Profiles of antibiotic susceptibilities of bacterial isolates and physico-chemical quality of water supply in rural Venda communities, South Africa

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Abstract

The Venda region of South Africa is predominantly rural and residents rely on untreated water sources for daily water needs. The physico-chemical quality of these water sources including antibiotic susceptibilities of enteric bacterial isolates which would guide clinicians in the empiric management of diarrhoea have received cursory attention. This study therefore sought to document the physico-chemical parameters and antibiograms of bacterial isolates from water sources in order to determine the safety for human consumption and to provide updated antibiotic data for empiric treatment of patients. Water samples were obtained on a weekly basis from Ngwedi, Mutale, Tshinane, Mutshindudi and Mudaswali Rivers and Makonde, Mudaswali and Thamathama Fountains between August 2000 and July 2002. Physico-chemical parameters such as turbidity, temperature, pH, lead, fluoride, cyanide, iron, sulphate, chromate and nitrate were determined. Antibiograms of bacterial isolates were ascertained using the disk diffusion method.

Results obtained revealed that all the physico-chemical variables of the water sources analysed were within normal recommended limits for safety of drinking water except for turbidity, which exceeded recommended limits and hence precludes the rivers from direct domestic use.

Antibiogram profiles showed multiple antibiotic resistances of *Salmonella*, *Shigella, Campylobacter, Aeromonas, Vibrio cholera, Enterobacter* and *Plesiomonas* to ampicillin, tetracycline, chloramphenicol, cotrimoxazole and erythromycin. In contrast virtually all the enteric bacterial isolates showed marked susceptibilities to ciprofloxacin, nalidixic acid, gentamicin, ceftriazone and amikacin. These effective antibiotics are therefore indicated in the empiric treatment of diarrhoeal cases or water-borne diseases of bacterial aetiology.

Keywords: water quality, microbial, physico-chemical, antibiograms, enteric bacteria, rural communities, Venda region

Introduction

The multifarious uses of water for drinking, bathing, washing and cooking are well known. Water meant for human consumption should be free from pollution, safe and acceptable. Indeed, the microbial quality of water sources should not exceed the maximum limits specified in the water quality guidelines (DWAF, 1996; WRC, 1998). However, the microbial quality of river water sources in rural communities in the Venda region, Limpopo Province, South Africa have been reported to be poor, unsafe and not acceptable for human consumption (Obi et al., 2002). Several bacterial enteropathogens namely *Campylobacter jejuni/coli, Salmonella, Shigella, Plesiomonas, Aeromonas, Vibrio cholerae* and *Escherichia coli* were also isolated from the river water sources (Obi et al., 2002). These enteric bacterial pathogens are variously incriminated in cases of diarrhoea, which accounts for a substantial degree of morbidity and mortality in different age groups worldwide (Black, 1993; Nath et al., 1993; Pracho an O'Ryan; 1994, Obi et al., 1997, 1998; El-Sheikh and El-Assouli, 2001).

Isolation of pathogens from water sources connotes a serious public health risk for consumers. To further compound this problem, enteric bacterial pathogens have been widely reported to demonstrate resistance to several antibiotics (Hoge et al., 1998; Obi

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et al., 1998; McArthur and Tuckfield, 2000; Engberg et al., 2001; Ash et al., 2002). For example, in 1984, 82% of *Campylobacter* strains from Lagos, Nigeria, were sensitive to erythromycin, and 10 years later only 20.8% were sensitive (Coker and Adefeso, 1994). In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from 0% before 1991 to 84% in 1995 (Hoge et al., 1998). Strains of *S. typhi* with multiple resistances to chloramphenicol, ampicillin and trimethoprim have led to several outbreaks (Rowe et al., 1997). In the United States, several rivers were reported to be reservoirs of antibiotic resistant bacteria (Ash et al., 2002). Antibiotic resistance may occur spontaneously, by selective pressure or because of antibiotic abuse by humans or over use in animals (White et al., 2000). Although antibiotic resistance is common, antibiotics are still indicated in the management of diarrhoea. Antibiotics shorten the duration of diarrhoea, decrease stool output and may mitigate complications (Black, 1993). However, in spite of the poor water quality in rural Venda region, data on antibiotic susceptibilities of bacterial isolates from river water sources are lacking. Such data, if available, will be useful in the empiric management of patients with diarrhoea in the region because antibiograms vary with time and geographical region.

Another gap in the corpus of research activities on water sources in the Venda region is the paucity of data on physico-chemical profiles, which are also indices of water quality. Monitoring of physico-chemical profiles is essential because of their effects on human health, when safety limits are exceeded (WRC, 1998). For example, chromium is associated with the occurrence of nasal

septum, skin ulcers as well as gastrointestinal and lung cancers. Beyond maximum acceptable limits of fluoride (0 to 1.0 mg·ℓ^{-1}), slight mottling of dental enamel, tooth damage and crippling skeletal fluorosis may occur. The target water quality range (TWQR) of lead is 0 to 10 µg/c (WRC, 1998). Above this limit, there may be increased danger of behavioural changes due to neurological impairments (WRC 1998). Data on profiles of water sources will be of value in ascertaining how the water supply may influence infection and disease among consumers (Nevondo and Cloete, 1999).

In this study, the physico-chemical quality of water sources and the antibiograms of bacterial isolates from these sources are reported.

Materials and methods

Study areas

The study sites were rural communities in the Venda Region of the Limpopo Province, South Africa. The main water sources in the rural communities were identified and sampled. They comprised Vuwani, Mutshindudi, Tshinane, Ngwedi, Mutale and Mudaswali Rivers. Makonde, Thamathama and Mudaswali fountains were also included in the study.

Sample collection

The collection of water samples from the river water sources mentioned above was done weekly over the period, August 2000 to July 2002. Water samples were collected aseptically into 1 ℓ Nalgene containers and transported on ice to the base laboratories in the Department of Microbiology, University of Venda for Science and Technology and the Department of Microbiology and Biochemistry, University of Fort-Hare. Physico-chemical analyses were conducted within 24 h after collection.

Microbiological profiles

Campylobacter, Aeromonas, Plesiomonas, Salmonella, Shigella, Vibro were identified using standard methods (*Standard Methods*, 1998). Briefly, for the isolation of *Campylobacter*, Skirrow's and Butzler's media were employed. Plates were incubated at 42°C under microaerophilic conditions for 72 h. Organisms were considered to be *Campylobacter* if they were S-shaped, Gram negative bacteria, motile, oxidase-positive, grew at 42°C but not at 25° C and sensitive to nalidixic acid. For the isolation of *Aeromonas* and *Plesiomonas* spp., specimens were inoculated onto xylose deoxycholate citrate agar (XDCA), incubated at 37°C for 24 h. Non-xylose fermenting colonies on XDCA were screened for oxidase production. Oxidase-positive colonies were further confirmed as belonging to *Aeromonas* or *Plesiomonas shigelloides*. For the isolation of other enteropathogens, specimens were inoculated on appropriate media such as MacConkey agar (MCA), deoxycholate citrate agar (DCA) and TCBS. Specimens were also inoculated into enrichment broths, such as selenite F broth to enhance the isolation of *Salmonella* and *Shigella* spp, whereas alkaline peptone water (APW), pH 8.6 was employed for the enrichment of *Vibrio cholerae*, *Plesiomonas* and *Aeromonas* spp. The APW was subcultured onto thiocitrate bile salt (TCBS) agar, whereas XDCA and selenite F broth cultures were subcultured onto DCA and Shigella-Salmonella (SS) agar. All inoculated media (enrichment and subculture) were incubated at 37°C for 24 h. Biochemical tests were employed for definitive identification.

Physico-chemical analyses of water quality

Inorganic water quality parameters namely: lead, fluoride, phosphate, chromate, nitrate, nitrite and sulphate were determined by the standard photometric method (1992) using the Nova 60 photometer (Merck). The temperature and pH were measured using a pH meter (Merck). Turbidity was determined using the microprocessor turbidity meter (Hanna Instruments).

Antibiogram determination

Antibiograms were ascertained on Mueller Hinton agar (Merck) using the Kirby-Bauer disk diffusion method (Bauer et al., 1966; Obi et al., 1998). Antibiotics employed and their concentrations included ampicillin (B-lactam) – 10 µg; gentimicin (aminoglycosides) – 10 µg; tetracycline (tetracyclines) – 30 µg; nalidixic acid (quinolones) – 30 µg; ciprofloxacin (fluoroquinolanes) – 10 µg; chloromphenicol (phenicols) – 30 µg; amikacin (aminoglycosides) – 30 µg; erythromycin – 15 µg and ceftriazone – 30 µg.

Results

Physico-chemical quality

Results on physico-chemical parameters showed that turbidity readings were highest in Mutale River (8.40 NTU), followed by Ngwedi River (6.12 NTU) and Tshinane River (4.90 NTU). Turbidity values recorded for Makonde Fountain, Mudaswali Fountain and Thamathama Fountain were 2.20, 2.74 and 3.50 NTU respectively (Table 1). The South African target water quality range for turbidity in domestic water use is 0 to 1 NTU (DWAF, 1996). The cyanide, iron, fluoride, lead, phosphate, sulphate and nitrate levels for the various river water sources were below the concentrations stipulated for no risk (Table 1). The fluoride level for the various water sources ranged between <0.01 and 0.11 mg·ℓ^{-1}. The limit for no risk of fluoride is 0-1.0 mg·ℓ^{-1} (WRC 1998). The sulphate level for Ngwedi, Mutale, Mutshindudi, Mudaswali and Tshinane rivers were 23 mg·ℓ^{-1}, 49 mg·ℓ^{-1}, 14 mg·ℓ^{-1}, 19 mg·ℓ^{-1} and 17 mg·ℓ^{-1} respectively wereas the level for Makonde, Mudaswali and Thamathama fountains were 20.3 mg·ℓ^{-1}, 27 mg·ℓ^{-1} and 16 mg·ℓ^{-1} respectively. The South African guideline for sulphate in domestic water is 0 to 200 mg·ℓ^{-1}

The levels of nitrate observed for all the water sources ranged between 1.0 mg·ℓ^{-1} and 3.2 mg·ℓ^{-1}. The limit for risk of nitrate is 0-6 mg·ℓ^{-1} (WRC, 1998). The chromate levels detected for the various water sources ranged between 0.01 mg·ℓ^{-1} to 0.05 mg·ℓ^{-1}. The limit for no risk of chromate is 0 to 0.50 mg·ℓ^{-1} (WRC, 1998) (Table 1).

For the pH readings, the values between 6.00 pH units and 8.00 pH units recorded for the water sources were within the acceptable pH values of no risk (6.00 to 9.00 pH units) (WRC, 1998). The recommended temperature limit for no risk is <25°C (WRC, 1998). The temperature readings for all the water sources investigated were <25°C (Table 1).

Antibiotic susceptibility

Antibiogram results of bacterial enteropathogens presented in Table 2 revealed marked susceptibilities (over 90%) of *Campylobacter, Salmonella* and *Escherichia coli* to nalidixic acid, ciprofloxacin and amikacin. All 100% of *Salmonella* species were susceptible to Amikacin. Eighty-eight percent of *Campylobacter* species and over 90% of *Escherichia coli*, *Salmonella, Plesiomonas* and *Shigella* species were susceptible to gentamicin. *Aeromonas*, *Enterobacter*

TABLE 1
The means of physico-chemical analysis of water samples obtained from different water sources in the Venda region, South Africa

Physical and chemical parameters	Limit for no risk	Ngwedi River	Makonde fountain	Mutale River	Mutshindudi River	Mudaswali River	Mudaswali fountain	Thamathama fountain	Tshinane River
Turbidity (NTU)	0 - 1	6.12	2.20	8.40	6.54	5.00	2.74	3.50	4.90
Cyanide (mg·ℓ^{-1})	0 - 0.1	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Fluoride (mg·ℓ^{-1})	0 – 1.0	<0.01	0.01	0.11	0.09	0.02	<0.01	0.04	0.01
Lead (µg·ℓ^{-1})	0 - 10	0.23	0.04	0.04	0.01	0.09	0.16	0.04	0
Phosphate (mg·ℓ^{-1})	0 -1	<0.5	0.8	0.5	0.4	0.1	<0.5	0.4	0.9
Sulphate (mg·ℓ^{-1})	0 - 200	23	20.3	49	14	19	27	16	17
Chromate (mg·ℓ^{-1})	0 – 0.50	0.02	0.01	0.05	0.02	0.04	0.04	0.04	0.04
Nitrite (mg·ℓ^{-1})	NS	0.04	0.06	0.04	0.02	0.09	0.01	0.09	0.06
Nitrate (mg·ℓ^{-1})	0 – 6	1.0	2.0	3.2	2.3	2.0	2.9	3.0	2.7
pH (pH units)	6.0 – 9.0	6.00	6.00	7.00	6.60	7.00	8.00	6.00	6.80
Temp (°C)	<25^{0}C	24.1	22.84	23.3	21.92	20.86	22.0	21.60	23

Key:
mg·ℓ^{-1}: milligrams/litre   Temp: Temperature   NTU: Nephelometric Turbidity Unit
°C: degrees Celsius   µg·ℓ^{-1}: micrograms/litre

and *Vibrio cholerae* demonstrated over 90% susceptibilities to ciprofloxacin and over 85% of the three isolates were in addition susceptible to amikacin. In general, over 80% of all bacteria isolated were susceptible to nalidixic and gentamicin, ciprofloxacin, amikacin and ceftiriazone with the exception of *Vibrio cholerae* with susceptibility rates of 73%, 60%, 53% to nalidixic acid, ceftriazone and gentamicin respectively and *Campylobacter* species with 62% susceptibility to ceftriazone (Table 2).

At least 20% of bacterial isolates demonstrated antibiotic resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphericol. A total of 35% of the *Campylobacter* isolates were resistant to erythromycin whereas 20% to 30% of *Campylobacter, Salmonella, Shigella, Plesiomonas, Aeromonas* and *Enterobacter* isolates showed resistance to cotrimoxazole, tetracycline, ampicillin, erythromycin and chloramphenicol.

Discussion

Rural communities in the Venda region of South Africa are known to rely on river water sources, which are devoid of treatment for their domestic water needs (Obi et al., 2002). Majority of the river water sources harboured enteropathogens and were also reported to be of poor microbiological quality and unsafe for consumption (Obi et al., 2002). However, data on antibiograms of bacterial isolates and physico-chemical profiles of water sources were lacking.

In this study, the physico-chemical parameters as well as antibiotic susceptibility profiles of water sources were examined in order to establish the chemical and physical safety of water sources and to provide updated data on antibiograms of enteric pathogens for better management of patients requiring empiric antibiotic therapy.

Results revealed that the physico-chemical quality, except for turbidity, of the various water sources examined was acceptable. Consequently, the river water sources examined may not pose any health hazard to residents and consumers in terms of the physico-chemical parameters studied, except for the turbidity index. The high turbidity values preclude the rivers for direct domestic use and may also be problematic for flocculation and filtration purposes, with

consequent increase in cost of water treatment. This assertion simulates a previous one reported for the chemical quality of water supply in the Keiskamma River of South Africa (Fatoki et al., 2003). Cyanide, lead and other variables investigated are variously known to pose health risks when they exceed maximum recommended limits. They are reportedly carcinogenic and hepatotoxic (WRC, 1998) and could be responsible for loss of productive hours due to their debilitating and crippling effects (WRC, 1998). However, results revealed that consumers are not likely to experience the harmful effects of the chemical variables studied because the variables fall within the acceptable and recommended limits for safety (WRC, 1998).

The presence of enteric bacterial pathogens in water sources may spell health hazards such as diarrhoeal diseases, which accounts for a substantial degree of morbidity and mortality in adults and children (Black, 1993; Du Pont, 1995; Jousilahti et al., 1997; Wasfy et al., 2000; El-Sheikh and El-Assouli, 2001). Management of diarrhoea may require the administration of antibiotics. However, several bacteria are known to be resistant to a wide array of antibiotics. (Obi et al., 1995; Aseffa et al., 1997; Yurdakok et al., 1997; Obi et al., 1998; Wasfy et al., 2000).

Results presented showed multiple antibiotic resistance of all bacteria isolates to ampicillin, erythromycin, tetracycline, chloramphenicol and cotrimoxazole. Multiple antibiotic resistances refer to resistance to two or more classes of antibiotics. The multiple antibiotic resistances of *Salmonella, Shigella, Campylobacter, Aeromonas* and *Plesiomonas* demonstrated in this study accords with other findings (Robins-Browne et al., 1983; Coker and Adefeso, 1994; Asefa et al., 1997; Obi et al., 1997, 1998). Erythromycin used to be the drug of choice for campylobacteriosis but increasing resistance of *Campylobacter* to erythromycin is well known (Coker and Adefeso, 1994). In this study, 35% of *Campylobacter* isolates were resistant to erythromycin. Strains of *Salmonella*, particularly *Salmonella typhi*, accounted for several outbreaks in the United States and world-wide, partly due to resistance to chloramphenicol, ampicillin and trimethoprim (Rowe et al., 1997; Mermin et al., 1998). This resistance pattern simulates the 20% and 47% resistance rates of *Salmonella* obtained in this study to chloramphenicol and ampicillin respectively.

TABLE 2
Antibiograms of enteric bacterial isolates from water sources in the Venda region, South Africa

Bacteria	Antibiotic susceptibility no (%)									
	NA	GM	COT	CIP	TE	AP	ERY	CHL	AKC	CEF
Campylobacter spp (n = 26)	24 (92%)	23 (88%)	18 (69%)	25 (96%)	18 (69%)	15 (58%)	17 (65%)	16(62%)	25(96%)	16 (62%)
E. coli (n = 40)	36 (90%)	37 (92.5%)	30 (75%)	38 (95%)	28 (70%)	20 (50%)	21 (52.5)	18 (45%)	39 (97.5%)	37 (92.5%)
Salmonella spp (n = 30)	28 (93%)	29 (96.6%)	20 (66.6%)	29 (96.6%)	14 (46.6%)	16 (53%)	24 (80%)	24 (80%)	30 (100%)	28 (93%)
Shigella spp (n = 30)	26 (86.6%)	28 (93%)	19 (63%)	28 (93%)	16 (53.3%)	15 (50%)	16 (53.5%)	18 (60%)	29 (96.6%)	28 (93%)
Plesiomonas shigelloides (n = 20)	18 (90%)	18 (90%)	15 (75%)	19 (95%)	14 (70%)	13 (65%)	10 (50%)	11 (55%)	19 (95%)	18 (90%)
Aeromonas (n = 20)	18 (90%)	17 (85%)	14 (70%)	18 (90%)	15 (75%)	12 (60%)	9 (45%)	10 (50%)	18 (90%)	16 (80%)
Vibro cholerae (n = 15)	11 (73%)	8 (53%)	10 (66.6%)	14(93.3%)	7 (47%)	8 (53%)	6 (40%)	9 (60%)	13 (87%)	9 (60%)
Enterobacter spp. (n = 20)	17 (85%)	15 (75%)	16 (80%)	18 (90%)	12 (60%)	11 (55%)	10 (50%)	11 (55%)	17 (85%)	16 (80%)

Key:
NA = Nalidixic acid   TE = Tetracycline   *E. coli = Escherichia coli*   GM = Gentamicin   ERY = Erythromycin   Spp = Species
COT = Cotrimoxazole   CHL = Chloramphenicol   AP = Ampicillin   CIP = Ciprofloxacin   AKC = Amikacin
CEF = Ceftriazone

Antibiotic susceptibility profiles showed that all enteric bacterial isolates were markedly sensitive to nalidixic acid, gentamicin, ciprofloxacin, amikacin and ceftriazone. These drugs may therefore be of value for enteric infections requiring empiric (Author to check) antibiotic therapy. These reported susceptibilities are in harmony with reports of other investigators (Yurdakok et al., 1997; Wasfy et al., 2000). It should be noted that susceptibility of bacteria to antibiotics is not static and resistance may be due to antibiotic abuse, antibiotic overuse or may be chromosomally or plasmid mediated (Obi et al., 1998). Antibiotic usage must therefore be carefully regulated and monitored.

It is concluded that the physico-chemical parameters of water sources were within safe and acceptable limits. Although ciprofloxacin, nalidixic acid, gentamicin, amikacin and ceftriazone were demonstrated to be effective against the enteropathogens studied, periodic monitoring of antibiograms is necessary to detect any changing patterns. In addition, antibiogram updates may be useful in characterising isolates. The multiple resistances of isolates to some antibiotic classes are of great public health concern and calls for caution in the indiscriminate use of antibiotics on humans and animals.

Acknowledgments

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