https://dx.doi.org/10.4314/ijs.v26i3.13

PREVALENCE AND ANTIBIOGRAM OF *Escherichia coli* SPECIES ISOLATED FROM HOSPITAL WASTEWATER AND THE RECEIVING STREAM IN OWO, ONDO STATE

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(Received: 16th September, 2024; Accepted: 21st November, 2024)

ABSTRACT

Wastewater effluent from tertiary hospital is a significant reservoir of pathogenic organisms and can contribute immensely to the toxicity of receiving water bodies if not adequately treated before discharge. This current study was conducted to evaluate the prevalence and antibiogram profile of pathogenic *Escherichia coli* isolates recovered from wastewater samples collected from different wards of a tertiary hospital in Owo and the receiving stream. A total of 76 presumptive *E. coli* isolates was confirmed biochemically, of which 60 were randomly selected and screened by molecular technique. Thirty-eight (38) of the isolates were confirmed using polymerase chain reaction (PCR). High resistance was recorded against meropenem, amikacin, ceftazidime followed by ceftriaxone, ciprofloxacin and gentamicin. However, 100% susceptibility to tetracyclines, cotrimoxazole and vancomycin was likewise recorded. Three predominant antimicrobial resistance patterns detected were comprised ; GEN-CRX-CHL-CTR-CTX-CIP-CPZ-AMK-MEM with multiple antibiotic resistance (MAR) index of 0.75, CPX-CTR-CTX-CIP-CPZ-AMK-MEM with MAR index of 0.60 and GEN-CTX-CIP-CPZ-AMK-MEM with MAR index of 0.50. The study concludes that effluents discharged from the tertiary hospital serves as a reservoir for antibiotic-resistant *E. coli*. The MAR index values are of great public health significance, hence the need to regularly monitor and evaluate hospital effluent discharges and ensure proper treatment before being discharged into the environment.

Keywords: *E. coli*, Hospital Wastewater, Stream, Antibiogram, Multiple Antibiotic Resistance Index.

INTRODUCTION

Wastewater is obtained from domestic and industrial sources, and when untreated, may contain harmful chemicals and pathogens which can harm our living and non-living environment. Hospital wastewater is notable not only for the hazardous chemicals, drugs and antibiotic residues it contains, but also for the increasing antibiotic-resistant pathogens present (Uko *et al.*, 2017; Khan *et al.*, 2020; Top *et al.*, 2020; Chiemchaisri *et al.*, 2022). More improvement in hospital services has resulted in more wastes, including wastewater which when discharged improperly comes with health consequences (Chong and Jin, 2012; Nguyen *et al.*, 2017). Treatment of wastewater can be simple or complex; as there are many processes which may be employed based on the degree of pollutants in such wastewater. For instance, microbial contamination can be effectively removed using reverse osmosis and ultra-filtration; while microbial cultures can be used to break down organic components (Crini and Lichtfouse, 2019). Despite these numerous processes, there is shortage of expertise and equipment needed to treat hospital wastewater in developing countries (Uko *et al.*, 2017; Parida *et al.,* 2022), and this results in inappropriately treated hospital wastewater channeled to public sewage systems (Souza and Feris, 2016; Falodun and Oladimeji, 2019). Consequently,, many of these pathogenic microorganisms contaminate the environment, are resistant to antibiotics (Remschmidt *et al.*, 2017) and they threaten continual use of antibiotics by humans (Okeke *et al.*, 1999; Lien *et al.*, 2017; Szekeres *et al.*, 2017). Hospitals are good culturing environments for multiple antibiotic resistant (MAR) microorganisms (Adekanmbi *et al.*, 2020). If this situation is left unchecked, the cost of responsive antibiotics infection management will continue to rise; as different variants of disease-causing pathogens will keep developing (Dang *et al.*, 2008; Periasamy and Sundaram, 2013). that MAR *E. coli* is a major pathogen found in hospital wastewater (Khan *et al.*, 2009), implicating it in spreading antimicrobial resistance (Karczmarczyk *et al.*, 2011; Tajbakhsh *et al.*, 2015; Williams *et al.*, 2016).

Escherichia coli is a Gram-negative bacterium which is notable as a faecal contaminant (Ekhaise and Omavwoya, 2008; Eze *et al.*, 2016) and commonly found in hospital wastewater (Korzeniewska *et al.*, 2013; Kraupner *et al.*, 2021). *Eschericia coli* causes many human ailments (Galvin *et al.*, 2010) including urinary tract infections (Fasugba *et al.*, 2015) and bloodstream infections (Kuo *et al.*, 2017). Many of these ailments could be life-threatening due to increasing multiple antibiotic resistance (MAR) (Poirel *et al.*, 2018; Naeem *et al.*, 2019). The problem of antibiotic resistance in *E. coli* is the continuous need for developing antibiotics with the passing of resistant genes to progeny (Wu *et al.*, 2020). Even though this is more common among hospital isolates, many environmental isolates are increasingly becoming multiple antibiotic resistance (Mustapha and Imir, 2019). *Escherichia coli* is a prevalent MDR Gram-negative bacterium in hospital wastewater (Egbule, 2016; Akther *et al.*, 2018; Mustapha and Imir, 2019) with high potential of transmission among individuals visiting or working in health facilities (Adler *et al.*, 2012). With the lack of or insufficient treatment of hospital wastewater common in many developing countries, it is expected that MAR *E. coli* will find its way into other environments outside the hospital.

As most hospital effluents are introduced into aquatic systems without treatment, there is probable multiplication of antibiotic-resistant pathogens in aquatic system with following negative public health consequence. No study has reported occurrence of *E. coli* in wastewater effluent of tertiary hospital in Owo. In view of this, we report the first investigation of and antibiogram profile of *E. coli* isolates recovered from a tertiary hospital in Owo, Ondo State. This is a preliminary investigation of our larger study on evaluation of hospital wastewaters as potential reservoir of pathogenic and antibiotic–resistant bacteria in Ondo State environment. This study therefore evaluated the prevalence of multiple antibiotic resistant *E. coli* species isolated from wastewater from different wards in Federal Medical Centre, Owo, and the receiving stream.

MATERIALS AND METHODS

Sample Collection

Wastewater samples were collected from different wards waste drains of the Federal tertiary Hospital, Owo, Ondo State from four sources including three different Departments namely; Children Ward Department (CWD), Female Surgical Ward (FSW), National Health Insurance Scheme (NHIS) and the Receiving Stream (STM). Samples were aseptically collected in 1litre bottles and transported to the laboratory under cold storage for processing.

Isolation and confirmation of *E. coli*

The membrane filtration technique was used to quantify the presumptive *E. coli* in the water samples. Briefly, 100 ml of water samples were filtered through the membrane filters (90mm, 0.45μm pore, Millipore, Ireland). The filter papers were then transferred into Eosine Methylene Blue Agar (EMB) plates and incubated overnight at 37°C. Presumptive *E. coli* with characteristic green metallic sheen colonies were enumerated as colony forming units (CFU) per 100 ml. Three to five (3-5) colonies were picked randomly from each plate as presumptive *E. coli* and then purified on nutrient agar before storage at -4 °C. Thereafter, *E. coli* isolates were confirmed by Gram Staining, and the following biochemical tests; oxidase, citrate, methyl red, indole, and Voges-Proskauer tests. Biochemically confirmed isolates (98) were randomly selected for molecular confirmation using polymerase chain reaction (PCR) assay.

DNA template Preparation for PCR Amplification

The method of Adesiyan *et al.* (2021) was employed for the extraction of the deoxyribonucleic acid (DNA) from the overnight culture of the isolated *E. coli.* Colonies from the nutrient agar culture were transferred into 1X Tris-EDTA buffer (100 μL), and boiled for 10 min at temperature of 100 °C. The boilates were cooled and centrifuged at the rate of 10, 000 rpm for 10 min. The ensuing supernatant was stored as the DNA template and used for PCR analysis.

Molecular Identification of *E. coli* through PCR amplification of 16S rRNA gene

All presumptive *E. coli* isolates identified by phenotypic features were established as *E. coli* through 16S rRNA gene amplification according to Hassan *et al.* (2014) using the *E. coli* primer pair ECO-1/ GACCTCGGTTTAGTTCACAGA and ECO-2 / CACACGCTGACGCTGACCA with amplicon size 585. *Esherichia coli* strain ATCC 25922 was used as the reference strain. A 12.5 µL aliquot containing 6.25 uL of Master mix (Bio Labs), 0.25 µl each of the forward and reverse primers (Inqaba, Biotec), DNA template (2 µl) and nuclease-free water (3.75ul) (BioConcept). The reaction conditions included: Initial denaturation for 5 min at 95°C; then 35 cycles of denaturation 45 s at 94°C, annealing for 45s at 45°C, and extension for 1 min at 72°C; followed by 5 min of final extension at 72°C. Electrophoresis at 90 V for 5 min was done on 1.5% agarose gel and viewed with gel documentation equipment (UVitec transilluminator, Avebury).

Antibiotic Susceptibility Test

Susceptibility test of *E. coli* isolates was performed by disc diffusion assay. Fresh cultures (18-22 hour) of *E. coli* isolates were aseptically transferred into 5 mL of 0.85% sterile physiological saline with turbidity adjusted to 0.5 McFarland standards (equivalence of 1.5 × 10^8 CFU/100 mL). Bacterial suspension was spread uniformly on Muller-Hinton agar using sterile swabs, after which antibiotic discs were positioned on the agar and incubated for 18 to 24 h at 35 ± 2 °C. Plates were then examined for zones of inhibition after incubation. Zones were measured with ruler and interpreted as susceptible, intermediate or resistant (Table 2) according to CLSI guideline (2020). Twelve (12) antibiotics were selected for testing on confirmed *E. coli* isolates which included: gentamicin (30µg), Cefuroxine (30µg), Chloramphenicol (30µg), Ceptriaxone (30µg), Ceflazidime (30µg), Ciprofloxacin (30µg), Tetracyclin (30µg), Cotrimoxazone (30µg), Vancomycin (30µg), Amikacin (30µg), Ceptazidime (30µg), Meropenem (30µg).

Table 1: Interpretative chart of Antibiotics Resistance Pattern of *E. coli* species.

Class	Antimicrobial Agents	Code	Dose	Zone Diameter Interpretative Criteria		
				Resistant	**Intermediate**	**Sensitive**
Aminoglycosides	Gentamicin	GEN	10µg	≤ 12	13-14	≥15
Phenicol	Chloramphenicol	CHL	10µg	<12	13-17	>18
Cephalosporin	Cefuroxime	CRX	30µg	<14	15-17	>18
	Ceftriaxone	CTR	30µg	<19	20-22	>23
	Cefotaxime	CTX	30µg	<22	23-25	≥26
	Ceftazidime	CPZ	30µg	<17	18-20	21
Fluoroquinolones	Ciprofloxacin	CIP	5µg	<15	16-20	≥21
Tetracycline	Tetracycline	TET	30µg	<11	12-24	>15
Sulfonamides	Cotrimoxazole	COT	25µg	≤19	-	≥20
Aminoglycosides	Amikacin	AMK	30µg	≤14	15-16	≥17
Glycopeptide	Vancomycin	VAN	30µg	<14	15-16	≥17
Carbapenems	Meropenem	MEM	30µg	≤19	20-22	≥23

Antibiotics Resistance indexing

The rate of occurrences and profiles of antibiotic resistances were obtained from the antibiotic susceptibility test. Likewise, Multiple Antibiotic-Resistant (MAR) phenotypes for isolates exhibiting resistance to 3 or more classes of antibiotics at each sampled site were determined MAR index is significant indicator of risk source of pollution potentially harmful to human (Titilawo *et al.*, 2015) and it is expressed mathematically as

MAR index = N/V

Where N, is total antibiotics to which resistance was recorded

and V, number of tested antibiotics

RESULTS

Total Presumptive *E. coli*.

A total of 12 wastewater (WW) samples were collected from four (4) different Departments of the hospital (NHIS Ward, Children Ward (CWD), Female Surgical Ward (FSW), and stream (STM)) as well as 8 receiving stream water over a 4-month period. Total coliform counts were estimated from the wastewater samples using EMB (Eosine Methylene Blue) agar. NHIS ward samples had the highest number of positive colonies followed by the CWD, STM, and FSW ward respectively (Table 3).

Table 2 Total coliform counts on the EMB Agar.

Sample Area	Frequency of sampling	Number of Colonies on EMB Agar (100 CFU/ml)
Children Ward (CWD)	CWD 1	79
	CWD 2	32
	CWD 3	83
Stream	STM 1	113
	STM2	116
	STM 3	TNC
Female Surgical Ward (FSWD)	FSWD 1	TNC
	FSWD 2	13
	FSWD 3	29
National Health Insurance Scheme (NHIS)	NHIS 1	179
	NHIS 2	63
	NHIS 3	No growth

CWD- Children ward; STM- Stream; FSWD-Female Surgical ward; NHIS- National health Insurance Scheme
Note: filtered sample volume is 100ml TNC - too numerous to count

E. coli confirmation

After the membrane filtration, 25 isolates were randomly selected from the pool of isolates recovered from each sample area (i.e CWD, STM, FSWD and NHIS) totaling 100 in all and sub-cultured for biochemical test. A total of 76 *E. coli* isolates was confirmed biochemically out of which sixty (60) was randomly selected and subjected to PCR analysis. In total 63% (38/60) were confirmed positive as *E. coli*. The gel representative of the molecular confirmation is as shown in Plate 1.

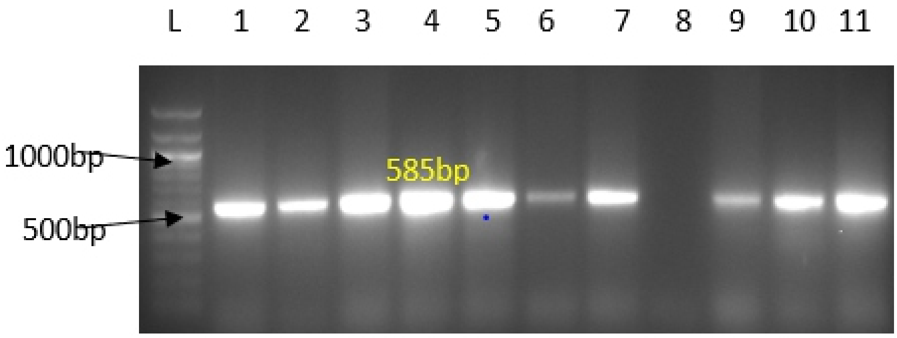


Plate 1: Amplification of 16S rRNA of *Escherichia coli*. L: 100bp ladder; Lane 2: positive control, Lane 8 negative control, Lane 1-7,9-11 confirmed *E. coli strains*

Antibiotics Resistance and Susceptibility Pattern of *E. coli*

The antibiotic resistance pattern of *E. coli* isolates is as presented in Table 4. In all the wastewater samples from the NIHS ward, CWD and FSW high Resistance was observed against Amikacin, Cefotaxime and Ceftazidime (100% resistance each) while variable resistance was observed against Gentamicin, Ceftriaxone, Ciprofloxacin, Cefuroxime, Chloramphenicol across other sample sources. However, some isolates show low or no resistance to Tetracycline, Cotrimoxazole, and Vancomycin among those recovered from NHIS, CWD, and FSW samples, while isolates recovered from STM samples showed some level of resistance to the antibiotics. The data indicates varying levels of resistance based on the sample source, with isolates having high resistance to some of the antibiotics observed consistently across all the sample types. This result thus suggests a high level of resistance to several commonly used antibiotics, indicating potential challenges for the treatment of infections from these sources with these antibiotics.

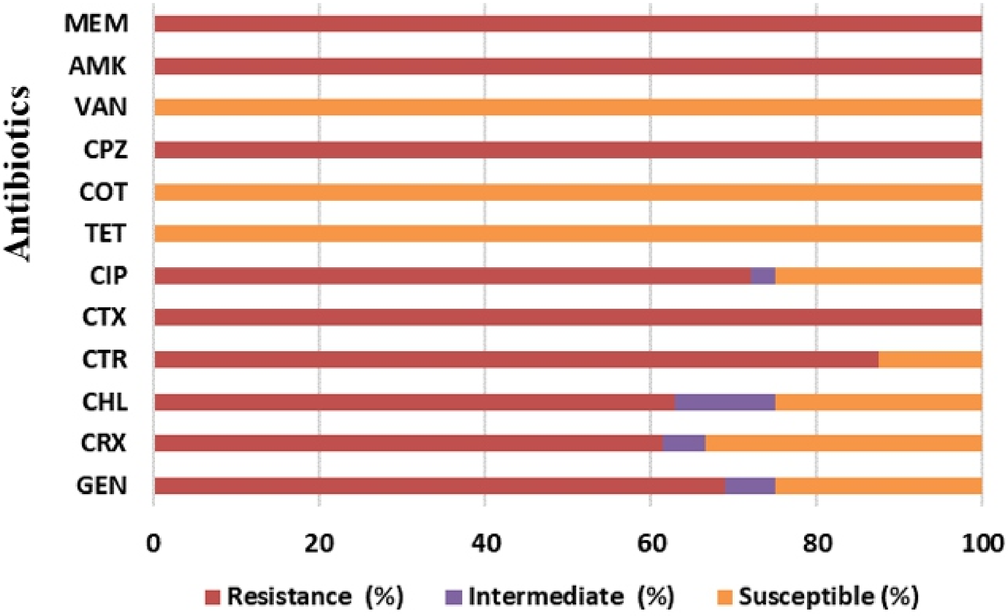
Table 3: Antibiotics Resistance Pattern of *E. coli* from waste water effluent and the receiving stream.

Class	**Antimicrobial Agents**	**Sample sources %**			
		NHIS (n=9)	**CWD (n=11)**	**FSW (n=4)**	**STM (n=14)**
Aminoglycosides	Gentamicin	78	82	25	79
	Cefuroxime	67	67	50	71
Phenicol	Chloramphenicol	67	67	50	71
Cephalosporin	Ceftriaxone	78	100	75	86
	Cefotaxime	100	100	100	93
	Ceftazidime	100	100	100	93
Fluoroquinolones	Ciprofloxacin	78	82	25	79
Tetracycline	Tetracycline	0	0	0	42
Sulfonamides	Cotrimoxazole	0	0	0	42
Aminoglycosides	Amikacin	100	100	100	72
Glycopeptide	Vancomycin	0	0	0	29
Carbapenems	Meropenem	100	100	100	21

Combined Antibiogram Profile of recovered *E. coli* from all the samples

The general antibiogram profile of *E. coli* isolates from all the samples (wastewater effluent and the receiving stream) as shown in Figure 1 illustrates the percentage of *E. coli* isolates that are resistant (R), intermediate (I), or susceptible (S) to various the antibiotics assessed.

The result shows a high level of resistance to many antibiotics, particularly Cefotaxime, Ceftazidime, Amikacin, and Meropenem (100% resistance). However, all the *E. coli* isolates showed no resistance to Vancomycin, Tetracycline and Cotrimoxazole, with 100% susceptibility recorded.

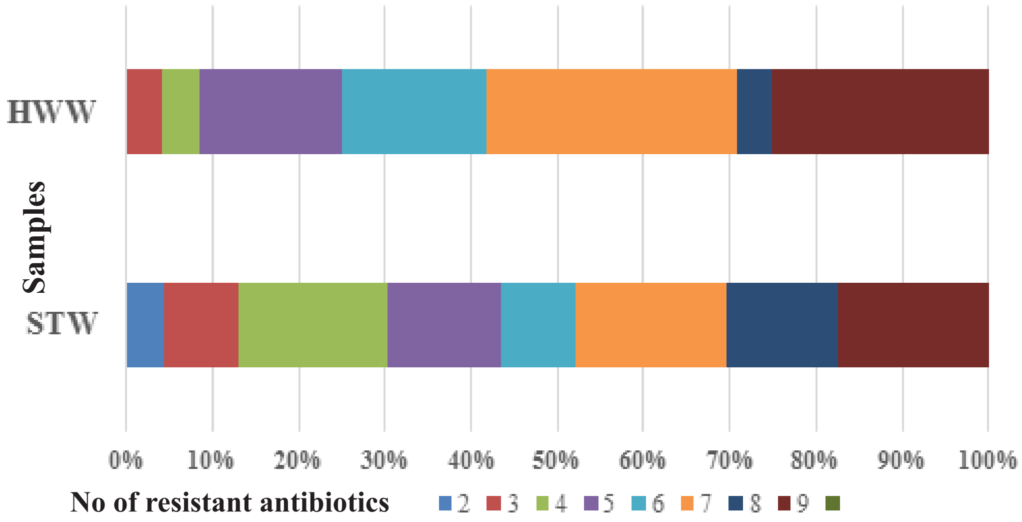


AMK amikacin, CHL chloramphenicol, CPZ Ceftazidime, CIP ciprofloxacin, CTX cefotaxime, GEN gentamycin, MEM meropenem, TET tetracycline, COT Cotrimoxazole VAN Vancomycin, Ceftriaxone CTR, Cefuroxime CRX

Figure 1: The Multiple Drug-Resistant Profile of *E. coli* Recovered from wastewater and stream samples.

The STW samples shows that 28.6% of isolates were resistant to seven antibiotics, 21.4% to eight, and 28.6% to nine. However, the HWW revealed that 29% of isolates were resistant to seven antibiotics, 4.2% to eight, and 25% to nine (Figure 2). Both sources of wastewater have a significant percentage of *E. coli* resistant to a high number of antibiotics, with HWW showing a highest resistance against seven and nine antibiotics (29% and 25% respectively) while STW samples had similar resistance pattern but more similar percentage resistance against seven, eight, and nine antibiotics.

This result reveals that isolates from HWW show more resistance at the high end, while isolates from STW show a broader spread of antibiotic resistance (Figure 2).



**** HWW- Hospital waste water; STW- Stream water*

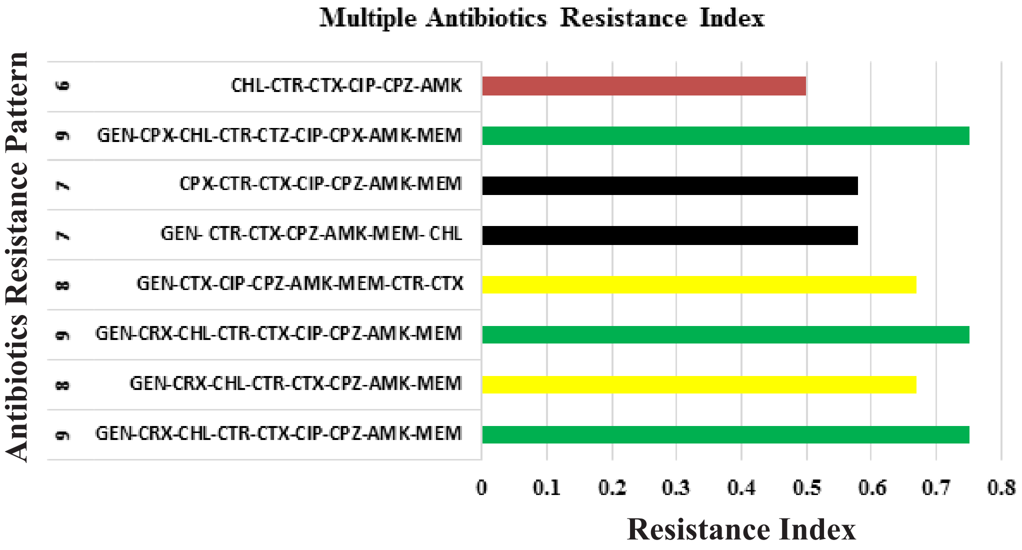
Figure 2. Percentage resistance to antibiotics recorded in E. *coli* isolates from Federal Medical Centre Owo

Multiple Antibiotic Resistance Pattern and Index of *E. coli* Isolates

The different notable and highest combinations of antibiotics to which the *E. coli* isolates. are resistant, as well as their MAR index are shown in figure 3. The MAR index ranges from 0.50 to 0.75, with most patterns having an index of 0.75 indicating resistance to 9 out of the 12 antibiotics tested. This high MAR index suggests that the *E. coli* isolates from this environment have probably been exposed to significant levels of antibiotics, leading to resistance.

Also, many of the combinations of resistance patterns are repeated, suggesting common exposure pathways or similar selective pressures in the environment.

Resistance to critical antibiotics such as Cefotaxime (CTX), Meropenem (MEM), and Ciprofloxacin (CIP) is consistent across multiple patterns, highlighting the critical public health challenge posed by these resistant isolates.



AMK amikacin, CHL chloramphenicol, CPZ Ceftazidime, CIP ciprofloxacin, CTX cefotaxime, GEN gentamycin, MEM meropenem, TET tetracycline, COT Cotrimoxazole VAN Vancomycin, Ceftriaxone CTR, Cefuroxime CRX

Figure 3: Multiple Antibiotics Resistance Pattern of *E. coli* isolates from waste water effluent of FMC, Owo

DISCUSSION

Escherichia coli, a common Gram-negative bacterium was isolated from hospital wastewater and the receiving stream in this study.

This organism has been reported to be associated with hospital wastewater in Nigeria (Bolaji *et al.*, 2011) and other sources (Aziz, *et al.*, 2014; Mahmood *et al.*, 2024). Different parts of the hospital as sampled revealed varied colony count of *E. coli*, with the NHIS Department of FMC Owo having the highest colony count among the sampled locations. This was followed by the samples collected from the children ward (CWD), and the stream in that order. This indicates that locations opened more to the public had higher counts of the organisms.

The widespread occurrence of *E. coli* isolates within the hospital departments as revealed by the polymerase chain reaction (PCR) analysis may be due to low level of sanitation and hygiene practices. The lower prevalence of *E. coli* in stream water samples may be due to the constant flow and mixing of the water, which likely dilutes bacterial concentrations. Nonetheless, the presence of *E. coli* in both HWW and STM is anticipated, as it naturally exists as part of the microbiome in the digestive tracts of both the sick and healthy individuals and with wide dissemination in the environment (Kagambèga *et al.*, 2023). Hospital wastewater is thought to be a source of antibiotic-resistant pathogens and a major factor in the environmental spread of antibiotic-resistant bacteria.

Escherichia colii isolates from hospital wastewater are known to cause nosocomial infections (Kaur *et al.*, 2020). Thus, these wastewaters pose a health risk to individuals who work or visit hospitals, as well as neighboring communities. It is therefore necessary to treat wastewater such that disease-causing pathogens are considerably eliminated.

Globally, the prescription and usage of antibiotics without consideration has led to a sharp rise in resistance in *E. coli* isolates (Guessennd *et al.,* 2013; Garba *et al.*, 2023). Majority of the *E. coli* isolates exhibited drug resistance); and were resistant to at least two classes of antibiotics (Doyle *et al.*, 2015). Majority of the isolates were resistant to Amikacin, Meropenem, Ceftriaxone followed by Gentamicin and Ciprofloxacin; which is at variance to previous studies where *E. coli* isolates showed resistance towards β-lactam, tetracycline, and cephalosporins (Olukosi *et al.*, 2016). Treating infection caused by Enterobacteriaceae commonly employs third-generation cephalosporins (Garba *et al.,* 2023). Consequently, resistance to this group of antibiotics has increased globally. Despite studies reporting tetracycline resistance from environmental samples (Olowe *et al.*, 2013; Ejere *et al.*, 2014; Di Francesco *et al.*, 2020), *E. coli* isolates from this study showed no resistance against tetracycline; which could be attributed to different sources of isolates and antibiotic usage in this environment.

MARI analysis in this study shows that 85% of the *E. coli* isolates had MARI values greater than 0.2. This high MARI suggests that the *E. coli* isolates from this environment have probably been exposed to significant levels of antibiotics, leading to resistance. Several combinations of resistance patterns are repeated, suggesting common exposure pathways or similar selective pressures in the environment. Resistance to critical antibiotics like Cefotaxime (CTX), Meropenem (MEM), and Ciprofloxacin (CIP) is consistent across multiple patterns, highlighting the critical serious public health challenge posed by these resistant isolates.

This result is slightly similar to that of Chigor *et al.* (2010), who recorded MARI in 80% of isolates recovered from surface water sources in Zaria. On the other hand, this result is consistent with studies by Ogbolu *et al.* (2013) and Edward *et al.* (2020), which reported that pathogenic Gram-negative bacteria in Nigerian hospital environments had significant levels of resistance, with 80% and 99.8% of *E. coli* isolates exhibiting a MARI > 0.2. This study also supports the findings of Adekanbi *et al.* (2020), who found that hospital wastewater and sludge included multiple antibiotic -resistant *E. coli* with MARIs ranging from 0.63 to 1.0. There is a considerable possibility of antibiotic contamination in the area, which makes the high resistance index here worrisome (Krumperman, 1983). The observations in this study indicates a possible significant exposure of *E. coli* in this environment to considerable level of antibiotics and antibiotic residues, which can ultimately lead to selection pressure (Baker *et al*., 2018).

As hospital effluents were considered a reservoir for antibiotic-resistant pathogens (Kizny *et al*., 2017; Park *et al.,*2020), playing a key role in the spread of antimicrobial-resistant bacteria in the environment. The display of high resistance to antibiotics in this study calls for concern as the presence of high concentration of antibiotic residues could select or support the proliferation of antibiotic resistant bacteria.

The result of this study is a public health concern especially in Nigeria where wastewater is discharged directly into adjoining water bodies or environment without subjecting it to prior treatment. The water in the receiving stream may be used for irrigation of vegetable crops as this is a common practice among farmers in Nigeria and elsewhere (Michael *et al.,* 2014; Soré *et al.,* 2020). Hence this could potentially enter the food chain or water supply leading to high prevalence of antibiotic resistance *E. coli* in the immediate environment of the hospital center. This could result to difficulty in therapeutic treatment, increased hospitalization costs due to prolonged patient hospitalizations as well as higher mortality and morbidity (French, 2005: Adesiyan *et al*., 2021). Likewise, the high concentration of *E. coli* in the hospital wastewater may offer inducement for horizontal transfer of resistance genes between *E. coli* and other bacteria in the environment (Guessennd *et al*., 2013; Drieux *et al*., 2016).

CONCLUSION

Effluents discharged from the hospital are a potential reservoir for antibiotic-resistant *E. coli* and possibly other Enterobacteriaceae as revealed in this study. There is therefore a need to regularly monitor and evaluate hospital effluent discharges and ensure proper treatment before being discharged into the environment. This finding underscores the need for stringent monitoring of antimicrobial use, wastewater management, and potential risks to public health from environmental contamination with resistant bacteria.

FUNDING

The research received no external funding

CONFLICT OF INTEREST

The authors declare no conflict of interest affiliated to this article

AUTHORS CONTRIBUTIONS

A. I. M and A. O.: Investigation, Methodology, Data Analysis; A. I. M and F. Y. M: Conceptualization, Supervision, Original Draft Preparation; A. I. M., F. Y. M., A. K.O., E. A. E., O. M and A. O.: Data Analysis, Validation, Writing, Review & Editing

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