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DETECTION AND ANTIBIOTIC SUSCEPTIBILITY OF PATHOGENIC *ESCHERICHIA COLI* ISOLATED FROM THE FINAL EFFLUENT OF TWO WASTEWATER TREATMENT PLANTS IN THE EASTERN CAPE PROVINCE, SOUTH AFRICA

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Abstract

Wastewater effluent is a significant reservoir for pathogenic and antibiotic-resistant *Escherichia coli*, which can pose substantial acute risks if released into surface water without adequate treatment. This study examines the presence of pathogenic *E. coli* strains in treated effluents and determines their antibiotic profiles.. A culture-based approach was employed using *E. coli* differential chromogenic agar for isolation via the membrane filtration method. Confirmation of presumptive *E. coli* strains was achieved by PCR. The confirmed isolates were also genotyped to identify the species present. Antibiotic profiling of the confirmed isolates was performed using the CLSI-recommended first-line antibiotics for *E. coli*. Out of eight pathotypes screened, four were confirmed: Uropathogenic *E. coli* (UPEC) at 5.7%, Enteroaggregative *E. coli* (EAEC) at 2.3%, Neonatal Meningitis *E. coli* (NMEC) at 1.1%, and Enteropathogenic *E. coli* (EPEC) at 0.6%. The antibiotic susceptibility patterns showed that UPEC had low resistance to meropenem (100%), cefotaxime (100%), and gentamicin (88.9%), but high resistance to tetracycline (74.1%), ampicillin (74.1%), and cephalothin (66.7%). EAEC, NMEC, and EPEC exhibited high sensitivity (100%) to meropenem, gentamicin, and cefotaxime, with varying degrees of resistance to ampicillin, tetracycline, and cephalothin. The study indicates that the two treatment plants discharge effluents containing pathogenic *E. coli*, serving as reservoirs for these bacteria. The study highlights the inadequacy of the plants to produce effluents of acceptable quality.

Keywords: Antibiotics resistance, Uropathogenic *E. coli* (UPEC), Enteroaggregative *E. coli* (EAEC), Neonatal Meningitis *E. coli* (NMEC), Enteropathogenic *E. coli* (EPEC), wastewater, South Africa

Introduction

Wastewater treatment plants play a vital role in managing and treating polluted water. Proper management of this wastewater is crucial to preventing environmental pollution, which could pose a risk to public health (West and Mangiameli, 2000; Elleuch *et al.*, 2018). Conventional wastewater treatment systems reduce the quantity of enteric bacteria. However, poorly treated wastewater at any stage of the treatment process can compromise the effectiveness of disinfectants used to deactivate these organisms (Anastasi *et al.*, 2012; Chatterjee, Ghangrekar and Rao, 2017). The incomplete removal of pathogens and antibiotic-resistant bacteria from wastewater has resulted in the introduction of treated but contaminated effluents into natural water resources, thereby increasing the risk of infection (Dolejska *et al.*, 2011). *E. coli* strains with virulence characteristics typical of uropathogenic strains have been reported to survive sewage treatment processes and have been found in environmental waters receiving effluent discharges from sewage treatment plants (STPs) (Anastasi *et al.,* 2010, 2012).

Aquatic environments are natural reservoirs of antibiotic-resistant bacteria, and wastewater treatment plants (WWTPs) are among the primary sources of these microorganisms. The presence of antibiotic resistance genes in water environments is a global concern, with a dramatic increase observed in recent years. A broad range of antibiotic resistance genes has been detected in wastewater effluents, surface water, river water, groundwater, and even drinking water (Dolejska *et al.*, 2011; Osińska *et al.*, 2019). Multi-drug resistance has been demonstrated in *E. coli* (Shariff *et al.*, 2013). In this context, studies from various provinces in South Africa have shown the presence of pathogenic and antibiotic-resistant *E. coli* in wastewater effluents and water bodies (Olaniran, Naicker and Pillay, 2009; Omar and Barnard, 2010; Phokela, Ateba and Kawadza, 2011).

The present study is a follow-up to previous research by Osuolale and Okoh (Osuolale and Okoh, 2015a, 2015b, 2017) aimed at assessing the quality of treated effluent discharged from wastewater treatment plants in the Eastern Cape, South Africa. This study is part of a broader investigation that included three other WWTPs, although those sites were used for viral rather than bacterial sampling (Osuolale and Okoh, 2017). The discharged water bodies were tested for pathogenic *E. coli* and their antibiotic profiles. The study areas are distinctive due to their semi-rural and semi-urban characteristics. Our research aims to provide insights into the presence of pathogenic *E. coli* in treated effluent

Materials and Methods

Sampling and Sampling Sites/Locations

The Amalinda Central wastewater treatment works is located at a geographical coordinate of 33° 00’ 59”S latitude and 27° 51’ 48”E longitude. This medium-sized plant has a treatment capacity of 5 million liters per day (ML/day) and primarily receives household waste. It employs the Bio-filter/PETRO (pond-enhanced treatment and operation) process for treating the influent (DWAF, 2009), with the final effluent discharged into the Umzonyana stream.

The Dimbaza WWTP is situated at 32° 51' 14.75"S latitude and 27° 14' 7.12" E longitude. This medium-sized plant receives municipal domestic sewage and runoff water. It operates an activated sludge system with a design capacity of approximately 8 ML/day (DWAF, 2009). The plant treats an average dry weather flow of 7,000 cubic meters per day and an average wet weather flow of 21,000 cubic meters per day. The final effluent is discharged into the Mdizeni stream, a tributary of the Keiskamma River (Linder and Lundéhn, 2002).

Samples were collected monthly from the final treated effluent (FE) over a 12-month period, from September 2012 to August 2013. The samples were collected in sterile 1.7-liter Nalgene bottles, with 10% sodium thiosulfate added to the bottles to neutralize the chlorine's effect on the target organisms. The samples were stored and transported in chiller boxes to the Applied and Environmental Microbiology Research Group (AEMREG) laboratory at the University of Fort Hare, Alice, South Africa, for analysis. All samples were processed within six hours of collection. The sampling frequency and number of samples followed the guidelines recommended in the *Quality of Domestic Water Supplies Volume 2: Sampling Guide* (DWAF, DHE and WRC, 2000).

Bacteriological analysis of the effluent samples for isolation was conducted using membrane filtration, following the method described by (Sans, 2011). *E. coli* coliforms were isolated using chromogenic agar (Conda, Madrid), which differentiates *E. coli* from other members of the Enterobacteriaceae family. *E. coli* colonies are easily distinguishable by their dark blue-green color. The filters were placed on the agar and incubated at 37°C for 24 hours, with the procedure performed in triplicate. Target colonies were counted and reported as CFU/100 ml. After 24 hours of incubation, counts within the suitable range (0-300 colonies) were recorded using manual counting, and the results per dilution plate count were documented.

Genotypic identification of *E. coli*

Isolation of genomic DNA and Genotypic identification of E. coli spp.

Purified presumptive *E. coli* isolates were grown in Luria Broth (LB) overnight for crude DNA extraction. Genomic DNA was extracted using the ZR Fungal/Bacterial DNA MiniPrep kit from Zymo Research, following the manufacturer's instructions. The extracted genomic DNA was either immediately used for molecular identification of the isolates or stored at -20°C until needed for the PCR reaction. Primers specific for the *uidA* gene in *E. coli*, which have been previously developed and validated for their specificity to fecal pollution, were used in the polymerase chain reaction (PCR) to confirm the identity of the isolates. The primers used were forward (5'-AAAACGGCAAGAAAAAGCAG-3') and reverse (5'-ACGCGTGGTTAACAGTCTTGCG-3'), targeting the *uidA* gene with a 147 bp expected amplicon (Dungeni, van Der Merwe and Momba, 2010).

The PCR conditions were as follows: an initial denaturation at 94°C for 2 minutes, followed by 30 cycles of 94°C for 1 minute, 62.7°C for 90 seconds, and 72°C for 1 minute, with a final extension at 72°C for 5 minutes. The primers specific to the pathotypes are listed in Table 1. PCR amplification was performed using a MyCycler thermal cycler (Bio-Rad). The PCR mixture contained 2× PCR Master Mix, 100 µM of each primer (1 µl of forward and 1 µl of reverse), and 5 µl of template DNA from each bacterial strain, bringing the final reaction volume to 25 µl. Gel electrophoresis was performed on the PCR products using a 2% w/v agarose gel at 100 V for approximately 90 minutes. The gel images were captured digitally and analyzed using the Uvitec, Alliance 4.7 system. The chromosomal DNA of a positive control was used as a reference to ensure primer accuracy and specificity.

Table 1. Primer pairs, expected amplicon size for characterization of *E. coli* pathotypes

Target strains	Target genes	Primer sequence (5'→3')	Amplicon size (bp)	References
EPEC	eae	TCA ATG CAG TTC CGT TAT CAG TT GTA AAG TCC GTT ACC CCA ACC TG	482	(Vidal *et al.*, 2005)
ETEC	lt	GCA CAC GGA GCT CCT CAG TC TCC TTC ATC CTT TCA ATG GCT TT	218	(Vidal *et al.*, 2005)
EIEC	ipaH	CTC GGC ACG TTT TAA TAG TCT GG GTG GAG AGC TGA AGT TTC TCT GC	933	(Vidal *et al.*, 2005)
EAEC	Eagg	AGA CTC TGG CGA AAG ACT GTA TCATG GCT GTC TGT AAT AGA TGA GAA C	194	(Omar and Barnard, 2010)
DAEC	daaE	GAA CGT TGG TTA ATG TGG GGT AA TAT TCA CCG GTC GGT TAT CAG T	542	(Vidal et al., 2005)
UPEC	pap	GACGGCTGTACTGCAGGGTGTGGCG ATATCCTTTCTGCAGGGATGCAATA	328	(Abe *et al.*, 2008)

NMEC	IbeA	ibeA-F- TTACCGCCGTTGATGTTATCA ibeA-R- CATTAGCTCTCGGTTCACGCT	171	(Watt and Lanotte, 2003)

Control isolates

E. coli ATCC 8973, along with six *E. coli* strains purchased from DSMZ, were used in the study. These strains included Enteroinvasive *E. coli* (EIEC, DSM 9025), Enteroaggregative *E. coli* (EAEC, DSM 10974), Enterotoxigenic *E. coli* (ETEC, DSM 10973), Uropathogenic *E. coli* (UPEC, DSM 4618), Enteropathogenic *E. coli* (EPEC, DSM 8695), and Neonatal *E. coli* (NMEC, DSM 10819), which were employed as positive controls.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed using the standard disc diffusion method on Mueller-Hinton agar (MH) (Conda, Madrid) as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012b). Fresh colonies, approximately 18 hours old, from nutrient agar culture plates were picked and suspended in 5 ml of sterile normal saline in test tubes. The turbidity of the suspension was adjusted to match the 0.5 McFarland standard. Sterile swabs soaked in the bacterial suspensions were used to inoculate the MH agar plates by uniformly spreading the bacteria across the agar surface. The selection of antimicrobials was based on the type of organism being tested and the source of the isolates (CLSI, 2012b). The antibiotics were chosen to represent different classes of antibacterial drugs to better assess the response of the examined strains. The antimicrobial susceptibility of *E. coli* isolates was tested using the following antibiotic discs: ampicillin (10 µg), cefotaxime (30 µg), gentamicin (10 µg), meropenem (10 µg), tetracycline (30 µg), and cephalothin (30 µg) (Davies Diagnostics, SA) (CLSI, 2012b).

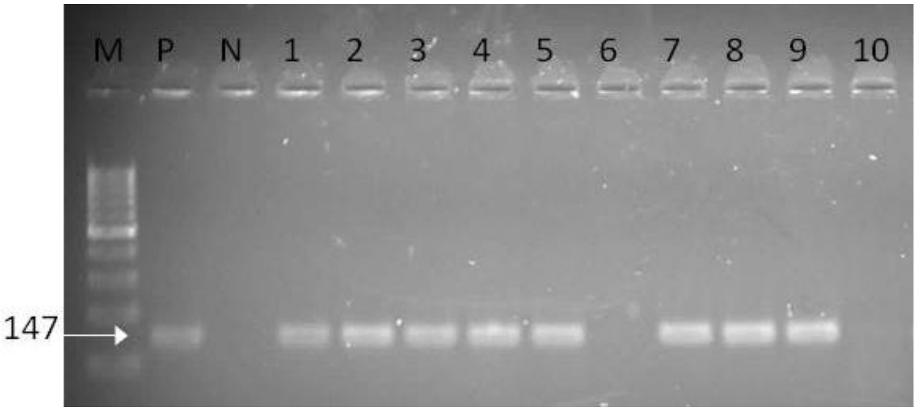
Results

A total of 943 *E. coli* isolates were collected from the final effluent of both wastewater treatment plants. Out of these, 476 isolates (50.5%) tested positive for pathogenic *E. coli* based on PCR detection of virulence genes (Figure 1). At the Amalinda Central WWTP, 270 out of 406 isolates (66.5%) were confirmed as pathogenic *E. coli*, whereas at the Dimbaza WWTP, 206 out of 437 isolates (47.1%) tested positive. These findings indicate a higher prevalence of pathogenic *E. coli* at the Amalinda Central WWTP compared to the Dimbaza WWTP. This variation in detection rates could be attributed to differences in treatment efficiency, influent microbial load, or environmental conditions at each facility. The presence of a significant proportion of pathogenic *E. coli* in the final effluent underscores potential public health risks associated with the discharge of treated wastewater into receiving water bodies. At the Amalinda Central WWTP, a total of 406 presumptive *E. coli* isolates were identified, while 437 isolates were collected from the Dimbaza WWTP (Table 2). Over the study period, a total of 476 *E. coli* isolates from both plants were confirmed (Figure 1). Among these, approximately 5.7% (27 isolates) were identified as Uropathogenic *E. coli* (UPEC). Of the UPEC isolates, 77.8% (21 isolates) were from the Amalinda Central WWTP, and 22.2% (6 isolates) were from the Dimbaza WWTP (Table 3). Figure 2 (below) illustrates the PCR confirmation of the *pap* gene for UPEC. Enteroaggregative *E. coli* (EAEC) was the next most frequently detected pathotype, accounting for 2.3% (11 isolates) of the total confirmed *E. coli* isolates (Figure 3). Of the confirmed EAEC isolates, 81.8% (9 isolates) were from the Amalinda Central WWTP, while 18.2% (2 isolates) were from the Dimbaza WWTP. Other confirmed pathotypes include Neonatal Meningitis-associated *E. coli* (NMEC) See figure 4, which was detected only at the Amalinda Central WWTP, and

Enteropathogenic *E. coli* (EPEC), which was detected only at the Dimbaza WWTP. No isolates of Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), or Diffuse-adhering *E. coli* were detected at either plant. The results of the *E. coli* pathotyping are presented in Table 3 below.

Table 2. *E. coli* confirmation of the presumptive isolates

Site	Number of isolates	Number of positive isolates (PCR)
Amalinda Central WWTP	406	270
Dimbaza WWTP	437	206
Total	943	476

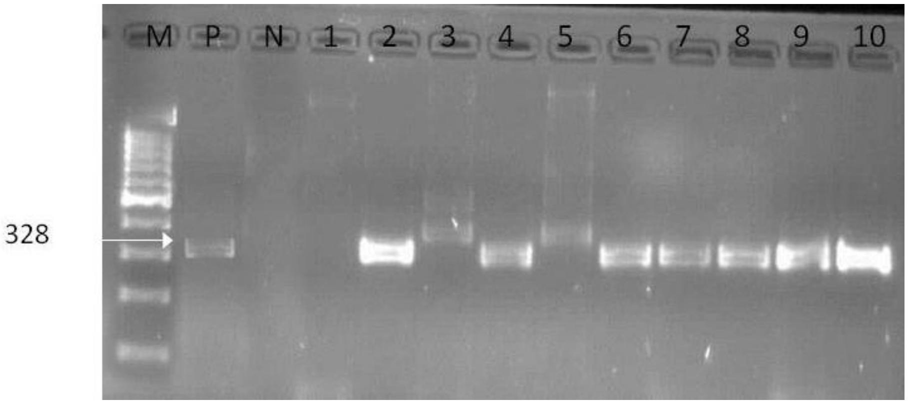


M: Molecular weight marker (100bp), P: *Escherichia coli* ATCC 8973 (Positive control), N: Negative control; Lanes 1-10: *E. coli* isolates

Figure 1: ***Representative agarose gel electrophoresis image of PCR-amplified virulence genes from subset of 10 E. coli isolates.;.***

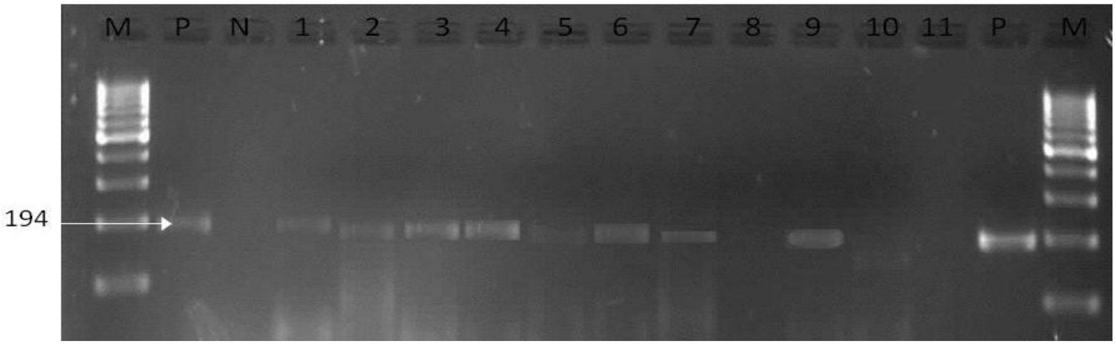
Table 3. Result of *E. coli* pathotyping

Pathotypes	Amalinda Central WWTP (n = 270)	Dimbaza WWTP (n=206)
Enteropathogenic *E. coli* (EPEC)	0	3
Enterotoxigenic *E. coli* (ETEC)	0	0
Enteroinvasive *E. coli* (EIEC)	0	0
Enteroaggregative *E. coli* (EAEC)	9	2
Neonatal meningitis- associated *E. coli* (NMEC)	5	0
Uropathogenic *E. coli* (UPEC)	21	6
Diffuse-adhering *Escherichia coli*	0	0



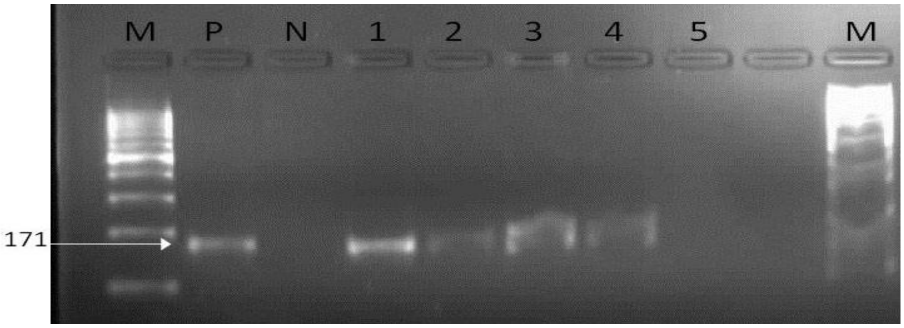
M: Molecular weight marker (100bp), P: Escherichia coli (UPEC) DSM 4618 (Positive control)
N: Negative control; Lanes 1-10: *E. coli* isolates

Figure 2: Agarose gel electrophoresis of representative *pap* gene amplification products of UPEC



M: Molecular weight marker (100bp), P: *Escherichia coli* (EAEC) DSM 10974 (Positive control), N: Negative control; Lanes 1-11: *E. coli* isolates

Figure 3: Agarose gel electrophoresis of representative *EAgg* gene amplification products of EAEC



M: Molecular weight marker (100bp), P: *Escherichia coli* (NMEC) DSM 10819 (Positive control), N: Negative control; Lanes 1-5: *E. coli* isolates

Figure 4: Agarose gel electrophoresis of representative *ibe* gene amplification products of NMEC

Antimicrobial susceptibility testing

The antimicrobial susceptibility profiles of the pathogenic *E. coli* isolates recovered from the final effluent of Amalinda Central and Dimbaza WWTPs were evaluated using six different antibiotics representing five antibiotic classes. The results (Table 4) revealed that all *E. coli* pathotypes exhibited 100% susceptibility to meropenem and cefotaxime, indicating the absence of carbapenem-resistant *E. coli* and Extended-spectrum beta-lactamase (ESBL)-producing strains. The Uropathogenic *E. coli* (UPEC) isolates demonstrated high resistance to several antibiotics, including meropenem (100%), cefotaxime (100%), and gentamicin (88.9%). Conversely, they exhibited significant resistance to tetracycline (74.1%), ampicillin (74.1%), and cephalothin (66.7%). Other *E. coli* pathotypes, including Enteroaggregative *E. coli* (EAEC), Neonatal Meningitis-associated *E. coli* (NMEC), and Enteropathogenic *E. coli* (EPEC), displayed high sensitivity (100%) to meropenem, gentamicin, and cefotaxime. EAEC showed 63.6% resistance to tetracycline and 54.5% resistance to both ampicillin and cephalothin. NMEC had intermediate sensitivity (80%) to cephalothin and exhibited 60% resistance to tetracycline and 40% resistance to ampicillin. EPEC displayed 100% resistance to both ampicillin and cephalothin, and 66.7% resistance to tetracycline. All tested pathotypes showed resistance to two or three antibiotics, primarily ampicillin, tetracycline, and cephalothin.

Table 4. Antimicrobial susceptibility testing of *E. coli* pathotypes

Pathotypes	**n = 46, Susceptibility profile (%)**																	
	Meropenem			**Gentamicin**			**Cefotaxime**			**Ampicillin**			**Tetracycline**			**Cephalothin**		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
UPEC	100	-	-	88.9	7.4	3.7	100	-	-	14.1	11.1	74.1	25.9	-	74.1	3.7	29.6	66.7
EAEC	100	-	-	100	-	-	100	-	-	36.4	-	63.6	36.4	-	63.6	-	45.5	54.5
NMEC	100	-	-	100	-	-	100	-	-	60	-	40	40	-	60	20	80	-
EPEC	100	-	-	100	-	-	100	-	-	-	-	100	33.3	-	66.7	-	-	100

Note: - S (susceptible), I (Intermediate), R (Resistant)

Discussion

This study investigated the incidence of four pathogenic *E. coli* strains in the final effluent discharged into surface water. A total of eight pathogenic *E. coli* pathotypes were identified. Of these, five can cause invasive intestinal infections, watery diarrhea, and dysentery in humans and animals, while the remaining three are associated with extra-intestinal infections, caused by extra-intestinal pathogenic *E. coli* (ExPEC) (Bekal *et al.*, 2003). Four of the eight identified pathotypes are detailed in Table 3. Both invasive and extra-intestinal pathotypes were detected.

In contrast to a previous study by (Osode, 2010), which identified Enterohemorrhagic *E. coli* (EHEC) and Enteroaggregative *E. coli* (EAEC) at Dimbaza WWTP, with Enteroinvasive *E. coli* (EIEC) confirmed at another plant, this study found Enteropathogenic *E. coli* (EPEC), Uropathogenic *E. coli* (UPEC), and EAEC at Dimbaza WWTP. The detection rate of pathotypes at Dimbaza WWTP was lower compared to Amalinda Central WWTP, except for EPEC, which was exclusively found at Dimbaza. This discrepancy may be attributed to the efficiency of the treatment plant, which might have resulted in the low detection of pathogenic *E. coli*. Alternatively, the *E. coli* strains present at these sites might lack virulence genes, as suggested by the absence of identified pathotypes in the isolated and confirmed *E. coli*. A similar observation was reported by (Masters *et al.*, 2011).

At the Amalinda Central WWTP, three pathogenic *E. coli* pathotypes were identified in higher concentrations compared to Dimbaza

WWTP. The identified pathotypes, as shown in Table 3, are of significant public health concern. In addition to Enteroaggregative *E. coli* (EAEC), which was also identified by (Osode, 2010) in WWTP effluent in the Eastern Cape, Enteropathogenic *E. coli* (EPEC) and Uropathogenic *E. coli* (UPEC) were major findings at Amalinda Central WWTP. Of the 476 confirmed *E. coli* isolates tested, UPEC constituted about 5.7%, followed by EAEC at 2.3%, Neonatal *E. coli* (NMEC) at 1.1%, and EPEC at 0.6%.

Similar studies have reported varying results. For instance, (Verma et al. 2008) in India found a high incidence of UPEC and EPEC in treated final effluent, though at lower concentrations. (Anastasi *et al.*, 2010, 2012) demonstrated that some *E. coli* strains with uropathogenic properties could survive sewage treatment and be released into the environment. (Diallo *et al.*, 2013) observed that EPEC was more common in city wastewater compared to slaughterhouse wastewater, where ExPEC prevalence was not significantly influenced by the treatment process and EPEC was less frequent in final effluents.

(Masters *et al.*, 2011) reported the presence of EAEC in water and investigated its associated virulence genes. They found EAEC in conjunction with EPEC, suggesting a potential source of fecal contamination. Additionally, (Hamelin *et al.*, 2007) documented the presence of EAEC, EPEC, UPEC, and NMEC in river water receiving urban municipal wastewater. (Siziwe Koba, 2013) identified ETEC, EIEC, and EPEC in one river and EAEC in both rivers studied in the Eastern Cape. One of the studied sites, Amalinda Central WWTP, demonstrated a notable diversity of *E. coli* pathotypes. This finding aligns with a similar study by (Adefisoye and Okoh, 2016)), which exhibited comparable trends in the quantity and types of pathogenic *E. coli* detected. The presence of these pathogenic strains has been associated with both human and non-human extra-intestinal infections in past research (Bekal *et al.*, 2003). Agricultural products and aquaculture have been reported to pose a high risk of diarrhea, and individuals in direct contact with wastewater are more vulnerable to disease compared to those not exposed (Trang *et al.*, 2007)..

In the Eastern Cape and Limpopo Provinces of South Africa, pathogenic *E. coli* strains—excluding NMEC and UPEC—have been isolated from diarrhea patients, with EAEC being the predominant cause of infection (Bisi-Johnson et al. 2011; Samie et al. 2007). The environmental presence of these pathogenic strains raises significant public health concerns due to their potential consequences (Clements *et al.*, 2012).

For routine reporting and primary testing, the choice of antibiotic panels was based on CLSI recommendations (CLSI, 2012b). The study included antibiotics from various classes, specifically: ampicillin (penicillin class), gentamicin (aminoglycosides), tetracycline, meropenem (carbapenems), and cephalothin and cefotaxime (first and third-generation cephalosporins) (CLSI, 2012b).

The antibiotic profiles of the pathogenic *E. coli* pathotypes revealed lower effectiveness of ampicillin, tetracycline, and cephalothin (a first-generation cephalosporin). These antibiotics represent major classes commonly used in first-line treatments. Although our study did not test other tetracycline class members, susceptibility to doxycycline and minocycline can be inferred from tetracycline susceptibility. Organisms resistant or intermediate to tetracycline may still be susceptible to doxycycline, minocycline, or both (CLSI, 2012a). On average, the pathogenic isolates exhibited higher resistance levels compared to their susceptibility, with a 60% resistance rate to tetracycline.

Cefotaxime was selected in this study to identify the presence of Extended-Spectrum Beta-

Lactamase (ESBL) producers among the *E. coli* isolates (CLSI, 2012b). None of the isolates exhibited ESBL production, as demonstrated by their 100% susceptibility to cefotaxime (Table 4). The emergence of carbapenem-resistant *E. coli* is a global concern, given that carbapenems are considered one of the last lines of defense for treatment (Nordmann et al. 2012). Our study showed that none of the pathogenic *E. coli* isolates were resistant to the carbapenem class, specifically meropenem (Table 4). Nontongana et al. (2014) observed resistance to some antibiotics in a study on river water in the Eastern Cape. Similarly, a study conducted in Durban on wastewater treatment plants found high resistance among *E. coli* isolates to ampicillin, amoxicillin, doxycycline, and tetracycline (Pillay and Olaniran, 2016). Multiple resistance patterns, as reported by Kinge, Ateba and Kawadza, (2010) and Mulamattathil et al. (2014) from wastewater, surface water, and water treatment plants, were consistent with the resistance patterns observed in our study, particularly against ampicillin and tetracycline. While our study did not detect any carbapenem-resistant *Enterobacteriaceae* (CRE), particularly *E. coli*, in South Africa, there have been reported cases of other *Enterobacteriaceae* members, such as *Klebsiella*, showing resistance to carbapenems (Brink *et al.*, 2012). Recent reports underscore the urgent need for institutions to curb the indiscriminate use of antibiotics and implement restrictive measures to prevent the looming threat of CRE in South Africa (Coetzee and Brink, 2011).

The presence of antibiotics in surface water and wastewater has been documented, with Matongo *et al.* (2015) reporting that insufficiently treated wastewater contributes significantly to surface water contamination. Additionally, improper use and disposal of pharmaceutical products and wastes by humans also contribute substantially to the pharmaceutical load in rivers. Understanding the resistance patterns of pathogenic bacterial strains across different regions of South Africa is crucial for effective drug management and policy implementation to address the growing issue of microbial resistance. This knowledge will guide the appropriate and prudent use of antibiotics. Developing and enforcing comprehensive institutional and organizational antibiotic policies will be instrumental in controlling these infections (Shariff *et al.*, 2013).

We have previously reported that the operational challenges of these wastewater treatment plants often lead to the discharge of inadequately treated effluent into receiving surface waters (Osuolale and Okoh, 2015b, 2015a, 2017). As time races against South Africa in addressing its water challenges, the global community is increasingly emphasizing the importance of safe wastewater management and reuse, as highlighted by the UN's World Water Day. Antibiotic stewardship is crucial for the responsible management of antibiotic use, and it is imperative for the Department of Water Affairs to reassess its approach to handling wastewater and antibiotic waste. Effective management practices are necessary to minimize environmental impacts and address public health concerns.

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Author Contributions

Olayinka Osuolale conducted all sampling and experiments and wrote the manuscript; and Anthony Okoh supervised the project.

Competing financial interests

The authors declare they have no actual or potential competing financial interests.

References

Abe, C.M., Salvador, F.A., Falsetti, I.N., Vieira, M.A.M., Blanco, J., Blanco, J.E., Blanco, M., Machado, A.M.O., Elias, W.P., Hernandes, R.T. and Gomes, T.A.T. (2008) 'Uropathogenic Escherichia coli (UPEC) strains may carry virulence properties of diarrhoeagenic E. coli.', *FEMS immunology and medical microbiology*, 52(3), pp. 397–406. Available at: https://doi.org/10.1111/j.1574-695X.2008.00388.x.

Adefisoye, M.A. and Okoh, A.I. (2016) 'Identification and antimicrobial resistance prevalence of pathogenic Escherichia coli strains from treated wastewater effluents in Eastern Cape, South Africa', *MicrobiologyOpen*, 5(1), pp. 143–151. Available at: https://doi.org/10.1002/mbo3.319.

Anastasi, E.M., Matthews, B., Gundogdu, A., Vollmerhausen, T.L., Ramos, N.L., Stratton, H., Ahmed, W. and Katouli, M. (2010) 'Prevalence and persistence of Escherichia coli strains with uropathogenic virulence characteristics in sewage treatment plants.', *Applied and environmental microbiology*, 76, pp. 5882–5886. Available at: https://doi.org/10.1128/AEM.00141-10.

Anastasi, E.M., Matthews, B., Stratton, H.M. and Katouli, M. (2012) 'Pathogenic Escherichia coli found in sewage treatment plants and environmental waters.', *Applied and environmental microbiology*, 78(16), pp. 5536–5541. Available at: https://doi.org/10.1128/AEM.00657-12.

Bekal, S., Brousseau, R., Masson, L., Prefontaine, G., Fairbrother, J. and Harel, J. (2003) 'Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays.', *Journal of clinical microbiology*, 41, pp. 2113–2125. Available at: https://doi.org/10.1128/JCM.41.5.2113-2125.2003.

Bisi-Johnson, M.A., Obi, C.L., Vasaikar, S.D., Baba, K.A. and Hattori, T. (2011) 'Molecular basis of virulence in clinical isolates of Escherichia coli and Salmonella species from a tertiary hospital in the Eastern Cape, South Africa.', *Gut pathogens*, 3, p. 9. Available at: https://doi.org/10.1186/1757-4749-3-9.

Brink, A., Coetzee, J., Clay, C., Corcoran, C., van Greune, J., Deetlefs, J.D., Nutt, L., Feldman, C., Richards, G., Nordmann, P. and Poirel, L. (2012) 'The spread of carbapenem-resistant Enterobacteriaceae in South Africa: Risk factors for acquisition and prevention', *South African Medical Journal*, 102(7), pp. 599–601.

Chatterjee, P., Ghangrekar, M.M. and Rao, S. (2017) 'Disinfection of secondary treated sewage using chitosan beads coated with <scp>ZnO</scp> -Ag nanoparticles to facilitate reuse of treated water', *Journal of Chemical Technology & Biotechnology*, 92(9), pp. 2334–2341. Available at: https://doi.org/10.1002/jctb.5235.

Clements, A., Young, J., Constantinou, N. and Frankel, G. (2012) 'Infection strategies of enteric pathogenic E. coli', *Gut Microbes*, pp. 0–16. Available at: https://doi.org/10.4161/gmic.19182.

CLSI (2012a) *Performance Standards for Antimicrobial Disk Susceptibility Tests ; Approved Standard — Eleventh Edition*.

CLSI (2012b) *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement*. Clinical and Laboratory Standards Institute.

Coetzee, J. and Brink, A. (2011) 'The emergence of carbapenem resistance in Enterobacteriaceae in South Africa', *Southern African Journal of Infectious Diseases*, 26(4), pp. 239–240.

Diallo, A.A., Brugère, H., Kérourédan, M., Dupouy, V., Toutain, P.L., Bousquet-Mélou, A., Oswald, E. and Bibbal, D. (2013) 'Persistence and prevalence of pathogenic and extended-spectrum beta-lactamase-producing Escherichia coli in municipal wastewater treatment plant receiving slaughterhouse wastewater', *Water Research*, 47, pp. 4719–4729. Available at: https://doi.org/10.1016/j.watres.2013.04.047.

Dolejska, M., Frolkova, P., Florek, M., Jamborova, I., Purgertova, M., Kutilova, I., Cizek, A., Guenther, S. and Literak, I. (2011) 'CTX-M-15-producing Escherichia coli clone B2-O25b-ST131 and Klebsiella spp. isolates in municipal wastewater treatment plant effluents.', *The Journal of antimicrobial chemotherapy*, 66(12), pp. 2784–2790. Available at: https://doi.org/10.1093/jac/dkr363.

Dungeni, M., van Der Merwe, R.R. and Momba, M.N.B. (2010) 'Abundance of pathogenic bacteria and viral indicators in chlorinated effluents produced by four wastewater treatment plants in the Gauteng Province, South Africa', *Water SA*, 36(5), pp. 607–614. Available at: https://doi.org/10.4314/wsa.v36i5.61994.

DWAF, D. of W.A. (2009) 'Green Drop Report'.

DWAF, DHE and WRC (2000) *Quality of Domestic Water Supplies Volume 2 : Sampling Guide*.

Elleuch, B., Bouhamed, F., Elloussaief, M. and Jaghbir, M. (2018) 'Environmental sustainability and pollution prevention', *Environmental Science and Pollution Research*, 25(19), pp. 18223–18225. Available at: https://doi.org/10.1007/s11356-017-0619-5.

Hamelin, K., Bruant, G., El-Shaarawi, A., Hill, S., Edge, T.A., Fairbrother, J., Harel, J., Maynard, C., Masson, L. and Brousseau, R. (2007) 'Occurrence of virulence and antimicrobial resistance genes in Escherichia coli isolates from different aquatic ecosystems within the St. Clair River and Detroit River areas.', *Applied and environmental microbiology*, 73, pp. 477–484. Available at: https://doi.org/10.1128/AEM.01445-06.

Kinge, C.N.W., Ateba, C.N. and Kawadza, D.T. (2010) 'Antibiotic resistance profiles of Escherichia coli isolated from different water sources in the Mmabatho locality, Northwest Province, South Africa', *South African Journal of Science*, 106(1/2), p. 6 Pages. Available at: https://doi.org/10.4102/sajs.v106i1/2.14.

Linder, S. and Lundéhn, C. (2002) *Metals in Sewage Sludge in the Eastern Cape*. Master Thesis. CHALMERS UNIVERSITY OF TECHNOLOGY. Available at: https://odr.chalmers.se/server/api/core/bitstreams/97b29da0-fd6e-4b22-a36a-17a56783f651/content.

Masters, N., Wiegand, A., Ahmed, W. and Katouli, M. (2011) 'Escherichia coli virulence genes profile of surface waters as an indicator of water quality', *Water Research*, 45, pp. 6321–6333. Available at: https://doi.org/10.1016/j.watres.2011.09.018.

Matongo, S., Birungi, G., Moodley, B. and Ndungu, P. (2015) 'Occurrence of selected pharmaceuticals in water and sediment of Umgeni River, KwaZulu-Natal, South Africa', *Environmental Science and Pollution Research*, 22(13), pp. 10298–10308. Available at: https://doi.org/10.1007/s11356-015-4217-0.

Mulamattathil, S.G., Bezuidenhout, C., Mbewe, M. and Ateba, C.N. (2014) 'Isolation of Environmental Bacteria from Surface and Drinking Water in Mafikeng, South Africa, and Characterization Using Their Antibiotic Resistance Profiles', *Journal of Pathogens*, 2014, pp. 1–11. Available at: https://doi.org/10.1155/2014/371208.

Nontongana, N., Sibanda, T., Ngwenya, E. and Okoh, A. (2014) 'Prevalence and Antibiogram Profiling of Escherichia coli Pathotypes Isolated from the Kat River and the Fort Beaufort

Abstraction Water', *International Journal of Environmental Research and Public Health*, 11, pp. 8213–8227. Available at: https://doi.org/10.3390/ijerph110808213.

Nordmann, P., Dortet, L. and Poirel, L. (2012) 'Carbapenem resistance in Enterobacteriaceae: Here is the storm!', *Trends in Molecular Medicine,* pp. 263–272. Available at: https://doi.org/10.1016/j.molmed.2012.03.003.

Olaniran, A.O., Naicker, K. and Pillay, B. (2009) 'Antibiotic resistance profiles of Escherichia coli isolates from river sources in Durban, South Africa', *World Journal of Microbiology {&} Biotechnology*, 25, pp. 1743–1749. Available at: https://doi.org/10.1007/s11274-009-0071-x.

Omar, K.B. and Barnard, T.G. (2010) 'The occurrence of pathogenic Escherichia coli in South African wastewater treatment plants as detected by multiplex PCR', *Water SA*, 36(2), pp. 172–176.

Osińska, A., Korzeniewska, E., Harnisz, M., Niestępski, S. and Jachimowicz, P. (2019) 'The occurrence of antibiotic-resistant bacteria, including Escherichia coli , in municipal wastewater and river water', *E3S Web of Conferences*. Edited by B. Kaźmierczak, P. Jadwiszczak, M. Kutyłowska, and U. Miller, 100, p. 00061. Available at: https://doi.org/10.1051/e3sconf/201910000061.

Osode, A.N. (2010) *Assessment of the prevalence of virulent Escherichia coli strains in the final effluents of wastewater treatment plants in the Eastern Cape province of South Africa*. University of Fort Hare.

Osuolale, O. and Okoh, A. (2015a) 'Assessment of the physicochemical qualities and prevalence of Escherichia coli and vibrios in the final effluents of two wastewater treatment plants in South Africa: Ecological and public health implications', *International Journal of Environmental Research and Public Health*, 12(10), pp. 13399–13412. Available at: https://doi.org/10.3390/ijerph121013399.

Osuolale, O. and Okoh, A. (2015b) 'Incidence of human adenoviruses and Hepatitis A virus in the final effluent of selected wastewater treatment plants in Eastern Cape Province, South Africa', *Virology Journal*, 12(1). Available at: https://doi.org/10.1186/s12985-015-0327-z.

Osuolale, O. and Okoh, A. (2017) 'Human enteric bacteria and viruses in five wastewater treatment plants in the Eastern Cape, South Africa', *Journal of Infection and Public Health*, 10(5), pp. 541–547. Available at: https://doi.org/10.1016/j.jiph.2016.11.012.

Phokela, P.T., Ateba, C.N. and Kawadza, D.T. (2011) 'Assessing antibiotic resistance profiles in Escherichia coli and Salmonella species from groundwater in the Mafikeng area, South Africa', *African Journal of Microbiology Research*, 5, pp. 5902–5909. Available at: https://doi.org/10.5897/ajmr11.934.

Pillay, L. and Olaniran, A.O. (2016) 'Assessment of physicochemical parameters and prevalence of virulent and multiple-antibiotic-resistant Escherichia coli in treated effluent of two wastewater treatment plants and receiving aquatic milieu in Durban, South Africa', *Environmental Monitoring and Assessment*, 188(5). Available at: https://doi.org/10.1007/s10661-016-5232-4.

Samie, A., Obi, C.L. and Dillingham, R. (2007) 'Enteroaggregative Escherichia coli in Venda, South Africa: Distribution of Virulence-Related Genes by Multiplex Polymerase Chain Reaction in Stool Samples of', *The American journal of tropical medicine and hygiene,* 77(1), pp. 142–150.

Sans, S.A.N.S. (2011) 'Drinking water - Part 1: Microbiological, physical, chemical, aesthetic and chemical determinands'. Pretoria: SABS Standards Division.

Shariff, A.R.V.A., Shenoy, S.M., Yadav, T. and Radhakrishna, M. (2013) 'The antibiotic susceptibility patterns of uropathogenic Escherichia coli, with special reference to the fluoroquinolones.', *Journal of clinical and diagnostic research : JCDR*, 7(6), pp. 1027–1030. Available at: https://doi.org/10.7860/JCDR/2013/4917.3038.

Siziwe Koba (2013) *Assessment of the incidence of E . coli in Tyume and Bufallo rivers in the Eastern Cape Province of South Africa By Siziwe Koba A thesis submitted in fulfilment of the requirements for the award of a degree of Doctor of Philosophy (PhD) (Microbiology)*. University of Fort Hare.

Trang, D.T., Hien, B.T.T., Mølbak, K., Cam, P.D. and Dalsgaard, A. (2007) 'Epidemiology and aetiology of diarrhoeal diseases in adults engaged in wastewater-fed agriculture and aquaculture in Hanoi, Vietnam', *Tropical Medicine and International Health*, 12, pp. 23–33. Available at: https://doi.org/10.1111/j.1365-3156.2007.01938.x.

Verma, T., Ramteke, P.W. and Garg, S.K. (2008) 'Quality assessment of treated tannery wastewater with special emphasis on pathogenic E. coli detection through serotyping', *Environmental Monitoring and Assessment*, 145, pp. 243–249. Available at: https://doi.org/10.1007/s10661-007-0033-4.

Vidal, M., Kruger, E., Durán, C., Lagos, R., Levine, M., Prado, V., Toro, C. and Vidal, R. (2005) 'Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic Escherichia coli associated with enteric infections', *Journal of clinical microbiology*, 43(10). Available at: https://doi.org/10.1128/JCM.43.10.5362.

Watt, S. and Lanotte, P. (2003) 'Escherichia coli strains from pregnant women and neonates: intraspecies genetic distribution and prevalence of virulence factors', *Journal of clinical microbiology*, 41(5). Available at: https://doi.org/10.1128/JCM.41.5.1929.

West, D. and Mangiameli, P. (2000) 'Identifying process conditions in an urban wastewater treatment plant', *International Journal of Operations & Production Management*, 20(5), pp. 573–590. Available at: https://doi.org/10.1108/01443570010318931.