Antibiotic resistance profiles of *Escherichia coli* isolated from different water sources in the Mmabatho locality, North-West Province, South Africa

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ABSTRACT

The antibiotic resistance profiles of *Escherichia coli* (*E. coli*), isolated from different water sources in the Mmabatho locality were evaluated. Water samples were collected from the local wastewater- and water-treatment plants, the Modimola Dam and homes in the area, and then analysed for the presence of *E. coli*, using standard methods. Presumptive isolates obtained were confirmed by the analytical profile index test. Antibiotic susceptibility testing was performed by the disc diffusion method. Of the 230 *E. coli* isolates tested, marked antibiotic resistances (over 70%) were observed for erythromycin, tetracycline, ampicillin, chloramphenicol and norfloxacin. Multiple antibiotic resistance patterns were also compiled. Overall, the phenotype T-Ap-E was frequent for *E. coli* isolated from the local wastewater and water-treatment plants, Modimola Dam and tap water. Cluster analysis performed showed a unique antibiotic resistance pattern which suggested a link between isolates from all sampling points. The findings indicated that improper wastewater treatment may have a potential impact on the dissemination and survival of *E. coli*, as well as other pathogenic bacteria in water for human and animal consumption. This may result in water- and food-borne disease outbreaks with a negative effect on antibiotic therapy.

INTRODUCTION

Escherichia coli (E. coli) is an organism that occurs universally in sewage and, because it is a faecal coliform, it plays an important role in the sanitary analysis of water.[1] Its presence in water indicates the presence of faecal contamination and the likelihood of other pathogenic microbes.[1] Five pathogenic strains of *E. coli* are frequently isolated from humans and animals suffering from diarrhoea.[2] These differ from other commensals in that they express virulence factors, which are molecules directly involved in pathogenesis, but which are also important for normal metabolic functions.[3] These pathogenic strains include:

- The enterotoxigenic *E. coli* strain, which causes traveller and infantile diarrhoea and is the main cause of haemolytic-uraemic syndrome associated with food-borne infections.[4]
- The enteroinvasive *E. coli* strain, which produces shigellosis-like diseases in children and adults.
- The enteropathogenic *E. coli* strain, which is the major cause of acute infantile diarrhoea in developing countries.
- The enteroaggressive *E. coli* strain, which produces persistent gastroenteritis and diarrhoea in infants and children,[5,6] and is prevalent in developing countries.
- The enterohemorrhagic *E. coli* strain, which is the major cause of sporadic outbreaks of haemorrhagic colitis.[7,8,9]

Antibiotic resistance in *E. coli* has been globally identified in isolates from environmental, animal and human sources.[10] This is a consequence of the use of antimicrobials in medicine and their application in animal husbandry, which have brought about phenotypic changes, often due to chromosomal mutations.[11] Studies have shown that many pathogenic organisms have developed some degree of resistance to antimicrobials and they confer resistance through different mechanisms, with a negative impact on veterinary and human medicine.[10,12,13] These mechanisms of resistance include the alteration of receptor-binding sites of drugs, a decreased intake of drugs by altering the entry or active efflux of the drug, the destruction or inactivation of the drug, and development of resistant metabolic pathways.[13]

The surfacing of antibiotic resistance usually results from the misuse of antibiotics as growth-promoters in animal production, for therapy and prophylaxis.[14] Because humans consume these animal products, there is a probability of the spread of resistant strains from animals to humans and thus healthy individuals can become carrier hosts for multiple antibiotic-resistant bacteria.[15] This may enhance the risk of developing haemolytic-uraemic syndrome, a disease more severe in children infected with *E. coli* O157:H7.[16] Several studies have revealed that *E. coli* is resistant to a number of antibiotics.[17,18,19,20] Adding to the consequences of microbial resistance to antibiotics on human health, contamination of surface water bodies with resistant bacterial strains from human activities and livestock operations has also been reported.[21] The objective of this study was to isolate *E. coli* organisms from water collected from different water sources in the Mmabatho locality in order to test their resistance to commonly used antibiotics.

METHODS

Collection of samples

Sampled sites were the inlet, primary, secondary, tertiary digesters and effluent from the local wastewater-treatment plant; the local water-treatment plant inlet and outlet; inlet, midpoint and outlet of the Modimola Dam; and tap water from a few homes in Units 8, 10 and 12 in the Mmabatho locality of the Mafikeng District.

Water samples were collected weekly over a period of two months (July to September 2006). Samples were collected aseptically in sterile 500 mL Schott Duran bottles, transported on ice to the Microbiology Laboratory at the Department of Biological Sciences, University of the North-West (South Africa) and plated out within 24 h.

Identification of *E. coli* isolates

Analyses of water samples were performed according to the standard method[22] for total and faecal coliform counts on m-Endo (Merck , Johannesburg, South Africa) and m-FC (Merck, Johannesburg, South Africa) agar plates incubated at 37 °C and 44.5 °C for 24 h, respectively. *Escherichia coli* ATCC® 25922 was used as a positive control.[23] Characteristic metallic-sheen and blue-coloured colonies on m-Endo and m-FC agar plates were selected and purified by streaking on nutrient agar (Biolab, Johannesburg, South Africa) plates. Plates were incubated at 37 °C for 24 h and stored for further use. Isolates were Gram-stained according to standard methods[24] and all Gram-negative isolates were subjected to primary and secondary biochemical identification. The primary biochemical tests performed were the triple sugar iron (TSI) agar, Simmons citrate agar, and oxidase tests, while the secondary biochemical test performed was the analytical profile index (API) 20E test. All tests were performed according to manufacturer's instructions (BioMérieux, France).

Antibiotic susceptibility test

Antibiotic susceptibility tests were performed on all *E. coli* positive isolates by the disc diffusion method, as previously described.[25] Bacterial suspensions of isolates were prepared and aliquots of 100 µL plated out on Mueller Hinton agar (Merck, Johannesburg, South Africa). Antimicrobial discs (Mast Diagnostics, Sefton, UK) impregnated with kanamycin (30 µg), streptomycin (300 µg), erythromycin (15 µg), tetracycline (30 µg), ampicillin (10 µg), norfloxacin (10 µg) and chloramphenicol (30 µg) were placed on the Mueller Hinton agar plates and incubated at 37 °C for 24 h. After incubation, the inhibition zone diameters were measured and classified using reference values.[26] Multiple antibiotic resistant (MAR) phenotypes were generated for isolates that showed resistance to three or more antibiotics.[27] MAR indices were evaluated as previously described.[28]

Cluster analysis

Susceptibility data for *E. coli* isolates from the different samples were determined using Ward's method and Euclidean distances on Statistica Software (version 7.0).

RESULTS

Antibiotic resistance data

A total of 230 *E. coli* isolates were obtained following biochemical characterisation (Table 1). Antibiogram results of *E. coli* isolates (Table 2) revealed resistance to more than one antibiotic, similar

TABLE 1
The proportion of isolates obtained for the various biochemical tests

Biochemical test		Oxidase test	TSI					SCT	API 20E
Sample source		Oxidase	Lactose	Glucose	Sucrose	Gas	H_2S	Citrate	*E. coli*
		-ve	(+ve)	(+ve)	(+ve)	(+ve)	(+ve)	(+ve)	
Wastewater	Inlet	1 (40/40)	1	1	1	1	0	1	0.75 (30/40)
	Primary	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Secondary	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Tertiary	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Effluent	1 40//40)	1	1	1	1	0	1	0.5 (20/40)
Modimola Dam	Inlet	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Midpoint	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Outlet	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
Tap water	Unit 8	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Unit 10	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)
	Unit 12	1 (40/40)	1	1	1	1	0	1	0.5 (20/40)

TSI: triple sugar iron agar test, SCT: Simmons citrate agar test, API: analytical profile index, +ve: positive, -ve: negative.

TABLE 2
The percentage antibiotic resistance of *E. coli* isolated from the different sampling sites

	Antibiotics						
Sampling site	K (30)	C (30)	Nor (10)	T (30)	Ap (10)	E (15)	S (300)
Wastewater (Inlet)	15	20	15	70	80	85	5
Wastewater (Primary)	50	55	45	75	75	95	5
Wastewater (Secondary)	25	35	35	85	45	90	0
Wastewater (Tertiary)	60	75	80	95	70	90	15
Wastewater (Effluent)	15	50	50	65	30	95	10
Modimola Dam (Inlet)	0	45	30	80	60	100	0
Modimola Dam (Midpoint)	20	80	40	80	35	95	10
Modimola Dam (Outlet)	0	5	15	50	50	95	0
Inlet water	10	30	30	75	20	100	0
Outlet water	0	20	0	65	10	75	0
Tap water (Unit 8)	0	50	0	5	65	60	5
Tap water (Unit 10)	10	5	0	60	35	90	0
Tap water (Unit 12)	15	40	0	35	70	50	5

TABLE 3
The predominant multiple antibiotic resistant (MAR) phenotypes for *E. coli* isolated from the different sampling sites

Wastewater inlet isolates (N = 30)		
MAR phenotypes	**Number observed**	**Percentage**
T-Ap-E-S	1	3.3
T-Ap-E	6	20
K-C-Nor-T-Ap-E	2	6.7
K-C-T-E	1	3.3
C-Nor-T-Ap-E	1	3.3

Wastewater primary digester isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
T-Ap-E	3	15
K-C-Nor-T-Ap-E	6	30
C-Nor-T-Ap-E	2	10
K-C-T-Ap-E	3	15
K-E-S	1	5

Wastewater secondary digester isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
K-C-Nor-T-Ap-E	2	10
Nor-T-Ap-E	2	10
C-T-Ap-E	1	5
K-C-T-E	1	5
C-Nor-T-Ap	1	5
Nor-T-Ap-E	1	5
K-T-Ap-E	1	5
Nor-T-E	1	5
T-Ap-E	1	5
C-T-E	1	5

Wastewater tertiary digester isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
C-Nor-T-Ap-E-S	3	15
K-C-Nor-T-Ap-E	10	50
K-C-Nor-T-Ap	1	5
K-C-Nor-T-E	1	5
Nor-T-E	1	5

Wastewater effluent isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
T-Ap-E	1	5
K-C-Nor-T-Ap-E	1	5
Nor-T-E	2	10
C-Nor-T-E	3	15
K-C-Nor-T-E	1	5
K-C-Nor-E	1	5
C-T-E	1	5
C-Nor-T-Ap-E-S	1	5
C-Nor-T-Ap	1	5

Dam inlet isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
C-T-Ap-E	4	20
C-T-E	3	15
Nor-T-Ap-E	6	30

TABLE 3 (CONTINUES....)
The predominant multiple antibiotic resistant (MAR) phenotypes for *E. coli* isolated from the different sampling sites

Dam inlet isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
T-Ap-E	1	5
C-Ap-E	1	5

Dam midpoint isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
C-Nor-T-E	4	20
C-Nor-T-Ap	1	5
K-C-Nor-T-Ap-E	1	5
K-C-T-E	1	5
C-T-E-S	1	5
C-T-E	3	15
C-T-Ap-E	4	20
K-Nor-E-S	1	5
C-Nor-E	1	5

Dam outlet isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
C-Nor-T-Ap-E	1	5
Nor-T-Ap-E	2	10
T-Ap-E	6	30

Inlet water isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
Nor-T-Ap-E	1	5
C-Nor-T-E	4	20
K-C-Nor-T-Ap-E	2	10
T-Ap-E	1	5

Outlet water isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
T-Ap-E	2	10
C-T-E	4	20

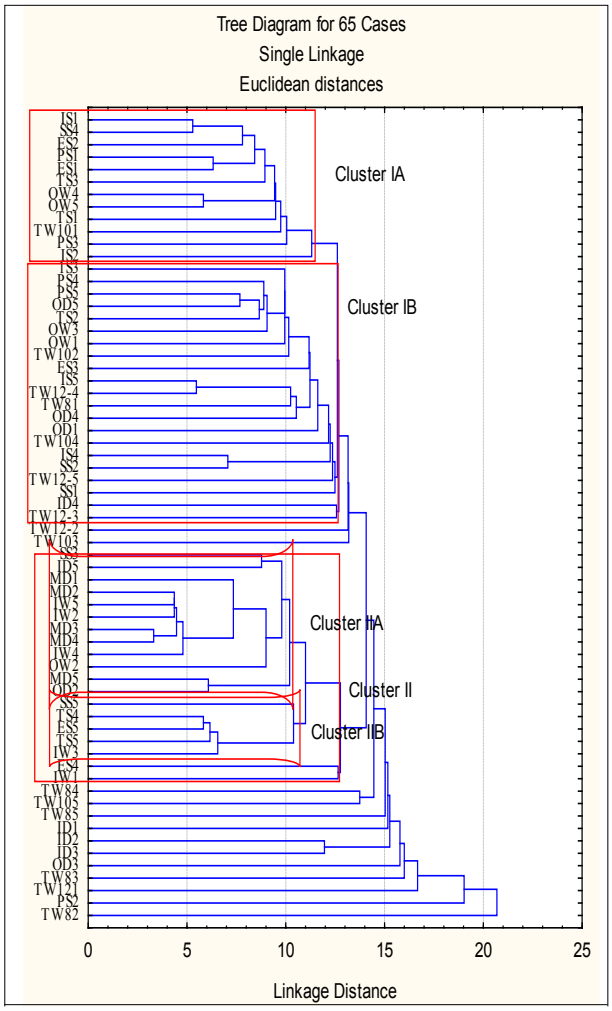
Tap water (Unit 8) isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
C-Ap-S	1	5
C-Ap-E	6	30
C-T-Ap	1	5

Tap water (Unit 10) isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
T-Ap-E	5	25
K-C-T-E	1	5

Tap water (Unit 12) isolates (N = 20)		
MAR phenotypes	**Number observed**	**Percentage**
C-T-Ap	1	5
K-Ap-E	1	5
C-T-Ap-E	2	10
T-Ap-E	1	5
C-Ap-E	2	10
K-C-T-Ap-E-S	1	5

TABLE 4
The percentage representation of *E. coli* isolated from different sampling areas within the various clusters

Sampling site	Cluster I		Cluster II	
	Cluster IA	Cluster IB	Cluster IIA	Cluster IIB
	N = 12	N = 21	N = 11	N = 5
Wastewater (Inlet)	2 (16.7%)	3 (14.3%)	0	0
Wastewater (Primary)	2 (16.7%)	2 (9.5%)	0	0
Wastewater (Secondary)	1 (8.3%)	2 (9.5%)	0	1 (20%)
Wastewater (Tertiary)	2 (16.7%)	1 (4.8%)	0	2 (40%)
Wastewater (Effluent)	2 (16.7%)	1 (4.8%)	0	1 (20%)
Inlet Dam	0	1 (4.8%)	1 (9.1%)	0
Midpoint Dam	0	0	5 (45.5%)	0
Outlet Dam	0	3 (14.3%)	1 (9.1%)	0
Inlet water	0	0	3 (27.3%)	1 (20%)
Outlet water	2 (16.7%)	2 (9.5%)	1 (9.1%)	0
Tap water (Unit 8)	0	1 (4.8%)	0	0
Tap water (Unit 10)	1 (8.3%)	2 (9.5%)	0	0
Tap water (Unit 12)	0	3 (14.3%)	0	0



Bacterial designation prefixes are based on the type of sample and area of collection. The tree was constructed using Ward's method and Euclidean distances in Statistica, version 7. Designation: IS = Inlet wastewater, PS = Primary wastewater, SS = Secondary wastewater, TS = Tertiary wastewater, ES = Effluent wastewater, ID = Inlet dam, MD = Midpoint dam, OD = Outlet dam, IW = Inlet water, OW = Outlet water, TW 8 = Tap water Unit 8, TW 10 = Tap water Unit 10, TW 12 = Tap water Unit 12.

FIGURE 1
Dendogram showing the relationship between *E. coli* isolated from water samples obtained from the Mmabatho locality based on inhibition zone diameter (IZD) data

to reports by other researchers.[15,29,30] Marked multiple antibiotic resistances (over 70%) were observed for erythromycin, tetracycline and ampicillin, chloramphenicol and norfloxacin. Multiple antibiotic resistance refers to the resistance of two or more classes of antibiotics. A large proportion (70%–95%) of *E. coli* isolated from wastewater samples obtained from the different sampling sites was resistant to chloramphenicol, norfloxacin, tetracycline, ampicillin and erythromycin. Similarly, a large proportion (80%–100%) of *E. coli* isolated from the Modimola Dam was resistant to chloramphenicol, tetracycline, and erythromycin. Furthermore, a large proportion (65%–100%) of *E. coli* isolated from the local water-treatment plant was resistant to tetracycline and erythromycin. Lastly, a 50%–90% resistance to chloramphenicol, tetracycline, ampicillin and erythromycin was observed for *E. coli* isolated from tap water. However, all tap water isolates were susceptible to norfloxacin. Susceptibility of a few isolates to streptomycin and kanamycin was also observed.

MAR phenotypes were compiled for all isolates obtained (Table 3). The predominant phenotypes from wastewater sites were T-Ap-E (20%, inlet), K-C-Nor-T-Ap-E (30%, primary), Nor-T-Ap-E and K-C-Nor-T-Ap-E (both 10%, secondary), K-C-Nor-T-Ap-E (50%, tertiary), and C-Nor-T-E (50%, effluent).

Similarly, the predominant phenotypes obtained for the local water-treatment plant were C-Nor-T-E and C-T-E at 20%, from the inlet and outlet, respectively. Also, predominant phenotypes from the Modimola Dam inlet, midpoint and outlet were Nor-T-Ap-E at 30%, C-Nor-T-E and C-T-Ap-E both at 20%, and T-Ap-E at 30%, respectively. C-Ap-E, T-Ap-E and C-T-Ap-E were the predominant phenotypes in tap water at 30%, 25% and 10% for Units 8, 10 and 12, respectively. Overall, T-Ap-E was a common phenotype observed for *E. coli* isolated from the local wastewater- and water-treatment plants, Modimola Dam and tap water.

A total of 65 *E. coli* isolates were randomly selected from all sampling sites and subjected to cluster analysis using the antibiotic inhibition zone diameter data. Two major clusters were generated, each subdivided into two minor clusters (IA, IB and IIA, IIB) as shown in Figure 1. Further analysis of the clusters was performed for patterns of associations of the isolates from the different sources as shown in Table 4. The analysis obtained was used as a tool in determining the uniqueness between the antibiotic resistance patterns of *E. coli* isolates from different areas. The largest cluster (Cluster IB) showed *E. coli* isolated from all sampled areas. The second largest (Cluster IA) represented *E. coli* isolated from wastewater, the local water-treatment plant (outlet) and tap water (Unit 10). Cluster IIA (the third largest cluster) represented *E. coli* isolated from the Modimola Dam

(inlet, midpoint and outlet) and the local water-treatment plant (inlet and outlet). The smallest cluster (Cluster IIB) represented mostly *E. coli* from wastewater (secondary, tertiary and effluent digesters) and the local water-treatment plant (inlet).

DISCUSSION

The Enterobacteriaceae family has been linked to well-known antibiotic-resistant gene pools. These genes are transferred into the normal flora of humans and animals, [31] where they exert a strong selective pressure for the emergence and spread of resistance in both pathogenic and commensal bacteria. Eventually they find their way into the environment via wastewater, manure and sewage sludge.[32] Based on the antibiotic-resistance patterns, we observed that all isolates tested were resistant to tetracycline (5%–95%), ampicillin (10%–80%), chloramphenicol (5%–80%) and erythromycin (50%–100%). The multiple antibiotic resistances of *E. coli* demonstrated in this study accord with those found in other studies. [15,21,28,30,33,34,35,36,37,38]

Antimicrobial drugs have a widespread use in human and veterinary medicine, animal husbandry, aquaculture, agriculture and food technology.[14] Therefore, animal feedstuffs are possible vehicles for transmission of resistant bacteria that could colonise the intestinal tract[39] and negatively impact the health and economy of the affected communities. As observed from the cluster analysis performed, cluster IB contained isolates from all the sampling stations. This was a cause of concern because it showed a link between the resistant isolates from the local wastewater-treatment plant, Modimola Dam, the local water-treatment plant and tap water supplied to homes, suggesting that there had been a previous exposure of these isolates to the antibiotics tested. Hence, there might be a risk of antibiotic-resistant gene transmission within the population, which might have a negative effect on antibiotic therapy.

CONCLUSION

The high percentage of phenotypes of *E. coli* isolates that were MAR to chloramphenicol, tetracycline, ampicillin, and, particularly to erythromycin, suggested that there has been a misuse of these drugs, which has resulted in these water sources posing a potential threat to humans in the area. The indiscriminate use of antibiotics in humans and animals is cause for great concern. The high antibiotic resistance also indicates a negative impact on therapy with these classes of antibiotics. The periodic monitoring of antibiotics to detect any changing patterns would be necessary for effective treatments. Strict quality control measures also should be put in place to ensure proper treatment of water and wastewater in these and other treatment plants. This would ensure the discharge of properly treated wastewater into water bodies to prevent the occurrence and spread of water- and food-borne diseases. A further study to evaluate the extent of antibiotic resistance transmission and the impact of such transmission on the effectiveness of antibacterial use in human medicine is imperative.

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