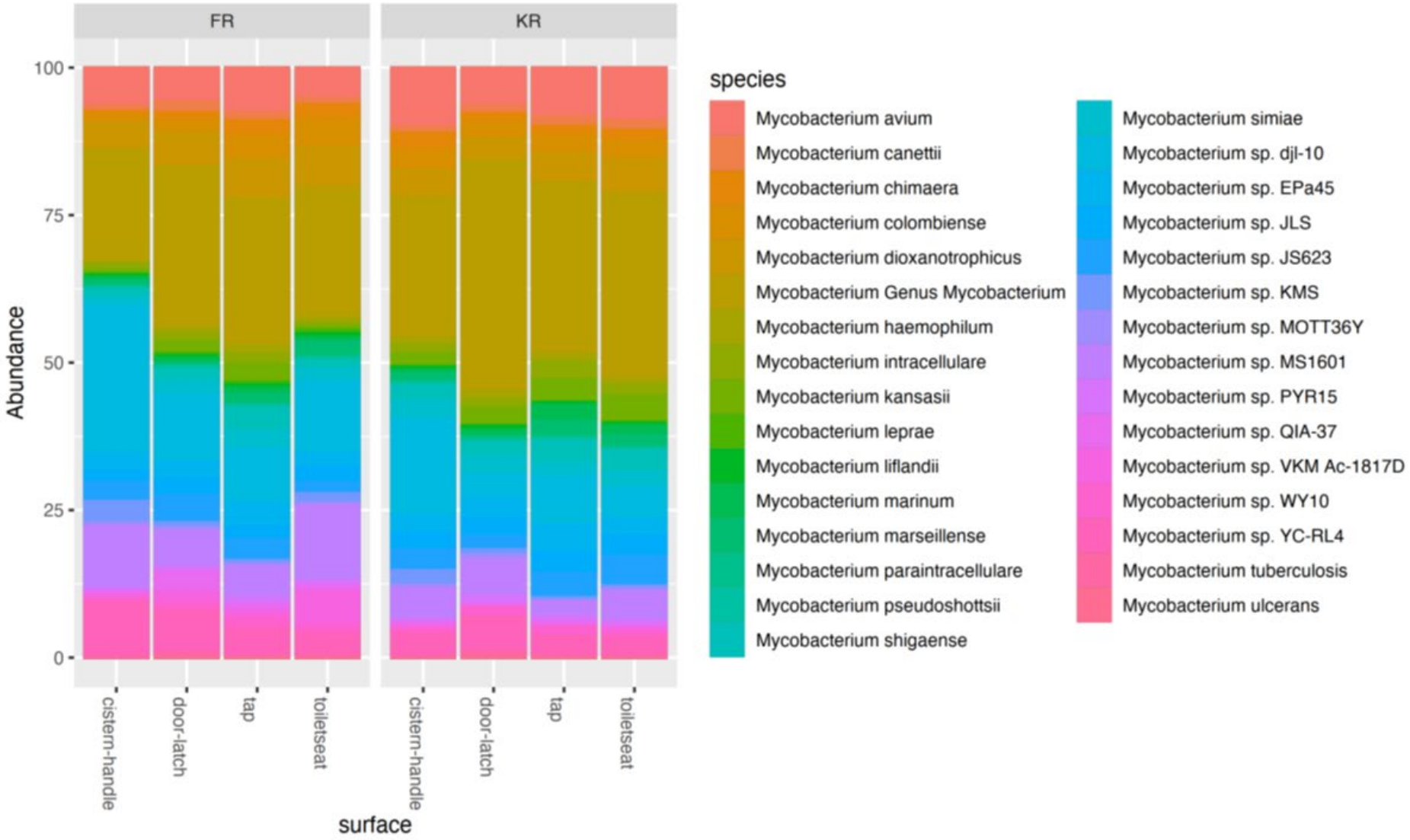

Figure 10. The abundance of *Mycobacterium* species on the contact surfaces within the female toilets.

3.7 *E. coli* concentration of contact surfaces in the CABs

E. coli concentration on the contact surfaces varied, although not significantly. Irrespective of the study location (either Kennedy or Foreman Road), the highest concentration of *E. coli* was detected on contact surfaces within the female toilets (**Figure 11**). For instance, the highest concentration of *E. coli* was on the cistern handle (6.01 Log10 CFU/cm^2), floor surface in front of toilet (6.23 Log10 CFU/cm^2) and tap handle (6.25 Log10 CFU/cm^2), all within female toilets. The difference between the male and female toilets in relation to the concentration of *E. coli* was not statistically significant (p-value ≥ 0.05). However, when the different contact surfaces are compared irrespective of gender and location, the highest mean concentration of *E. coli* were detected on the cistern handles (5.7 Log10 CFU/cm^2), internal pull latch (5.8 Log10 CFU/cm^2), external door handle (5.7 Log10 CFU/cm^2) and tap handle in shower cubicle (5.7 Log10 CFU/cm^2).

The detection of *E. coli* on almost all key contact surfaces in our study shows the potential for these surfaces to act as possible avenues or routes of pathogen transmission. Similar studies have found the handle, floor, latch of toilet door handle and tap handle to be the most contaminated (Flores et al., 2011; De Alvis et al., 2012; Sabra, 2013; Verani et al., 2014; Abiose, 2019; McGinnis et al., 2019), which corroborates our findings. For instance, the study by Fankem et al. (2006) observed that the most contaminated surfaces in public toilet facilities found in airports, bus terminals and universities were toilet seats, sinks, floors and napkin dispensers. However, that study represented different physical environments where the prevalence of contamination can be expected to be much lower (3-21%) compared to in our study. Furthermore, the toilet facilities in their study were in areas that perhaps had lesser user numbers or frequency of use compared to the CABs located in the informal settlements. In our study area, the CABs serve as the main source of sanitation for the inhabitants in these settlements. Sabra (2013) reported a higher occurrence of contamination of contact surfaces within female public toilets as s in our study. They demonstrated that over 91% of toilet handles were contaminated.

Several reasons could account for the surface-associated contaminations. These include direct deposition of faeces on these surfaces, unclean hands, and soil. For instance, surfaces such as the cistern handle, the tap handle and latch of the toilet door could have been contaminated through unclean hands. The study by De Alwis et al. (2012) observed that contamination of door handles in male toilets were highly contaminated as compared to female toilets. This was followed by a survey of the users and reported that over 50% of the males using these toilets did not wash their hands. Therefore, unclean hands could have accounted for the high contamination of surfaces regularly touched by hands. The contamination of the toilet seats and floors next to the toilets could be due to the faecal matter. Flushing of toilets has been reported to play a role in toilet seat contamination due to the generation of droplets or aerosols that may contain some faecal matter (Flores et al., 2011). Studies have shown that droplets or aerosols generated after multiple flushing could still contain bacteria, although in reduced concentrations. For instance, Johnson et al. (2017) reported a 3 Log10 in bacterial indicators after one flush, 1-2 Log10 after two flushes and thereafter, less than 1 log10. These reports,

therefore, support our hypothesis that the contamination of the toilet seats is primarily due to the presence of *E. coli* in faecal matter that is deposited either directly on these toilet seats or due to droplets or aerosols generated during flushing. One of the other most contaminated surfaces was the floor, which could be attributed to the soil from footwear (Flores et al., 2011). During the study we observed that children were playing on the floor within these toilets, this, therefore, highlights a significant health risk. In addition to soil being the main source of contamination of floors, it could also account for the contamination of the cistern handles, in addition to unclean hands (Flores et al., 2011). This conclusion was based on caretaker observation that some people used their foot for flushing of the toilets and the presence of a similar bacterial community on the toilet floors and the cistern handles. It has also been reported that some persons within the study area wipe faeces with their hands and smear these faeces on walls either due to habit, cultural or religious reasons. This was corroborated by our findings, were faecal contamination of the CAB walls was commonly observed. This practice could have contributed to the contamination of the contact surfaces.

This indicates the key contact surfaces that could potentially lead to pathogen transmission in the CABs. Although these CABs are cleaned daily, the focus may be on larger surfaces, like the floor or toilet seats, but not small contact surfaces such as the cistern handle, door latch (either internal or external) or the taps. These surfaces could therefore be harbouring large concentrations of potentially pathogenic microbes, as inferred from the concentration of *E. coli* on these surfaces.

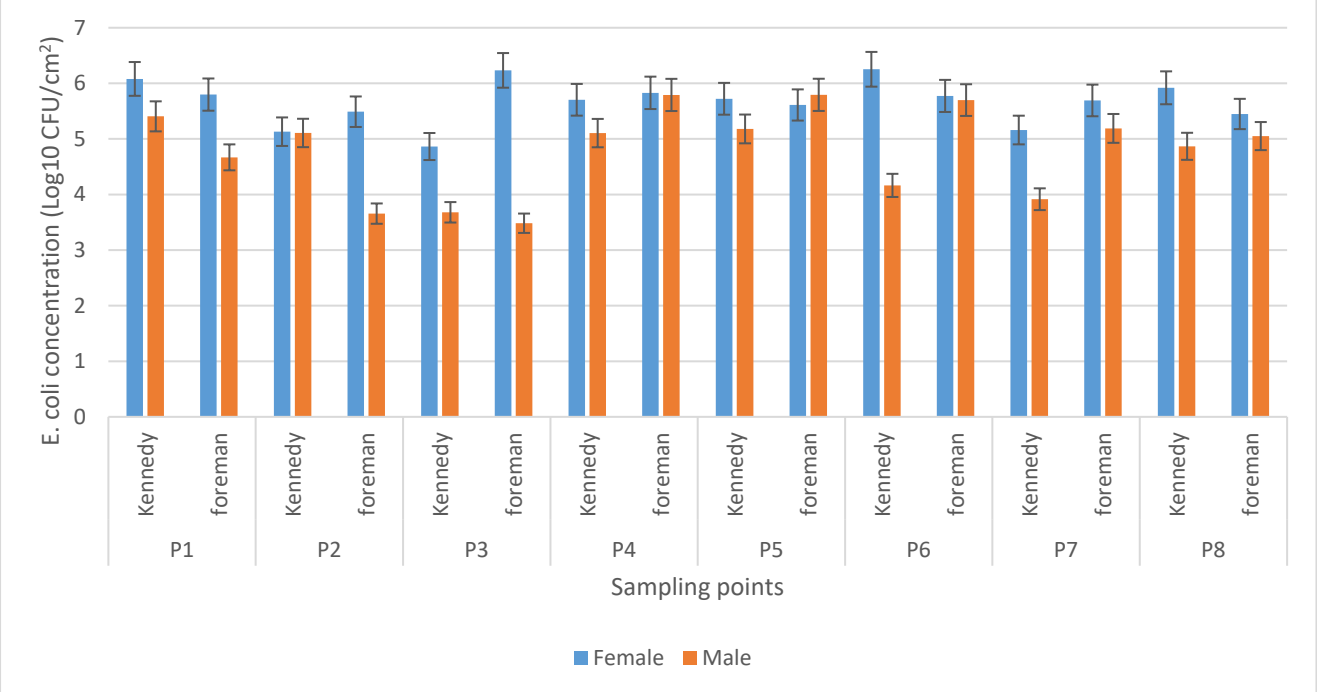

Figure 11. Concentration of *E. coli* on key contact surfaces* in community ablution blocks (CABs) within the two settlements.

*P1: Cistern handle; P2: Toilet seat; P3: Floor surface in front of toilet; P4: Internal pull latch; P5: External door handle; P6: Tap handle in shower cubicle; P7: Internal common floor surface; P8: Tap handle in wash basin

3.8 Potential risks of infection with pathogenic *E. coli* on the contact surfaces

The potential risks of infection with pathogenic *E. coli* based on the concentration on these contact surfaces varied in a similar fashion to the variation in the *E. coli* concentration measured and discussed above. **Table 1** presents the calculated median risks of infection. Briefly, considering only daily risks, at least two people out of 100 users of the CABs may be infected when they touch surfaces such as the cistern handle, external door latch and tap handle in both shower and washbasin. However, the daily risks are higher for the people that come into contact with the internal door latch of the toilet cubicles (three out of 100 people - 3.7×10^{-3} per person). Considering that users of the CABs will not touch only one surface at a time, the risks of infection will increase if the combined exposure to multiple surfaces is considered.

Based on the earlier user survey conducted in the study areas, it was observed that the populations within these informal settlements use the CABs more than once a day. We modelled the frequency of exposure in a day and assessed the risks thereof (daily risks). The risks of infection increased; for instance, the risks of infection with contact with the internal door latch increased to almost four out of 100 people exposed being infected. This increase in the risk of infection was observed in the rest of the other contact surfaces (Table 1). Multiple exposures over the course of the year also lead to a statistically significant increase in the risks of infection with pathogenic *E. coli*. As shown in Table 1, yearly exposure may result in almost every person relying on these contact surfaces been infected. This is due to the measured risks of either 1 or 9.9×10^{-1} per person per year. With the exception of the floor surface in front of the toilet cubicle, which had a risk of infection of 8.6×10^{-1} ($\pm 6.1 \times 10^{-3}$) per person per year. This could be due to the low likelihood of exposure via this route and the low concentration of *E. coli* on this surface as measured in the study.

It must be noted that the measured risks of infection do not take into consideration the possible reduction in risks achievable with proper hygienic practices such as hand washing. For instance, Friedrich et al. (2017) observed a reduction of *E. coli* contamination on hands from 1.4 (± 0.9) log10 CFU *E. coli* per hand to 1.2 (± 0.8) log10 CFU/hand. This shows that additional handwashing, perhaps at home, after using the CABs could potentially reduce the risks of infections calculated here by half. We, therefore, considered this as a major risk reduction intervention and modelled it separately under the suggested risk-reduction measures. Furthermore, it must be noted that the risks of infection could increase if the exposure to multiple surfaces is factored in, this, therefore, calls for the incorporation of other risks reduction interventions such as effective cleaning of the surfaces, especially the small contact surfaces with a high frequency of contact. Additionally, hand washing at home after use of the CABs could potentially reduce the risks further, as mentioned above. These were considered as options for risk reduction in the risk reduction section.

Table 1. Risk of infection (± 90% CI) with pathogenic *E. coli* due to one time, daily and yearly exposure to the contact surfaces* with the CABs.

	P1	P2	P3	P4	P5	P6	P7	P8
Onetime	1.9×10^{-2} ($\pm 7.1 \times 10^{-4}$)	1.1×10^{-2} ($\pm 1.4 \times 10^{-4}$)	3.7×10^{-3} ($\pm 1.5 \times 10^{-4}$)	2.5×10^{-2} ($\pm 3.0 \times 10^{-4}$)	1.6×10^{-2} ($\pm 1.9 \times 10^{-3}$)	2.1×10^{-2} ($\pm 2.1 \times 10^{-3}$)	9.7×10^{-3} ($\pm 1.8 \times 10^{-3}$)	1.8×10^{-2} ($\pm 2.0 \times 10^{-3}$)
Daily risks	2.7×10^{-2} ($\pm 1.0 \times 10^{-3}$)	1.6×10^{-2} ($\pm 2.1 \times 10^{-4}$)	5.4×10^{-3} ($\pm 1.9 \times 10^{-3}$)	3.6×10^{-2} ($\pm 4.7 \times 10^{-4}$)	2.4×10^{-2} ($\pm 2.6 \times 10^{-3}$)	3.1×10^{-2} ($\pm 2.7 \times 10^{-3}$)	1.4×10^{-2}($\pm 2.4 \times 10^{-3}$)	2.7×10^{-2} ($\pm 2.6 \times 10^{-3}$)
Yearly risks	1 ($\pm 4.3 \times 10^{-3}$)	9.9×10^{-1} ($\pm 7.4 \times 10^{-3}$)	8.6×10^{-1} ($\pm 6.1 \times 10^{-3}$)	1 ($\pm 4.8 \times 10^{-4}$)	9.9×10^{-1} ($\pm 3.1 \times 10^{-3}$)	1 ($\pm 3.8 \times 10^{-3}$)	9.9×10^{-1} ($\pm 5.4 \times 10^{-3}$)	9.9×10^{-1} ($\pm 1.8 \times 10^{-3}$)

*P1: Cistern handle; P2: Toilet seat; P3: Floor surface in front of toilet; P4: Internal pull latch; P5: External door handle; P6: Tap handle in shower cubicle; P7: Internal common floor surface; P8: Tap handle in wash basin

3.9 Semi-quantitative assessment of risks associated with the CABs

The risk associated with the external areas of these CABs was high irrespective of the settlement. For instance, we observed that all CABs were littered with solid waste around them. The same concerns apply for the other parameters assessed, which include pools of water, weeds/grass and faecal matter around the CAB. This resulted in high risk scores of 4, for all CABs (**Table 2**).

The health and safety risk scores for the internal structures and state of the CABs ranged from 7-14, indicating that all CABs within the two settlements pose some level of health and safety risks. Using the risk matrix presented above, four of the CABs in Kennedy Road and all CABs within Foreman Road poses medium risks and the remaining one at the former site poses a high risk to the users. The highest risk score (score of 14) was determined for a CAB within the Kennedy Road informal settlement. The risk posed by the internal conditions and structures was largely due to five main issues, as highlighted previously. These are described in detail under different categories as;

- ***Absence of soap in wash basins (for handwashing) and in showers*:** The lack of soap is a challenge for good hygienic practices like handwashing, which has been reported to be critical in limiting diarrhoeal infections (Luby et al., 2018; Null., 2018; Wolf et al., 2018). For instance, Luby et al. (2018) observed that 7-day diarrhoea was lowered in children who had access to combined water, sanitation and handwashing (3.5%). These findings indicate that access to sanitation coupled with good hygienic practices such as handwashing could potentially reduce health risks. Therefore, to ensure access and optimal hygiene practices, the provision of effective handwashing facilities with soap could be beneficial. As observed during the first part of the study, the *E. coli* concentration of surfaces such as the cistern handle, toilet door latch and tap handle was high. This could be due to the absence of soap, as mentioned previously, which could have led to the presence of the bacteria on the hands of the users and further transfer to these surfaces.

- ***Signs of faecal contamination on surfaces within the CAB*:** Several studies (Stenström, 2011; Pickering et al., 2012; Sclar et al., 2016; Baker et al., 2018) have reported the presence of pathogens in faeces, therefore the presence of faecal matter on contact surfaces within the CAB may play a role in the transmission of pathogens among users. This could be the reason for the high *E. coli* contamination observed in this study.

- ***Dirty diapers, newspapers, plastic, sand/mud and sanitary pads/tampons present inside toilets*:** Reports of sewer blockages was common in the CABs based on interactions with caretakers employed to clean these facilities as well as users. These blockages could be due to the presence of these materials. These materials may potentially block the sewer pipes and infrastructure resulting in sewer overflows, spillage and seepages which was seen in some of the CABs (**Table 3**). It is further noted that no refuse bins, receptacles and skips were found at any of the CABs assessed.

- ***The presence of flies within the CABs was also a major issue*:** this could be attributed to the faecal matter and blocked sewer pipes resulting in overflows. These flies could facilitate the transmission of pathogens from the CABs to households in proximity. This is therefore a major health risk, not only for the users but for the community in general. Siregar and Susanna (2020) reported the presence of *E. coli* on the common houseflies (*Musca domestica*), these could therefore potentially lead to diarrhoea. This was corroborated by Das et al. (2018) who reported a reduction in childhood diarrhoea corresponding with a reduction in fly population due to the use of insecticide sprays.

- ***Inadequate lighting in the CABs at night*:** This is both a health and a safety issue for the users. Without adequate lighting at night, these CABs may become crime scenes which may be a deterrent for their use. Additionally, due to the fear of attacks especially among women and children, the inhabitants may then choose other options for their sanitation needs. This could be open defaecation or the use of night buckets where faecal content is disposed into stormwater systems or open spaces within settlements. The practice of open defaecation and indiscriminate disposal of faecal matter and urine could expose the community to faecal-oral disease transmission.

Table 2: Risks associated with the external areas of the CABs.

	Kennedy 1		Kennedy 2		Kennedy 3		Kennedy 4		Kennedy 5		Foreman 1		Foreman 2		Foreman 3	
	0	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
Solid waste around CAB		1		1		1		1		1		1		1		1
Pools of water around CAB		1		1		1		1		1		1		1		1
Weeds/grass around CAB		1		1		1		1		1		1		1		1
Faecal matter around CAB		1		1		1		1		1		1		1		1
Risk Score		**4**		**4**		**4**		**4**		**4**		**4**		**4**		**4**

Key: 0: No risks; 1: Risks

RISK MATRIX	
Risk category	**Risk Score**
Low risk	0-1
Medium risk	2-3
High risk	4

Table 3. Risk scoring for major health and safety challenges associated with the internal areas of the CABs.

	Kennedy 1		Kennedy 2		Kennedy 3		Kennedy 4		Kennedy 5		Foreman 1		Foreman 2		Foreman 3	
	0	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**	**0**	**1**
Functional wash hand basin inside CAB	0			1	0			1	0		0		0		0	
Functional wash trough outside CAB	0		0		0		0		0		0		0		0	
Functional showers	0			1		1	0		0			1		1	0	
Soap in the shower		1		1		1		1		1		1		1		1
Stagnant water in the shower	0			1		1		1	0		0		0			1
Functional flush toilets	0			1		1		1	0	1		1	0		0	
Toilet seats are intact and functional	0			1		1		1		1	0			1		1
Soap available for handwashing		1		1		1		1		1		1		1		1
Signs of faecal contamination on floors, ceilings, windows, doors, handles, toilet seats and bowls		1		1		1		1		1		1		1		1
Dirty diapers, newspapers, plastic, sand/mud and sanitary pads/tampons present inside CAB:		1		1		1		1		1		1		1		1
Faecal and urine odour in the toilets		1		1		1		1		1		1	0		0	
Adequate ventilation	0		0		0		0		0		0		0		0	
Presence of flies and other insects		1		1		1	0			1	0		0		0	
Fresh water leakages inside the CAB	0			1	0		0		0			1	0			1
Greywater leakages inside the CAB	0		0		0		0		0		0		0		0	
Sewerage overflows inside the CAB	0			1		1	0		0			1	0		0	
Toilet cubicle doors are functional	0		0		0			1		1		1	0		0	
Roof of CAB intact with no defects	0		0		0		0		0		0		0		0	
Walls of CAB intact with no defects	0		0		0		0		0		0		0		0	
Electrical dangers in using the toilets (e.g. no bare electrical connections)	0		0		0		0		0		0		0		0	
Adequate lighting in the facility at night (inside facility).		1		1		1		1		1		1		1		1
Risk Score		7		14		12		11		10		11		7		8

Key: 0: No risks; 1: Risks

RISK MATRIX	
Risk category	**Risk Score**
Low risk	0-6
Medium risk	7-13
High risk	14-21

4. CHALLENGES FACED BY CARETAKERS OF THE CABs

4.1 Socio-economic and demographic profile of caretakers

All three caretakers in Foreman Road and three of the five caretakers in Kennedy Road were female. All caretakers reside within the settlement in which the CABs are located and are therefore available to work on weekends and during holidays. The duration of employment as a caretaker varies; for instance, two caretakers at Kennedy Road have been employed for between 5 and 6 years. The rest of the caretakers (irrespective of settlement) have been employed for between 3 and 4 years. This suggests that the years of experience was not significantly different across caretakers, with most being employed for a time period of 3-5 years. Most of the caretakers in Kennedy Road (80%) have had some secondary school education compared with those at Foreman Road, where only two of three had completed primary education only. These limited levels of education may have the potential to affect their understanding of hygiene and management training they received including related health consequences in respect of an inadequately cleaned CAB.

4.2 Maintenance and operational duties

All caretakers interviewed stated that their respective CABs are not locked at any time during the day or night. Caretakers in Foreman Road settlement stated that they clean the CAB more than 4 times a day as compared with the Kennedy locality, where the majority of caretakers (80%) clean twice daily. This means that the Foreman Road CABs may potentially have a higher level of cleanliness than Kennedy Road. The caretakers indicated that adequate water supply is available for cleaning these CABs. As affirmed by the caretakers, each of them reported that they make an entry in a written-roster each time they complete their cleaning tasks for the day. Additionally, they reported that they receive cleaning materials including detergents, disinfectants and two lots of 48 toilet rolls on a monthly basis. Whilst they indicated that cleaning tools such as a mop, broom, and toilet brush are issued monthly, *Personal Protective Equipment* (PPE) such as safety boots and overalls are provided annually by the Municipality. According to all the caretakers, there is no supply of soap at the CABs for handwashing, implying that this may contribute to poor or inadequate hygiene amongst users and to potential faecal-oral transmission of diseases. This supports the findings made during the semi-quantitative assessment.

Based on the responses from the caretakers (**Table 4**) the main operational challenges faced by the caretakers are blocked toilets. These appear to occur monthly in Kennedy Road and weekly in Foreman Road. They indicated that most of these blockages were largely caused by newspapers, sanitary pads/tampons, cardboards, pieces of cement bags, plastics and children's toys. None of the CABs inspected contained refuse bins or receptacles within or in close proximity to these facilities. The absence of safe refuse containment and collection contributes to inadequate waste management and thus indiscriminate dumping. Dirty toilets, dirty floors and stagnant water are some of the frequent challenges faced by the caretakers. This could account for the presence of flies around the CABs (based on survey response) and could expose the caretakers to several health risks. Table 4 lists the frequency of challenges encountered by caretakers employed at the Kennedy Road and Foreman Road sites. The most frequently reported challenges (daily) for both settlements were; faeces on toilet surfaces and on floors inside cubicle; faeces, soil and waste on floors inside the CABs; faeces outside the CABs, stagnant water in the showers, broken sewer pipes, non-functioning toilets/urinals and stagnant greywater outside the CABs. These observations could account for the high level of microbial contamination reported on the contact surfaces within the CABs and the associated high risks of infections calculated. This calls for the implementation of risks reduction measures.

Table 4. Operational challenges faced by CAB caretakers at Kennedy Road (n=5) and Foreman Road (n = 3) informal settlements.

	Frequency of Challenge (Kennedy Road)				Frequency of Challenge (Foreman Road)			
	Daily	**Weekly**	**Monthly**	**Biannually**	**Daily**	**Weekly**	**Monthly**	**Biannually**
Blocked toilets			5 (100%)		Blocked toilets		3(100%)	
Toilets dirty (faeces on toilet surfaces and on floors inside cubicle)	5 (100%)				Toilets dirty (faeces on toilet surfaces and on floors inside cubicle)	3 (100%)		
Sewer surcharge from toilets and/or broken pipes			4 (80%)	1 (20%)	Sewer surcharge from toilets and/or broken pipes	3 (100%)		
Dirty (faeces, soil and waste) on floors inside CAB	5 (100%)				Dirty (faeces, soil and waste) on floors inside CAB	3 (100%)		
Toilets don't flush properly			5 (100%)		Toilets don't flush properly	3 (100%)		
Taps in wash hand basin not working			5 (100%)		Taps in wash hand basin not working			3 (100%)
Floor surface of shower facility dirty and/or water stagnant	5 (100%)				Floor surface of shower facility dirty and/or water stagnant		3(100%)	
Dirty area (faeces, waste) outside CAB	4 (80%)	1 (20%)			Dirty area (faeces, waste) outside CAB	3 (100%)		
Urinals not working			5 (100%)		Urinals not working	3 (100%)		
Stagnant greywater outside CAB	5 (100%)				Stagnant greywater outside CAB	3 (100%)		

At the Kennedy Road settlement, the caretakers reported that they received training from the Municipality on how to clean and maintain the CABs whilst at the Foreman Road settlement, the two caretakers that responded to this question said they had not received any training. However, all the caretakers, including the two from Foreman Road who said they had not received any training on how to clean and maintain the CABs, stated that they have been trained on how to repair small operational faults in the CABs. However, they also reported that they have not been supplied with the necessary tools for these repairs. These responses, therefore, indicate that training of caretakers was previously done by the Municipality. However, caretakers are unable to fix minor faults including water leaks which cost the municipality millions of rand in non-revenue losses. In an event or situation whereby caretakers are unable to fix or repair a defect, these faults are reported to the eThekwini Municipality Call Centre via a phone call, SMS or WhatsApp message. The fault is logged on the Municipality's fault logging system (Faultman). A unique Reference Number is generated and provided to the caretaker for tracking and/or following up on complaints logged. The Call Centre subsequently dispatches the fault to the relevant Superintendent's clipboard for action.

4.3 Hygiene and occupational risk for caretakers

According to caretakers at the Kennedy Road informal settlement, they are issued with rubber gloves in addition to the cleaning materials and consumables on a monthly basis. However, the one caretaker who responded to this question at Foreman Road said this is provided once every two months. The caretakers also reported that they are provided with other PPE including overalls, safety boots and masks. In relation to diarrhoea awareness, four (out of five) caretakers in Kennedy Road and one (out of three) caretakers in Foreman Road informal settlements respectively, indicated that they were aware of the term 'diarrhoea'. The caretakers who responded 'yes' to knowing what diarrhoea is gave several ways through which they could contract the disease. These included the following: not washing their hands with soap, not using gloves and masks during CAB cleaning, drinking dirty water and playing in dirty places or close to an unclean toilet. In addition, the caretakers also attributed diarrhoeal infections to the consumption of contaminated food and dirty hands. The caretakers listed germs (bacteria and viruses) and worms as the main causes of diarrhoea.

Only one caretaker from Foreman Road experienced diarrhoea for a duration of one day in the month preceding the survey. This caretaker did not think the diarrhoea was caused due to her work duties and responsibilities as a caretaker of a CAB. A caretaker within the Kennedy Road site reported they he/she had diarrhoea for a duration of three days in the month preceding the survey. Diarrhoea could be classified into four types, each reflecting a different pathogenesis, including acute watery diarrhoea, dysentery, persistent or prolonged diarrhoea and chronic diarrhoea (Vesikari and Torun, 1994). According to the *World Health Organization* (WHO), diarrhoea is defined as the "passage of 3 or more loose or liquid stools per day, or more frequent passage than is normal for the individual" (WHO, 2017). Based on the case definition and duration of symptoms, both caretakers experienced acute watery diarrhoea which is characterised by an abrupt onset of frequent, watery, loose stools without visible blood usually subsiding within 72 hours of onset (Vesikari and Torun, 1994). The identification of potential pathogens on the contact surfaces and the risks estimates reported earlier shows the possibility

of these infections coming from contact with contaminated surfaces within the CABs in their line of duty.

The diarrhoeal incidence caused the caretakers to miss work for at least two days. It is noted that only one caretaker in Foreman Road indicated that she was unable to find a substitute cleaner to manage and clean the CAB in her absence, hence the majority of CABs could be maintained during such periods of caretaker illness. No other health or safety issues relating to their work were reported by the caretakers.

The caretaker responses on the routes of transmission and causes of diarrhoea especially in relation to their duties as caretakers shows a good understanding of their occupational risk in respect of diarrhoeal infections. It is well known that good hygienic practices and access to safe water for drinking are the major interventions that could prevent and/or limit diarrhoeal infections. The caretakers, therefore, show a good understanding of this. This could account for the relatively low incidence of diarrhoea among the caretakers. In addition, the provision of adequate PPE and other materials may have contributed to the low incidence of diarrhoea.

5. PROPOSED INTERVENTIONS

The observed and calculated health and safety risks reported and discussed above calls for the incorporation of risks reduction measures. This section contains some of the proposed measures. The section is categorised into two sections, firstly the reduction of health risks and secondly measures to help caretakers to undertake their duties effectively as well as maintain a clean environment within the CABs.

5.1 Risk reduction interventions

5.1.1 Improved hygiene of users: There is a need for public education among users of the CABs to encourage hygiene practices such as the washing of hands with or even without soap to reduce contamination of their hands and possibly other surfaces.

5.1.2 Improved hygiene of users: There is a need for public education among users of the CABs to encourage hygiene practices such as the washing of hands with or even without soap to reduce contamination of their hands and possibly other surfaces.

5.1.3 Supply of soap: The municipal authorities provide detergents for cleaning, however the lack of soap for hand washing in these facilities has shown to pose a challenge. Therefore, the provision of soap for handwashing could potentially reduce the prevalence of contamination.

The incorporation of the risk reduction measures into the QMRA approach resulted in lesser risks estimates. We, therefore, modelled the possible reduction in risks when simple mitigation measures such as thorough cleaning of the surfaces and washing of hands with and without soap are implemented. A simple wipe of surfaces with soap led to about 1 Log10 bacterial reduction on surfaces (Tuladhar et al., 2012). A second wipe achieved a further 1-3 Log10 reduction. Therefore, regular wiping on these surfaces by either the users or caretakers could potentially reduce the contaminations further. This will subsequently lead to lower risks of infections. A further reduction in the risks is achievable if the users of these facilities washed their hands with running water; either with or without soap. Only 23% hands were contaminated after washing with only water, with a further reduction to only 8% hands contaminated when washed with soap as observed by Burton et al. (2011). Similar results were obtained in another study (Jensen et al., 2015), with a 1 (±0.4) Log10 CFU reduction achieved without soap and 1.7 (±0.8) Log10 CFU with soap. Incorporating these possible risk reduction strategies resulted in a reduced risk as presented in **Table 5**. For instance, the risk of infection due to contact with the internal pull latch of the toilet door reduced to about three out of a 1000 people been infected ($2.6x10^{-3}$ ± 2.2 $x10^{-4}$) per person per one-time exposure. This is a significant reduction from the risks calculated when these risk reduction measures are not incorporated (Table 1).

Table 5. Risk of infection (± 90% CI) with pathogenic *E. coli* due to one time, daily and yearly exposure to the contact surfaces* with the CABs after implementation of risks reduction measures.

		P1	P2	P3	P4	P5	P6	P7	P8
Onetime	Hand washing with water	$2.0x10^{-3}$ ($\pm 4.1 x10^{-4}$)	$1.6x10^{-3}$ ($\pm 2.4 x10^{-5}$)	$1.6x10^{-4}$ ($\pm 1.8x10^{-5}$)	$2.6x10^{-3}$ ($\pm 2.2 x10^{-4}$)	$1.8x10^{-3}$ ($\pm 2.1 x10^{-4}$)	$2.5x10^{-3}$ ($\pm 3.2x10^{-4}$)	$6.2x10^{-4}$ ($\pm 4.8 x10^{-5}$)	$1.9x10^{-3}$ ($\pm 2.4 x10^{-4}$)
	Hand washing with soap	$2.9x10^{-4}$ ($\pm 6.1 x10^{-5}$)	$1.2x10^{-4}$ ($\pm 1.4 x10^{-5}$)	$3.2x10^{-5}$ ($\pm 1.5x10^{-5}$)	$4.4x10^{-4}$ ($\pm 3.0 x10^{-5}$)	$1.6x10^{-4}$ ($\pm 1.9 x10^{-5}$)	$3.1x10^{-4}$ ($\pm 2.1 x10^{-5}$)	$9.1x10^{-5}$ ($\pm 1.8 x10^{-5}$)	$2.4x10^{-4}$ ($\pm 2.0 x10^{-5}$)
Daily risks	Hand washing with water	$3.7x10^{-3}$ ($\pm 1.8 x10^{-4}$)	$2.4x10^{-3}$ ($\pm 1.7 x10^{-4}$)	$6.3x10^{-4}$ ($\pm 2.9x10^{-4}$)	$7.6x10^{-3}$ ($\pm 3.4 x10^{-4}$)	$2.7x10^{-3}$ ($\pm 1.4 x10^{-4}$)	$5.1x10^{-3}$ ($\pm 2.1 x10^{-4}$)	$8.4x10^{-4}$ ($\pm 2.5 x10^{-4}$)	$3.1x10^{-3}$ ($\pm 1.4 x10^{-4}$)
	Hand washing with soap	$3.7x10^{-4}$ ($\pm 1.0 x10^{-5}$)	$2.1x10^{-4}$ ($\pm 2.1x10^{-5}$)	$4.8x10^{-5}$ ($\pm 1.9 x10^{-5}$)	$5.5x10^{-4}$ ($\pm 4.7 x10^{-5}$)	$2.8x10^{-4}$ ($\pm 2.6 x10^{-5}$)	$4.5x10^{-4}$ ($\pm 2.7 x10^{-5}$)	$1.2x10^{-4}$ ($\pm 2.4 x10^{-5}$)	$3.2x10^{-4}$ ($\pm 2.6 x10^{-5}$)
Yearly risks	Hand washing with water	$5.6 x10^{-2}$ ($\pm 4.6x10^{-3}$)	$2.1x10^{-2}$ ($\pm 7.6x10^{-3}$)	$1.0x10^{-2}$ ($\pm 6.3x10^{-3}$)	$8.6 x10^{-2}$ ($\pm 7.1 x10^{-3}$)	$2.3x10^{-2}$ ($\pm 3.2 x10^{-3}$)	$8.3 x10^{-2}$ ($\pm 3.9x10^{-3}$)	$1.3x10^{-2}$ ($\pm 5.5 x10^{-3}$)	$3.4x10^{-2}$ ($\pm 1.9 x10^{-3}$)
	Hand washing with soap	$4.2 x10^{-2}$ ($\pm 5.7x10^{-3}$)	$1.7x10^{-2}$ ($\pm 9.7x10^{-3}$)	$6.2x10^{-3}$ ($\pm 7.9x10^{-4}$)	$6.1 x10^{-2}$ ($\pm 3.4 x10^{-3}$)	$1.9x10^{-2}$ ($\pm 4.4 x10^{-3}$)	$5.9 x10^{-2}$ ($\pm 4.4x10^{-3}$)	$8.4x10^{-3}$ ($\pm 6.9 x10^{-4}$)	$2.9x10^{-2}$ ($\pm 2.3 x10^{-3}$)

*P1: Cistern handle; P2: Toilet seat; P3: Floor surface in front of toilet; P4: Internal pull latch; P5: External door handle; P6: Tap handle in shower cubicle; P7: Internal common floor surface; P8: Tap handle in wash basin.

5.2 Additional interventions

5.2.1 Upskilling of caretakers: The responses from the caretakers indicated there was previously some kind of training on cleanliness and maintenance of the CABs, however, some of them may have forgotten. Therefore, there is a need for regular upskilling. Additionally, the presence of solid waste, pools of water and faeces around the CABs was another major finding identified during the CAB risk assessment in specific relation to the external areas. It is therefore recommended that the caretakers are educated on the need to clean the surrounding areas of the CAB as part of their duties.

5.2.2 Provision of tools for minor repairs: Although these caretakers were trained on how to repair minor faults, per their own responses, they do not have the requisite tools to carry out this work. Therefore, the provision of these tools could reduce the issues surrounding greywater inside the showers and minor blockages and wasting of reticulated water via small leaks. This could potentially save the municipality costs relating to repairs.

5.2.3 Solid waste disposal: Provision of moveable refuse receptacles at CAB sites could also be explored as a medium-to-long-term strategy explored by the municipality to address the disposal of solid waste around and within the CABs.

5.2.4 Lighting: The provision of adequate and sustainable lighting around these CABs at night could be very helpful in reducing defaecation around these CABs.

5.2.5 Enhance monitoring and rapid response to complaints: A number of the CABs had non-functional taps, non-functional toilets, broken and/or missing toilet seats and leakages, thereby reducing their utility by the population. Therefore with a better monitoring and surveillance regime and rapid response to fault complaints, these issues could be addressed.

6. CONCLUDING REMARKS

The study shows that contamination of key contact surfaces within shared sanitation facilities is a common occurrence. This contamination could be due to several factors that pertain to hygiene practices and the general habits of the users of these facilities. These habits and practices could have led to faecal matter contamination either directly or from toilet flushing, from unclean hands and soiling from footwear. This poses potentially higher risks of infection, with almost everyone using these facilities at risk of infection over the course of a year due to reliance on these facilities. However, the incorporation of risk reduction strategies such as wiping of the surfaces and washing of hands could significant reduce these infection risks. The conditions in the immediate surroundings of these CABs also pose significant health and safety risks for the users requiring immediate action. Internally, the lack of soap for handwashing, faecal matter on the doors and walls, materials that could potentially cause sewer blockages, flies and lack of lighting are the main health and safety issues identified.

The findings of this study has implications for future practices and decision-making in relation to sanitation delivery within informal settlements and shared sanitation in general. Evaluation of sanitation facilities has largely focused on the use of these facilities and their physical state. However, this study presented a holistic approach that can be used to estimate risks associated with the use of these facilities. This approach could be added to the evaluation of sanitation facilities, to provide input on the potential risks. This would be very instrumental in ensuring that sanitation facilities do not become hotspots for disease transmission.

7. PROJECT OUTPUT

Therefore, in addition to this report the project yielded the following products or study ouput, namely:

7.1 A short documentary highlighting the significance of this study, background on the municipality's CAB initiative, the key findings and recommendations was prepared. The aim of the documentary is to explain the project in simple terms so that practitioners and engineers in the field will be able to understand the main study findings and implications for further research and future practice. This we hope will serve as dissemination tool to encourage other studies in the future to consider a video documentary to better explain their scientific projects. Copy of the final edited version will be made available to both the Water Research Commission and eThekwini Municipality upon completion.

7.2 A digital health educational pamphlet with WhatsApp usability has also been developed in both English and isiZulu versions for sharing and distribution to eThekwini informal communities. We are currently awaiting final permission/approval from eThekwini Communications Unit to release the posters into the public domain. Both versions of the digital poster will be made available to the *Water Research Commission* (WRC) and indeed distributed to community upon final permission granted by eThekwini Municipality.

7.3 Finally, the following scientific papers have been written on various aspects or objectives of the study and have been submitted to various journals, namely:

(i) **Ramlal P.S.***, T.A. Stenström T.A., Munien S, Buckley, C.A., Amoah, I.D. and Sershen. 2019. Relationships between shared sanitation facilities and diarrhoeal and soil-transmitted helminth infections: An analytical review. Journal of Water, Sanitation and Hygiene for Development, vol 9, 2 (2019) doi: 10.2166/washdev.2019.180 **[Status: Published]**

(ii) **Ramlal, P.S.***, Amoah, I.D., Pillay, S., Gounden, T., Ramsuran, N and Buckley, C.A. 2020. 'The role of shared sanitation in promoting access to sanitation and hygiene within the context of COVID-19: An assessment of the Community Ablution Blocks (CABs) in eThekwini, South Africa.' Alternation Journal, Interdisciplinary Journal for the Study of the Arts and Humanities in Southern Africa **[Status: Accepted and Under Review]**

(iii) **Ramlal, P.S.***, Lin, J., Buckley, C.A., Stenström, T.A., Amoah, I.D., Okpeku, M., Kanzi, A. and Ramsuran, V. 2021. 16S rRNA-based metagenomic profile of microbes on contact surfaces within shared sanitation facilities. Ecological Genetics and Genomics. [**Status: Published]**

(iv) **Ramlal, P.S.***, Lin, J., Buckley, C.A., Stenström, T.A. and Amoah, I.D., 2021. An assessment of the health risks associated with shared sanitation: A case study of the community ablution blocks in Durban, South Africa. Environmental Monitoring and Assessment. [**Status: Accepted and Under Final Review]**

(v) **Ramlal, P.S.***, Lin, J., Buckley, C.A., Stenström, T.A. and Amoah, I.D., 2021. Determining the role of sanitation and hygiene in diarrhoeal infections among inhabitants of informal settlements in the eThekwini Municipality of South Africa. International Journal of Hygiene and Environmental Health**. [Status: Submitted to Journal].**

Mr P.S Ramlal led the design of the manuscripts, acquired and analysed the literature and data, drafted the majority of the manuscripts. All co-authors provided important intellectual content, revised the draft manuscripts and reviewed the final manuscripts. Funder: WRC of South Africa has been cited in all manuscripts and copies of each published article will be submitted upon publication by respective journals.

Isaac Dennis Amoah, Leanne Pillay, Nashia Deepnarian, Oluyemi Awolusi, Kriveshin Pillay, **Preshod Ramlal**, Sheena Kumari, Faizal Bux. 2021. Detection of SARS-CoV-2 on contact surfaces within shared sanitation facilities and assessment of the potential risks for COVID-19 infections. International Journal of Hygiene and Environmental Health. **[Status: Published].** Please note that this is an additional publication to the study, done in collaboration with Institute of Water and Wastewater Technology, *Durban University of Technology* (DUT). However this study was funded by the DUT.

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APPENDIX I

Table S1. Surface area swabbed for each contact surface.

Sampling Point	Surface Area (cm^2)
1. Cistern Handle	10
2. Toilet Seat	25
3. Floor Surface in front of Toilet	25
4. Internal Pull Latch	10
5. External Door Handle	10
6. Tap Handle (Shower Cubicle)	15
7. Internal Common Floor Surface Area	25
8. Tap (Wash Hand Basin)	15

Table S2. Primer sets for *uidA* detection in *E. coli* isolates.

Primers	Sequences	Product size (bp)
uidA-F	5'ATGCCAGTCCAGCGTTTTTGC 3'	102 bp
uidA-R	5' AAAGTGTGGGTCAATAATCAGGAAGTG 3'	

The conditions for thermocycling were as follows:

Denaturation: 94°C for 5 minutes. 1cycle

Annealing: 94°C for 30 sec. 63°C for 30 sec.

Extension: 72°C for 1.5 minute. 30 cycles

Final extension: 72°C for 5 min.1cycle.

APPENDIX II

Community Ablution Block (CAB) Observational Checklist and Risk Assessment Protocol

Area of Observation:

Name of informal settlement:	

Date of Observation: _____

Assessment undertaken by:……

ID NO OF CABs:

MALE		FEMALE		ID NO:	

GPS Coordinates:	

EXTERNAL AREA OBSERVATION

Physical condition of areas in immediate vicinity of CABs	**0**	**1**	**Comments**
Solid waste around CAB			
Pools of water around CAB			
Weeds/grass around CAB			
Faecal matter around CAB			

Legend

0	Absent	Poses no health and safety risks
1	Present	Poses health and safety risks

INTERNAL OBSERVATION

	0	**1**	**Comments**
1 **Functional wash hand basin inside CAB**			
2 **Functional wash trough outside CAB**			
3 **Functional showers**			
4 **Soap in the shower**			
5 **No stagnant water in the shower**			

6 **Functional flush toilets**			
7 **Toilet seats are intact and functional**			
8 **Soap available for handwashing**			
9 **No signs of faecal contamination on:**			
(a) floors			
(b) ceilings			
(c) windows			
(d) doors / walls			
(e) handles			
(f) toilet seats			
(g) toilet bowel			
10 **No soiled materials present on inside of CAB:**			
(a) dirty diapers			
(b) newspaper/paper			
(c) plastic			
(d) sand/mud			
(e) Sanitary pads/tampons			
11 **No faecal and urine odour in the toilets**			
12 **Adequate ventilation**			
(a) Functional windows			
(b) Functional doors			
(c)Functional vents			
13 **No presence of flies and other insects**			
14 **No fresh water leakages inside the CAB**			
15 **No greywater leakages inside the CAB**			
16 **No sewerage overflows inside the CAB**			
17 **Toilet cubicle doors are functional**			
18 **Roof of CAB intact with no defects**			

[19] **Walls of CAB intact with no defects**			
[20] **No electrical dangers in using the toilets (e.g. no bare electrical connections)**			
[21] **Adequate lighting in the facility at night (inside facility).**			

Legend

0	YES	Poses no health and safety risks
1	NO	Poses health and safety risks

The level of risk from both the external and internal observations of each CAB will be used to generate a risk ranking. This ranking will be based on the total scores for each CAB. The risk ranking will then be generated

EXTERNAL AREAS	
Risk score	**Rank**
0-1	No risk
2-3	Moderate risk
4	High risk
INTERNAL AREAS	
0-6	No risk
7-13	Moderate risk
14-21	High risk

APPENDIX III

Questionnaire: Caretaker Survey

Interviewers should introduce themselves and describe their background and the purpose of the survey.

Name of informal settlement:	
CAB Identification No and location:	
Male:	Female:

Date:	

Fieldworker Name:	

CONFIDENTIAL

OCCUPATIONAL RISK AND SANITATION-RELATED DIARRHOEAL DISEASE ASSESSMENT QUESTIONNAIRE

SECTION A: SOCIO-ECONOMIC AND DEMOGRAPHIC PROFILE OF CARETAKER

A1. Sex:

	1 Male
	2 Female

A2. What is your level of education?

1 No formal education		2 Partial Primary		3 Primary Completed	
4 Partial Secondary		5 Secondary Completed		6 Certificate/Diploma	
7 Undergraduate Degree		8 Postgraduate Degree		9 Adult Based Education	

A3. What is your average monthly income?

_____

A4. How long have you been working as a caretaker for CAB?

	1 < 1 year
	2 1 – 2 years
	3 3 – 4 years
	4 5 – 6 years
	5 > 6 years

A5. Do you live in this community?

1 Yes		2 No	

A6. Do you work on public holidays?

1 Yes		2 No	

A7. If you are not working, sick, or absent for any other reasons, is there somebody else available to clean and manage your CAB(s) in your absence?

1 Yes		2 No	

SECTION B: MAINTENANCE AND OPERATIONAL DUTIES

B1. Do you lock the CAB (male and female facilities) at any time of day or night? **[If No, proceed to B2 below]**

1 Yes		2 No	

B1.1 If Yes (B1) above, what time(s) during the day or night?

1 Day-Time	2 Night-Time
From: to:	From: to:

B1.2. What are the main reasons for locking the CAB?

_____

_____

_____

B2. How many times per day do you clean the facility?

1 Once per day		2 Two times per day		3 Three times per day		4 Four times per day	
4 (If > 4 times per day, please specify):							

B3. Is there a duty roster that you have to complete after cleaning each CAB?

1 Yes		2 No	

B3.1. If Yes (B3) above, do you fill this roster after every cleaning routine or at the end of the day?

1 Yes		2 No	

B4. Is there sufficient water supply for cleaning toilets, showers and inside surfaces of CAB?

1 Yes		2 No	

B5. Do you receive cleaning materials?

1 Yes		2 No	

B5.1 What cleaning materials and equipment is supplied for cleaning and how often do you receive stock?

		a Daily	b Weekly	c Monthly	d Once every 2 months	e Quarterly	f Do not receive/not supplied	g Other (please specify)
	1 Detergents for cleaning							
	2 Disinfectants							
	3 Mop							
	4 Broom							
	5 Cloths for wiping internal surfaces							
	6 Toilet brush							

B6. How often does the Municipality supply toilet paper?

1 Daily	2 Weekly	3 Monthly	4 Once every 2 months	5 Quarterly	6 Other (please specify):

B6.1 How many individual rolls are supplied each time?

_____

B7. Is there a constant supply of soap at the facility for handwashing?

1 Yes		2 No	

B8. What do you think are the main operational problems members of the community experience with the CAB and how often do they experience them?

	Type of problem	a Daily	b Weekly	c Monthly	d Biannually	e Annually	f Never
	1 Blocked toilets						
	2 Toilets dirty (faeces on toilet surfaces and on floors inside cubicle)						
	3 Sewer surcharge from toilets and/or broken pipes						
	4 Dirty (faeces, soil and waste) on floors inside CAB						
	5 Toilets don't flush properly						
	6 Taps in wash hand basin not working						
	7 Floor surface of shower facility dirty and/or water stagnant						
	8 Dirty area (faeces, waste) outside CAB						
	9 Urinals not working						
	10 ,Stagnant greywater outside CAB						

B9. If there is a blockage in the toilet(s), what do you think usually causes this problem?

	1 Newspaper
	2 Cardboard
	3 Diapers
	4 Sanitary pads
	5 Tampons
	6 Plastic
	7 Sand/mud
	8 Pieces of cement bags
	9 Children's toys
	10 Other (please specify):

B10. Have you noticed any flies around the toilet or inside of CAB? if so when specifically?

1 Everyday		2 ≥2/week		3 1/week		4 1/month		5 Never	

B11. Have you noticed any of the following before your cleaning routine? **[You may choose more than one alternative]**

		a Urine	b Faeces	c Urine & faeces	d Water	e Soil	f Old newspaper
	1 Toilet seat						
	2 Toilet rim						
	3 Cistern handle						
	4 Floor surface around toilet						
	5 Door handle of toilet cubicle						
	6 Floor surface of shower facility						
	7 Internal common floor surface of CAB						
	8 Wash hand basin						

B12. Did you receive any training on how to clean and maintain CAB? **[If No, proceed to B13 below]**

1 Yes		2 No	

B12.1. If Yes (B12), who provided it? _____

B12.2. If 'YES' to (B12), did you receive training on the following?

TRAINING:	a YES	b NO
1 How to clean CABs with daily cleaning schedules		
2 How to repair burst pipes		
3 Plumbing		
4 First Aid		

B13. Do community members have to pay for using the CAB? **[If No, proceed to B14 below]**

1 Yes		2 No	

B13.1 If Yes (B12), who do they pay? _____

B13.2 How much do they pay? _____

B14. If you see a problem or malfunction with the CAB, what do you do?

1 I fix the problem myself		2 I call the Municipality		3 I am unable to do anything – I wait for contractors to fix problem		4 Other (please specify):

B15. Have you been supplied with tools/equipment and trained by the Municipality to fix and repair small operational defects?

1 Yes		2 No	

B16. How do you inform eThekwini Municipality of malfunctioning/defects/blockages in CABs?

1 I telephone eThekwini Water Services		2 I email, SMS or WhatsApp eThekwini Water Services		3 I visit eThekwini Water Services Department and lodge complaint		4 I inform ward councillor		5 Other (please specify):

B17. Do you use the CAB for your own personal needs? **[If Yes, proceed to C1 below]**

1 Yes		2 No	

B17.1 If No (B17), Why? _____

B17.2. If No in (B17), What alternative means (sanitation practice) do you use?

_____

SECTION C: HYGIENE AND OCCUPATIONAL RISK

C1. Do you use gloves when cleaning?

1 Yes, all the time		2 Yes, sometimes		3 No	

C1.1. If 'YES' (C1), how often do you receive new gloves?

Frequency	Tick relevant box
1Daily	
2Weekly	
3Monthly	
4Once every 2 months	
5Quarterly	
6Other (Specify in space below)	

C.2 What type of gloves do you use? _____

C3. What other personal protective equipment have been issued to you by the Municipality?

1Overall		2 Safety/Gum boots		3 Masks		4 Other (please specify):

C4. Do you walk barefoot when entering, using or cleaning the CAB?

1 Always		2 Once a month		3 Twice a week		4 Once a week		5 Never	

C5. Are you aware of the term diarrhoea?

1 Yes		2 No	

C5.1 If 'YES' to (C5), how do you think a person can contract diarrhoea?

_____

C6. What do you think causes diarrhoea? (you may choose more than one alternative)

	1 Germ / Bacterial/viral infections	
	2 Worm infection	
	3 Dirty hands	
	4 Eating spoiled/contaminated food	
	5 Other, please specify:	

C7. How many times in the last month did you experience diarrhoea? **[If you did not have diarrhoea, proceed to C8 below]**

1 Once	
2 Twice	
3Thrice	
4 More than thrice	
5 None	

C7.1. If you have had diarrhoea (C7) above, how long on average did each even last?

1 One day	
2 Two days	
3 Three days	
4 More than three days	
5 More than three days	
6 Other (please specify how long it lasted):	

C7.2. Did you miss any work days due to this event?

1 Yes		2 No	

C7.2.1: If "YES" (C7.2) above, how many days? _____

C7.3. If "YES" to C7, do you think you had this diarrhoea because you clean CABs?

1 Yes		2 No	

C8. Have you experienced any other health and/or safety risks directly related to you cleaning the CABs?

1 Yes		2 No	

C8.1. If "YES" in C8.1 above, what health condition(s) or injury was it?

_____

Thank caretaker after interview.