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Occurrence of multidrug resistant and extended spectrum β-lactamase (ESBL) - producing *Escherichia coli* in wastewater of two healthcare facilities in Ibadan, Nigeria

Abimbola O. Adekanmbi*, **Adedoyin O. Adeyemi** and **Oreoluwa M. Olajide**

Environmental Microbiology and Biotechnology Laboratory, Department of Microbiology, University of Ibadan, Nigeria

*E-mail address: bimboleen@yahoo.com

ABSTRACT

Most industries in developing countries of the world, especially hospitals and other clinical settings, lack wastewater treatment facilities, and as such, untreated wastewater from their operations are discharged into water bodies without any form of treatment. This study aimed at the antibiotic sensitivity profile and ESBL production in *E. coli* isolated from untreated hospital wastewater before discharge into the environment. Untreated wastewater from two hospitals, a State Government-owned hospital (SGH) and a privately-owned hospital (POH) with no wastewater treatment facilities were sampled for a period of four months. Isolation of *E. coli* was carried out using the pour plate technique on Eosin Methylene Blue agar, while identification was carried out using conventional methods. Determination of ESBL production was done by means of the Double Disc Synergy Technique and antibiotic sensitivity testing was carried out by employing the disc diffusion method. A total of fifty-eight (58) *E. coli* were obtained: SGH at 55 and POH at 3. Herein, in 100% of the total count, resistance was indicated for ampicillin and ertapenem, while 14%, 11%, 16% and 57% of the total count were resistant to ceftazidime, cefpodoxime, cefotaxime and amoxicillin-clavulanate, respectively. In addition, 94.8% showed resistance to tetracycline, 19% to ciprofloxacin, 6.9% to gentamycin, 39.7% to chloramphenicol and 55% and 47% to sulfamethoxazole-trimethoprim and nalidixic acid, respectively. Furthermore, 94.8% of all the isolates were multidrug resistant (MDR), while 29.3% were ESBL positive. Wastewater from the two hospitals under study contained ESBL positive and MDR *E. coli*, suggesting a need to forestall a potential threat to public health by treating the wastewater generated by both hospitals before discharge into the environment.

***Keywords*:** *Escherichia coli,* β-lactamase, wastewater, hospital-waste

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1. INTRODUCTION

The production of ESBL by Enterobacteriaceae has resulted in a rapid rise in antibiotics failure. These enzymes are known to hydrolyze the β -lactam ring in antibiotics such as penicillins, cephalosporins and monobactams (Oberoi *et al*., 2013) and are inactivated by β-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005). Of all the resistance mechanisms against β-lactam antimicrobials in gram-negative organisms, the production of β-lactamases is the most important (Canto'n *et al*., 2002). The widespread use and application of antimicrobial compounds in animal husbandry, clinical settings, and aquaculture have led to the uncontrolled dissemination of antibiotic resistant bacteria into the environment (Kolar *et al*., 2001; Gaskins *et al*., 2002; Molstad *et al*., 2002; and Kummerer, 2009a). These resistant agents gain entrance to the environment via the indiscriminate discharge of untreated or partially treated wastewater from domestic, clinical and industrial sources (Goni-Urriza *et al*., 2000; Chitnis *et al*., 2004; Kim and Aga, 2007).

Furthermore, contaminated wastewaters especially from clinical sources mostly contain partially metabolized or unused antibiotics and according to European Molecular Biology Organization (EMBO) report, antibiotics are excreted in varying amount by humans and animals into receiving water bodies via sewage and other wastewaters. The excretion from the use of some of the widely consumed classes of antibiotics e.g. tetracycline, phenicols, trimethoprim, beta-lactams, can exceed 50% of the administered dose and can act as a selective pressure for the development of resistance to antibiotics in bacteria (Berkner *et al*., 2014).

The transfer into the environment of bacteria with acquired resistance to antimicrobial agents poses both direct and indirect risks to man and human health. Exposure to antibiotic resistant bacteria (ARB) resulting in treatment failure constitutes the direct risks, while the asymptomatic carriage of ARB in the form of commensals inhabiting the gut, mucosa and skin account for the indirect risks, due to the possible exchange of resistance genes between commensals and pathogenic organisms (Blaak *et al*., 2015).

In developing countries, like Nigeria, wastewaters are discharged into receiving channels without any form of treatment. The discharge of untreated wastewater from healthcare facilities, has led to the spread of ARB, and this has exposed the human population to possible infection especially in areas where the receiving waters are used for domestic purposes. This has necessitated the need to explore hospital wastewater as a vehicle for the dissemination of ARB into the environment. This study therefore aimed at isolating *Escherichia coli* from untreated wastewater generated by two secondary hospitals in Ibadan, Nigeria and screening the isolated bacteria for ESBL production.

2. MATERIALS AND METHODS

2. 1. Description of the sample collection sites/sample collection

The study was carried out at two secondary hospitals in Ibadan, Nigeria. The two hospitals; a State Government-owned hospital (SGH) and a privately-owned hospital (POH) with no wastewater treatment facilities and discharge their wastewater directly inside an adjoining receiving river, which is being used for activities including laundry, agriculture, and other household purposes by people around the community. Wastewaters were collected from three sections of the two hospitals; General laboratory and Microbiology laboratory (SGH) and

Hospital wards (POH). Samples were collected in sterile sample containers fortnightly for a period of four months and transported on ice to the laboratory and analyzed within two hours of collection. Approval was sought from the two hospitals before access was granted for collection of their wastewater samples.

2. 2. Isolation and characterization of *Escherichia coli* from hospital wastewater

Isolation of *E. coli* from the hospital wastewater samples was carried out using the standard pour plate technique on Eosin Methylene Blue agar (Harrigan and MacCance, 1976). The plates were incubated overnight at 35±2 ºC after which presumptive, morphologically distinct colonies showing green-metallic sheen appearance of *E. coli* were subcultured onto fresh plates. Pure isolates were stored on glycerol stock and nutrient agar slants for further studies. The identities of the isolates were confirmed using sugar and biochemical tests including Gram staining, motility, oxidase test, citrate test, indole test, urease test, methyl red-voges proskaeur (MR-VP), catalase test and fermentation of sugars (Sneath, 1996).

2. 3. Susceptibility testing

The susceptibility of the isolates to antimicrobials was carried out using the Kirby-Bauer disc diffusion method. The antibiotics were purchased from Oxoid, UK and included: tetracycline (tetracycline), gentamycin (aminoglycosides), chloramphenicol (phenicols), ciprofloxacin (fluoroquinolones), sulfamethoxazole-trimethoprim (folate pathway inhibitors), nalidixic acid (quinolones), ertapenem (carbapenems), ampicillin (penicillin), amoxicillin-clavulanate (β-lactam/β-lactamase inhibitor) and cefotaxime, ceftazidime, cefpodoxime (3rd generation cephalosporin). The antibiotics were selected for the AST based on the panel of antibiotics recommended for Enterobacteriaceae in the CLSI guidelines (CLSI, 2018). An isolate was regarded as resistant if no zone of inhibition was observed around the particular antibiotic disc. The data obtained were used to determine the phenotypic pattern of resistance for each isolated organism. Bacteria showing resistance to more than two classes of antibiotics were classified as multi-drug resistant (MDR).

2. 4. Screening for ESBL production in selected isolates

The screening for ESBL production was carried out on isolates showing reduced susceptibility or resistance to any of the third-generation cephalosporins (ceftazidime, cefotaxime and cefpodoxime) using the Double Disk Synergy Test (DDST). The isolates were considered to be ESBL-positive when it showed a clear-cut increase or enhancement in zone diameter towards the amoxicillin-clavulanate (AMC) at the centre (CLSI, 2018).

3. RESULTS

A total of fifty-eight (58) non-duplicated *E. coli* were obtained from the hospital wastewater. The isolates were selected based on the point of isolation and the antibiotics susceptibility pattern to prevent unnecessary duplication of isolates. Of the total isolates, 55 (94.8%) were obtained from the wastewater of the SGH while the remaining 3 (5.2%) were obtained from wastewater generated by POH (Figure 1).

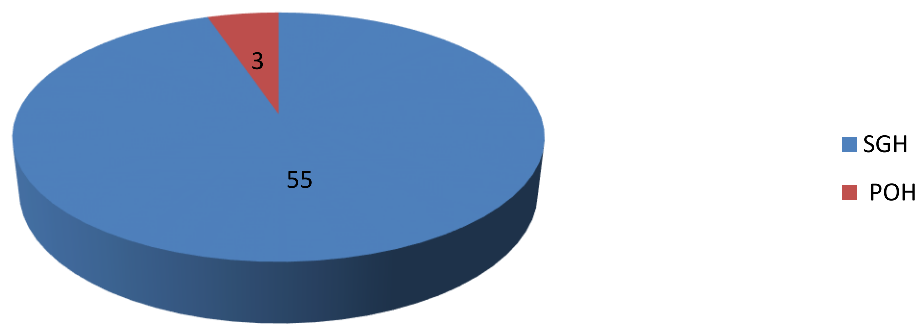

Figure 1. Frequency of *E. coli* obtained in untreated wastewater from the two hospitals

Table 1 is showing the resistance phenotype in *Escherichia coli* isolated from hospital wastewater samples. Fifty-five (55) of the total isolates representing 94.8% showed resistance to more than two antibiotics and were classified as MDR. One of the isolates showed resistance to all the twelve tested antibiotics.

Table 1. Resistance phenotype in *E. coli* isolated from hospital wastewater samples.

No. of Antibiotics	Resistance pattern	[a]No. of isolates	(%)
2	etp-ap	3	5.17
3	te-etp-ap	5	8.62
4	te-etp-ap-caz	3	5.17
	te-etp-ap-amc	6	10.34
	te-etp-ap-ctx	1	1.72
	te-etp-ap-cpd	1	1.72
	c-etp-ap-amc	1	1.72
5	te-sxt-etp-ap-caz	1	1.72
	te-etp-ap-amc-ctx	1	1.72
	te-etp-ap-amc-caz	1	1.72
	te-sxt-etp-ap-amc	1	1.72
	te-sxt-na-etp-ap	2	3.45
	te-c-sxt-etp-ap	1	1.72
	te-na-etp-ap-ctx	1	1.72
6	te-c-sxt-etp-ap-amc	3	5.17
	te-c-sxt-na-etp-ap	4	6.90
	te-c-na-etp-ap-amc	1	1.72
	te-sxt-na-etp-ap-amc	4	6.90
	te-etp-ap-amc-caz-cpd	1	1.72

7	te-cip-na-etp-ap-ctx-cpd te-c-cip-sxt-etp-ap-ctx te-c-sxt-na-etp-ap-amc te-cip-sxt-na-etp-ap-amc te-cip-sxt-na-etp-ap-ctx te-sxt-na-etp-ap-amc-cpd	1 1 4 1 1 1	1.72 1.72 6.90 1.72 1.72 1.72
8	te-c-cip-sxt-na-etp-ap-amc te-c-cip-sxt-na-etp-ap-ctx te-cn-c-sxt-na-etp-ap-amc	3 2 1	5.17 3.45 1.72
10	te-c-cip-sxt-na-etp-ap-amc-ctx-cpd	1	1.72
12	te-cn-c-cip-sxt-na-etp-ap-amc-ctx-caz-cpd	1	1.72

KEY:
[a] Number of isolates showing the unique phenotype of resistance indicated.
ap: ampicillin (10µg), caz: ceftazidime (30µg), cpd: cefpodoxime (10µg), ctx: cefotaxime (30µg), amc: amoxicillin-clavulanate (30µg), te: tetracycline (30µg), cip: ciprofloxacin (5µg), cn: gentamycin (10µg), c: chloramphenicol (30µg), sxt: sulfamethoxazole/trimethoprim (25µg), etp: ertapenem (10µg), na: nalidixic acid (30µg), +: positive, -: negative.

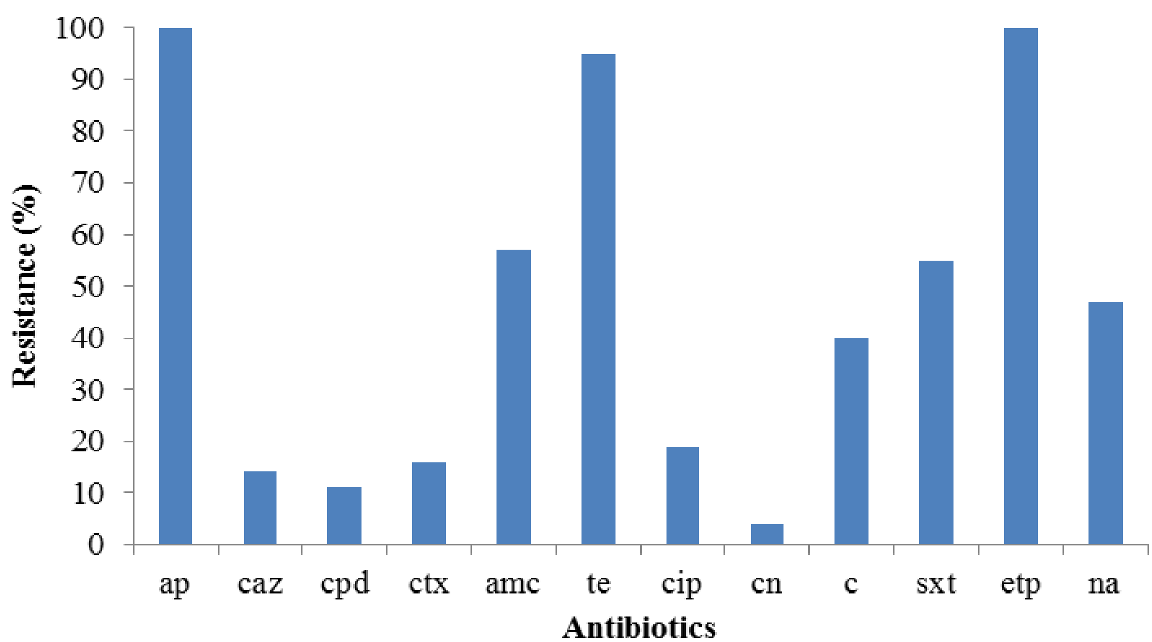

Figure 2. Percentage resistance of *E. coli* isolated from hospital wastewater to antibiotics

KEY:
ap: ampicillin (10µg), caz: ceftazidime (30µg), cpd: cefpodoxime (10µg), ctx: cefotaxime (30µg), amc: amoxicillin-clavulanate (30µg), te: tetracycline (30µg), cip: ciprofloxacin (5µg), cn: gentamycin (10µg), c: chloramphenicol (30µg), sxt: sulfamethoxazole/trimethoprim (25µg), etp: ertapenem (10µg), na: nalidixic acid (30µg)

Figure 2 shows the percentage resistance of *Escherichia coli* isolated from hospital wastewater to selected antibiotics. There was 100% resistance to ampicillin and ertapenem by all fifty-eight (58) *E. coli* isolated from hospital wastewater. Of the 58 isolates, 14%, 11%, 16% and 57% were resistant to ceftazidime, cefpodoxime, cefotaxime and amoxicillin-clavulanate respectively, while 55 of the 58 isolates representing 94.8% showed resistance to tetracycline, 11 (19%) to ciprofloxacin, 4 (6.9%) to gentamycin and 23 (39.7%) to chloramphenicol. 55% and 47% of the total isolates from the hospital wastewater were resistant to sulfamethoxazole-trimethoprim and nalidixic acid respectively.

Table 2 shows the percentage ESBL-producing *E. coli* isolated from the hospital wastewater samples. Of the fifty-eight (58) isolates obtained from the untreated hospital wastewater, seventeen were ESBL positive, with sixteen of them isolated from wastewater obtained from SGH (29.1%) and only one isolate from wastewater obtained from POH (33.3%).

Table 2. Percentage of ESBL-producing *E. coli* isolated from the hospital wastewater.

Sampling site	No. of *E. coli* isolated	No. of ESBL producers	% ESBL positive isolates
SGH	55	16	29.1
POH	3	1	33.3

4. DISCUSSION

The availability of drugs especially antibiotics to the general public in developing countries of the world is a major factor contributing to the increased prevalence of drug-resistant bacterial strains in the environment (Lateef, 2004). This coupled with the lack of treatment facilities in most industries especially hospitals, has led to the deposition of hazardous chemicals into receiving water bodies; this becoming a public health issue. This is because the wastewater generated from hospitals could be potentially hazardous to the environment because of the constituents including radioactive materials, chemical and pharmaceutical wastes, especially antibiotics together with certain pathogenic microorganisms (Sharpe, 2003).

In this study, a total of fifty-eight (58) *E. coli* strains isolated from different sections of two hospitals were obtained. *E. coli* and other members of the Enterobacteriaceae have been isolated from several hospital wastewater as earlier reported by other authors. Elmanama *et al*. (2006) reported the isolation of *E. coli* from wastewater generated by Al-Shifa hospital in Gaza. In the study, 30.5% of the total 154 bacteria obtained were identified to be *E. coli*. In addition, a study carried out by Katouli *et al.* (2012) reported the isolation of *E. coli* in untreated wastewater generated by hospital and other receiving inlet and outlet in South East Queensland, Australia. Moges *et al*. (2014) also reported the isolation of multi-drug resistant bacterial pathogens from hospital and non-hospital environments in which 12.3% of the total 65 isolates obtained from the former were identified as *E. coli*. Andy and Okpo (2018), in their study on effluent and waste dumpsite of hospitals in Calabar metropolis in Nigeria reported that *E. coli* constituted 20.7% of the total one hundred and seventy nine isolates obtained in their study.

In this study, all the fifty-eight isolates obtained were resistant to ampicillin and ertapenem, this observation is quite dissimilar to what was obtained by El Manama *et al*. (2006), in which they reported 42.6% resistance to piperacillin and 36.2% resistance to amoxicillin, two antibiotics in the same class with ampicillin (penicillins). In total disagreement with this study is the fact that they reported no resistance to imipenem, an antibiotic in the carbapenem class as ertapanem, same as Andy and Okpo (2018) and Brechet *et al*. (2014). The resistance to amoxicillin-clavunalate in this study was higher than what was obtained in the study carried out by Andy and Okpo (2018). In their study, 24.3% of the total *E. coli* were reported to show resistance to the antibiotic which is in sharp contrast with the resistance of 57% observed in this present study. In addition, Lien *et al*. (2017) reported a resistance of 60.7% and 46.7% respectively in *E. coli* isolated from untreated hospital wastewater obtained from rural and urban hospitals in Vietnam.

The resistance to gentamycin in *E. coli* from this study (6.9%) is however lower than what was reported by several other authors; 10.6% by El Manama (2006), 21.6% by Andy and Okpo (2018) and 20% by Sharmin *et al*. (2018). In this study, there was 14% resistance to ceftazidime while the resistance to cefotaxime and nalidixic acid were 16% and 47% respectively. These values are comparatively lower than ceftazidime (89%), cefotaxime (80%) and nalidixic acid (67%) reported by Brechet *et al*. (2014). The occurrence of antibiotic resistance in *E. coli* from wastewater is a recognized phenomenon. In a study carried out by Katouli *et al*. (2012) on *E. coli* strains isolated from untreated hospital wastewater, there was a high level of resistance to most commonly used antibiotics namely; aztreonam, gentamicin, amoxicillin-clavulanic acid, ceftazidime, with the least level of resistance observed towards ciprofloxacin; norfloxacin, nalixidic acid, nitrofurantoin and chloramphenicol. The percentage ESBL positive isolates in this study was 29.3% in the total isolates obtained from both hospitals in this study, with sixteen obtained from the SGH and only one from POH. This is higher than the percentage 11.4% and 8.6% respectively obtained in a study carried out on wastewater generated from a private and tertiary hospital in the same geopolitical zone as reported by Falodun and Oladimeji (2019). This could be attributed to several factors including the concentration of antibiotics in the respective wastewater. Further studies could be to investigate the correlation between concentration of antibiotics in the wastewater and the level of resistance in the isolates obtained.

5. CONCLUSION

The occurrence of multidrug resistant and ESBL producing bacteria in wastewater generated by the hospitals in this study further emphasized the need for a regulation in the use of antimicrobials in hospital settings and also an enforcement of wastewater treatment by regulatory agencies, to forestall a probable public health breakdown emanating from the introduction of multidrug resistant bacteria into receiving water bodies.

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