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Determination of Water Quality and Detection of Extended Spectrum Beta-Lactamase Producing Gram-Negative Bacteria in Selected Rivers Located in Ibadan, Nigeria

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Abstract

The present study is designed to determine the occurrence of Extended Spectrum β-Lactamase (ESBL)-producing Gram-negative bacteria in water samples from selected rivers in Ibadan, Nigeria. Water samples were collected from three rivers and physicochemical analysis carried out. Isolated Gram-negative bacteria were identified using conventional biochemical method. Antimicrobial susceptibility test of the isolates was by disc diffusion technique while ESBL detection was by double disc synergy method. Physicochemical analysis showed that turbidity ranged between 17.7- 164.7NTU; total suspended solids between 0.45 -1.3mg/L; total dissolved solids between 246 - 735mg/L. The conductivity, Biological Oxygen Demand and Chemical Oxygen Demand were between 367-1061mg/L, 267.8-385.2mg/L, and 395.8-563.3mg/L, respectively; oil and grease was between 272.8 - 2067.9mg/L. A total of 207 β-lactam resistant Gram-negative bacteria were isolated, out of which 37 (17.9%) produced ESBL; 9(24.3%) were from Yemetu, 14 (37.8%) from Kudeti and 14 (37.8%) from Alaro rivers. Among the ESBL-producers, 35.1% were *Klebsiella pneumonia*e, while 91.9%, 73.0% and 64.9% of ESBL isolates showed resistance to Cefotaxime, Cefepime and Aztreonam, respectively; while resistance to Ciprofloxacin and Gentamicin was 8.1% and 18.9% respectively. The present study reveals the need for continuous pollution monitoring and proper management program of the rivers to prevent indiscriminate discharge of wastes harboring ESBL-producing bacteria into water bodies.

Keywords: Rivers, Extended spectrum β-lactamase, Gram-negative bacteria, Antibiotics, Resistance.

1. Introduction

One of the factors responsible for the global emergence of antibiotic resistance among enteric bacteria during the recent decades is the misuse of antibiotics (Chitanand *et al*., 2010). More so, the occurrence of Extended-Spectrum β-Lactamase (ESBL) production has been due to the use of cephalosporin in both clinical practices and animal husbandry (Canton *et al*., 2008; Castanheira *et al*., 2008). These strains of bacteria produce beta-lactamase enzymes that cleave to the beta-lactam ring thereby disrupting the action of antimicrobials leading to the development of resistance to most beta-lactam antibiotics including the first, second, third and fourth generation cephalosporins. The increase in the number of ESBL producing Gram-negative bacteria is a threat to healthcare because infections caused by these strains of organisms are difficult to treat, leads to increased medical costs and limited therapeutic options (Harris *et al*., 2015; Upadhyay and Joshi, 2015). Production of ESBL is common with many species of Gram-negative bacteria but is mainly detected in the family *Enterobacteriaceae* (Falagas and Karageorgopoulos, 2009).

One of the main sources of transmission of pathogenic organisms including antibiotic resistant bacteria is water. Moreover, multiple antibiotic resistant bacteria have been isolated from different water sources, such as rivers, groundwater, drinking water and recreation water (Marti *et al*., 2013; Ramirez-Castillo *et al*., 2013). The possibilities of human exposure to water bodies contaminated with ESBL–producing bacteria when used for recreation, irrigation, drinking and other domestic purposes is very high (Zhang *et al*., 2015). In Nigeria, most studies on ESBL producing bacteria focused on isolates from clinical origin particularly *E. coli*. Meanwhile, there is dearth of information on isolates from environmental samples. The present study is, therefore, aimed at evaluating the water quality of selected rivers in Ibadan as well as determining the occurrence of ESBL production in Gram negative bacteria isolated from selected rivers within Ibadan metropolis. These rivers are used for domestic purposes, such as washing and bathing.

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2. Materials and Methods

2.1. Description of the Study Area

The sites of the present study include: Alaro, Yemetu and Kudeti Rivers. Alaro River (with geographical position of 0812901N, 00593332E) is located in an industrialized environment, in Ibadan South West Local Government Area of Oyo State. There is discharge of effluents from industries into the river. Yemetu River (with geographical position of 0723318N, 00354303E) is along Oje Street behind Adeoyo hospital, in Ibadan North Local Government Area and Kudeti River (with geographical position of 0721909N, 00353845E) is along Idi-arere Kudeti road, in Ibadan South East Local Government Area of Oyo state. Yemetu and Kudeti rivers are located within residential areas, where open defecation is highly practised and domestic wastes are dumped in the rivers and along the river banks.

2.2. Sample Collection

Water samples were randomly collected between the months of May and July, 2015 into sterile containers and transported in an ice pack to the Laboratory, Department of Microbiology, University of Ibadan, for microbiological analysis. The geographical position of the sampling sites was determined and recorded.

2.3. Physico-Chemical Analysis of the Water Samples

The physico-chemical analysis of the water samples was carried out using standard analytical methods. Parameters, such as temperature and pH, were determined on the field using thermometer and pH meters respectively while turbidity was determined using turbidity meter. Total dissolved solid, electrical conductivity and dissolved oxygen were determined using an Extech digital meter (Extech Instruments, USA). Chemical Oxygen Demand (COD), suspended solids, acidity, alkalinity, chloride, Biochemical Oxygen Demand (BOD); and oil and grease were determined using standard methods (APHA, 2005).

2.4. Isolation of Beta-Lactams Resistant Gram-Negative Bacteria

Isolation of beta-lactams resistant Gram-negative bacteria was carried out as described by Lu *et al*. (2010) using MacConkey agar supplemented with filter-sterilised solution of ampicillin. Pour plate method was used for the inoculation as the supplemented media were dispensed into Petri dishes containing 1ml of the appropriate dilution of the samples. The plates were incubated at 37^{o}C for 18-24 hours. Distinct colonies presumptive of the target organisms were picked and further sub-cultured to obtain pure cultures. The isolates were characterized and identified using conventional biochemical method (Cheesbrough, 2008)

2.5. Antibiotic Susceptibility Tests

Antibiotic susceptibility test was carried out using the disc diffusion technique as described by Bauer *et al*. (1966). The antibiotics discs used were Cefotaxime (CXM, 30mg), Ceftazidime (CAZ, 30mg), Cefepime (FEP, 30mg), Aztreonam (AZ, 30mg), Imipenem (IMP, 10mg), Amoxicillin-Clavulanate (AMX, 30mg), Ciprofloxacin (CIP, 5mg), Gentamicin (CN, 10mg) and Florfenicol (FFL, 30mg) (Oxoid, UK). The susceptibility test was carried out using an overnight culture suspension of the test isolates adjusted to 0.5 McFarland Standard. The culture suspensions were inoculated unto the surface of Mueller Hinton agar plates with sterile swab sticks. The antibiotic discs were carefully placed on the inoculated plates with the aid of sterile forceps and incubated at 35±2˚C for 18-24 hours. The zones of inhibition were measured and interpreted based on Clinical and Laboratory Standards Institute (2017).

2.6. Detection of ESBL-Producing Bacteria Using Double Disc Synergy Test (DDST)

All the Ceftazidime (30mg) and Cefotaxime (30mg) resistant isolates were selected for ESBL detection using double disc synergy test previously described by Lu *et al*. (2010). The test was carried out using discs of amoxicillin-clavullanate and discs of ceftazidime (30mg) and cefotaxime (30mg) which were placed around amoxicillin-clavullanate disc at a distance of 15 to 20 mm from each other (center to center). The plates were incubated at 37^{o}C and after 18-24 hours of incubation, the plates were observed. Isolates producing ESBL were those with zones of inhibition around any of the cephalosporin discs with a clear-cut increase towards the amoxicillin-clavullanate disc. ESBL-positive *Klebsiella pneumoniae* ATCC 700603 and ESBL negative *Escherichia coli* ATCC 25922 strains were used as control.

3. Results

The physico-chemical analysis of the water samples from the rivers showed that Kudeti River had the highest temperature values (29^{o}C) while the pH ranged between 9.3 and 9.4. Except for the pH and DO, Yemetu River had the highest values for all the physico-chemical tested parameters (Table 1).

Out of the 207 beta-lactam resistant Gram-negative bacteria isolated from the rivers, 79 (38.2%) were from Alaro river, 62 (30.0%) from Kudeti river and 66 (31.9%) from Yemetu river while *Klebsiella* spp. had the highest occurrence rate (Table 2). The results of the susceptibility profile of the Gram-negative bacteria showed that resistance of bacteria from Yemetu, Kudeti and Alaro Rivers to cefotaxime were 68%, 58%, and 48%, respectively, while to cefepime, it was 55% (Yemetu), 40% (Kudeti) and 41% (Alaro). However, resistance of the bacteria was 3% (Yemetu and Kudeti) and 1% (Alaro) to imipenem (Table 3).

Table 1. Results of the Physico-chemical analysis of the water samples

Physicochemical parameters	Sampling points		
	Alaro	Kudeti	Yemetu
Turbidity (NTU)	17.7	17.7	164.7
Temperature (°C)	26	29	28
pH	9.4	9.4	9.3
Alkalinity (mg/L)	18.7	16.1	24.9
Acidity (mg/L)	2.0	1.6	1.9
Total Solids (mg/L)	246.5	652.6	736.3
Total Suspended Solids (mg/L)	0.5	0.7	1.3
Total Dissolved Solids (mg/L)	246	652	735
Electrical Conductivity (µS/cm)	367	945	1061
Dissolved Oxygen (mg/L)	8.4	8.4	8.4
Biological Oxygen Demand (mg/L)	267.8	296.4	385.2
Chemical Oxygen Demand (mg/L)	395.8	424.5	563.3
Nitrate (mg/L)	33.7	38.2	45.7
Chloride (mg/L)	38.6	27.5	78.2
Oil and grease (mg/L)	272.9	1400.1	2067.9

Key: NTU – Nephelometric Turbidity Unit, µS/cm - micro-Siemens per centimeter, mg/L – Milligram per Litre

Table 2. Occurrence of Beta-Lactam resistant Gram-negative bacteria isolates obtained from the rivers n(%)

	Sampling sites			
Genus	Alaro	Kudeti	Yemetu	Total
Escherichia spp.	8(10%)	19(31%)	5(8%)	32 (15%)
Klebsiella spp.	31(39%)	14(23%)	27(41%)	72 (35%)
Enterobacter spp.	13(17%)	15(24%)	7(10%)	35 (17%)
Pseudomonas spp.	8(10%)	3(4%)	7(10%)	18 (9%)
Salmonella spp.	4(5%)	5(8%)	11(17%)	20 (10%)
Proteus spp.	15(19%)	6(10%)	9(14%)	30 (14%)
Total	79(38.2%)	62(30.0%)	66(31.9%)	207(100%)

Table 3. Susceptibility profile of Gram-negative bacteria isolated from the rivers to selected beta-lactam antibiotics

	Yemetu River, n=66(31.9%)		Kudeti River, n=62(30%)		Alaro River, n=79(38.2)	
Antibiotics	I+R	S	I+R	S	I+R	S
IMP	14(21%)	53(80%)	10(16%)	52(84%)	8(10%)	71(90%)
CXM	55(83%)	11(17%)	48(77%)	14(23%)	59(75%)	20(25%)
CAZ	32(48%)	34(52%)	27(44%)	35(57%)	29(37%)	50(63%)
FEP	38(58%)	29(44%)	26(42%)	36(58%)	33(42%)	46(58%)
AZ	38(58%)	28(42%)	27(44%)	35(56%)	36(46%)	43(54%)
AMX	47(71%)	19(29%)	39(63%)	23(37%)	51(65%)	28(35%)

Out of the beta-lactam resistant Gram-negative bacteria, 37 (17.9%) were positive for ESBL production. These isolates belonged to the following genera: *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Escherichia and Proteus* (Table 4). Exactly 37.8%, 24.3% and 37.8% ESBL producers from the rivers were from Alaro, Yemetu and Kudeti, respectively.

Furthermore, the resistance patterns of the ESBL-producing isolates to combinations of antibiotics showed that there were 19 different antibiotypes. The highest was 5(13.5%) in which three *K. pneumonia* and two *P. Mirabilis* showed resistant to a combination of both CXM and FEP. This was followed by 4(10.8%) antibiotypes that included a combination of CXM, FEP, AZ and FFC with one each of *K. pneumonia, P. mirabilis, E. aerogenes* and *P. putida;* one *P. mirabilis* also showed resistant to a combination of seven antibiotics that included CXM, FEP, AMX, AZ, CIP, FFC and CN (Table 5).

Table 4. Detection of ESBL-producing Isolates

		ESBL detected n (%)			
Isolates	Numbers screened	Alaro	Kudeti	Yemetu	Total ESBL detected n(%)
Escherichia spp.	32	_	2 (6.3)	_	2(6.3)
Klebsiella sp.	72	4(5.6)	5(6.9)	4(5.6)	13(18.1)
Enterobacter sp.	35	4(11.4)	3(8.6%)	1(2.9)	8(22.9)
Pseudomonas sp.	18	3(16.7)	3(16.7)	1(5.6)	7(38.9)
Salmonella sp.	20	_	_	_	-
Proteus sp.	30	3(10.0)	1(3.3)	3(10.0)	7(23.3)
Total	207	14(6.7)	14(6.7)	9(4.3)	37(17.9)

Table 5. Antibiotypes of ESBL-producing Gram–negative bacteria

Antibiotypes	*K. pneumoniae*	*P. fluorescens*	*P. mirabilis*	*E. coli*	*E. aerogenes*	*P. aeruginosa*	*P. putida*	Total n(%)
CXM AZ	2	0	0	0	0	0	0	2(5.4%)
CAZ AMX	1	0	0	0	0	0	0	1(2.7%)
CXM FEP	3	0	2	0	0	0	0	5(13.5%)
FEP AZ	1	0	0	0	0	0	0	1(2.7%)
CXM CAZ	0	0	0	0	1	0	0	1(2.7%)
CXM FFC	0	0	0	0	2	0	0	2(5.4%)
CXM FEP FFC	0	0	1	0	0	0	0	1(2.7%)
CXM FEP AZ	0	0	0	0	1	0	0	1(2.7%)
CXM CAZ AZ	1	0	0	0	1	0	0	2(5.4%)
CXM FEP AZ FFC	1	0	1	0	1	0	1	4(10.8%)
CXM CAZ FEP AZ	1	0	1	0	0	0	0	2(5.4%)
CXM FEP AMX AZ	0	0	0	1	0	0	0	1(2.7%)
CXM FEP AZ CIP FFC	1	0	0	0	0	0	0	1(2.7%)
CXM FEP AMX FFC CN	0	0	0	0	0	1	0	1(2.7%)
CXM CAZ FEP AZ FFC	0	1	0	0	1	0	0	2(5.4%)
CXM FEP AZ FFC CN	0	0	0	0	0	1	0	1(2.7%)
CXM FEP AMX AZ FFC CN	0	2	0	0	0	0	0	2(5.4%)
CXM CAZ FEP AZ FFC CN	0	1	0	0	0	0	0	1(2.7%)
CXM FEP AMX AZ CIP FFC CN	0	0	1	0	0	0	0	1(2.7%)

4. Discussion

The physico-chemical analysis of the water samples that showed pH values within the range 9.3 and 9.4 for the three rivers were within the international permissible limit of 6.5-9.5 (WHO, 2008). Likewise, the temperature of the rivers (26^{o}C-29^{o}C) was within the temperature permissible limit of less than 40^{o}C as recommended by the Federal Ministry of Environment of Nigeria (FMENV, 2001). While the turbidity (17.7 NTU) of the water samples collected from Alaro and Kudeti rivers were above the Standard Organisation of Nigeria (SON) permissible limits of 5.0 NTU (SON, 2007) the turbidity (164.7 NTU) obtained from Yemetu river was geometrically above the limit. The reason for this difference could be as a result of the location of the rivers. Yemetu River, for instance, receives inputs from a tertiary hospital, residents and the domestic wastes which could have led to the high turbidity value. The turbidity of both Alaro and Kudeti rivers were lower but comparably similar to the range of 24 and 28 NTU previously reported from another study conducted on a river in the same city of Ibadan (Adekambi and Falodun, 2015).

The pH range (9.3-9.4) of the rivers in the present study were higher compared to the pH range of 7.04 - 7.11 reported by Adekanmbi and Falodun (2015). The disparity in the pH values may be as a result of less anthropogenic activities impacting on the latter river compared to the rivers in the present study. These high pH values imply that the presence of basic salts (such as sodium and potassium salts) is likely to be prevalent in the river waters (John De Zuane, 1990). However, the pH range obtained in the present study is similar to a previous report from Turkey (Atici *et al*., 2008). The range of chloride quantity in the three rivers (38.6-78.2mg/l) is below the WHO permissible limit of 250mg/l chloride in water samples (WHO, 2008). Although, the range of Nitrate (33.7 - 45.7 mg/l) observed in the present study fell within the acceptable limit allowed (50 mg/l) in river water (WHO, 2008), the health implication associated with elevated concentrations of nitrate greater than 11mg/l in water is blue-baby syndrome (Methemoglobinemia) in children and Insulin-Dependent Diabetes Mellitus (IDDM) in adult when concentration exceeds 25 mg/l (Kostraba *et al*., 1992; Ward *et al*., 2005).

The total suspended solids (0.5-1.3 mg/l) and total dissolved solids (246-735mg/l) obtained from the present study were within the permissible limit of 30mg/l and 2000 mg/l, respectively (FMENV, 2001). However, the total suspended solids in the present study were lower while the total dissolved solids were higher compared to the 200mg/l and 320mg/l, respectively reported from the study carried out on another river (Ona River) in Ibadan, Nigeria (Osibanjo *et al*., 2011). The reason for the disparity could be as a result of an increasing measure of dissolved inorganic salts in Ona River. Moreover, the results of the biological oxygen demand (BOD) of the three rivers (267.8 mg/l-385.2 mg/l) were far above the permissible limit of 50 mg/l set by Federal Ministry of Environment (FMENV, 2001). Furthermore, the values of the Chemical Oxygen Demand (COD) of 563.3mg/l, 395.8 mg/l and 424.5mg/l obtained from Yemetu, Alaro and Kudeti Rivers, respectively, were all above the Federal Ministry of Environment permissible limit of 150 mg/l (FMENV, 2001) for surface waters. In addition, the value of the Dissolved Oxygen (DO) of 8.4 mg/l obtained from each river was above the permissible limit (5.0 mg/l) of the Federal Ministry of Environment (FMENV, 2001). Control

of indiscriminate discharge of wastes into these rivers is, therefore, imperative to forestall further deterioration of the river water.

The results of the oil and grease of the rivers (272.9-2067.86 mg/l) were far above the Federal Ministry of Environment permissible limit of 10mg/l (FMENV, 2001). The reason for this may be due to the urban runoff which conveys great amount of oil and grease from various auto-repair workshops within the vicinity of the sampling areas. For instance, close to Yemetu River bank is a large auto-repair workshop from where oil and grease discharges into the river. The results of the present study also showed that the most polluted of the three rivers was Yemetu River as revealed by the results obtained from the physicochemical analysis. The reason for the high level of pollution of the rivers could hence be largely attributed to anthropogenic activities that impacts on the river such as high practice of open defecation, improper disposal of wastes into the rivers as well as the release of industrial discharge into the Alaro River which is located in an industrialized locale.

Singal *et al*. (2005) and Reich *et al*. (2013) reported an increased prevalence of ESBL-producing *Enterobacteriaceae*. In the present study, 17.9% ESBL-producing Gram-negative bacteria predominantly of the family *Enterobacteriaceae* were detected and are similar to the recently reported 15.2% ESBL-producing bacteria of which, all the isolates belonged to the *Enterobacteriaceae* family from a study conducted on untreated hospital wastewater in the southern part of Nigeria (Egbule, 2016). In a study conducted in China, a higher prevalence of ESBL-producing isolates (69.6%), of the *Enterobacteriaceae*, from water samples collected from urban river, was reported (Lu *et al*., 2010). *Klebsiella pneumonia* having the highest occurrence rate among the ESBL-producing organisms in the present study is of great public health concern because it has been reported that the most common causative agent of nosocomial and community acquired infections are the members of the *Enterobacteriaceae* (Coque *et al*., 2008). The persistent and contagious nature of *Klebsiella* spp. may be as a result of resistance to harsh conditions due to the presence of capsules that gives protection to the cells (Paterson and Bonomo, 2003).

The observation from the present study that none of the ESBL-producing bacteria were resistant to imipenem except one of the *Enterobacter* species is similar and comparable to a previous study conducted in Malaysia in which all the 19 ESBL-producing bacteria from four different rivers were reported to be susceptible to imipenem (Tissera and Lee, 2013). It has been found that ESBL isolates are usually resistant to most β-lactam antibiotics and the implication of this is that few options are left for the treatment of ESBL-associated infections. Antibiotics susceptibility result that showed high resistance of the ESBL-producing isolates to cefepime, a fourth generation cephalosporin, is in agreement with previous reports of increasing emergence of resistance to fourth generation cephalosporins (Naumiuk *et al*., 2001; Grover *et al*., 2006). It was observed in the present study that all ESBL-producing *E. coli*, *P. mirabilis* and *Pseudomonas* spp. were resistant to cefepime, a fourth generation cephalosporin. Meanwhile, resistance to this antibiotic has been previously reported to be linked to the hydrolysis by blaCTX-M gene coded β-lactam enzyme (Paterson and Bonomo, 2003). However, ESBL-producing bacteria that exhibited high resistance to Cefotaxime (91.9%), Cefepime (73.0%), Aztreonam (64.9%) and Ceftazidime (37.8%), in the present study, is not in agreement with the total (100%) resistant to Cefotaxime and Ceftazidime and no resistant to Aztreonam reported from another study on surface water that included samples from various ponds, lakes and river in Dhaka city, Bangladesh (Nasreen *et al*., 2015). The reason for the differences may be as a result of the studied samples.

Gundogan and Yakar (2007) had previously reported a low resistance of the ESBL-producing isolates to ciprofloxacin and gentamicin which is also similar to the results of the present study. This, therefore, corroborates the assertion that ciprofloxacin and gentamicin can be effective in the treatment of infections caused by ESBL-producing bacteria. The resistance patterns of the ESBL producing isolates against the antibiotics tested in the present study showed that the majority were multidrug resistant (resistant to three or more classes of antibiotics); such multiple antibiotic resistance has been reported to be the outcome of the acquisition of resistance genes through genetic exchange and mutation as well as physiological mechanisms, such as the possession of specific proteins and efflux pump.

5. Conclusion

In conclusion, the present study shows that the studied rivers were not only contaminated with chemical impurities, they also contain ESBL-producing bacteria some of which harbor multidrug resistance features. These organisms could serve as potential risks of infection outbreaks on exposure; hence the need to put in place appropriate measures to prevent contamination of local surface waters. Furthermore, imipenem, ciprofloxacin and gentamicin showed good effect on the ESBL-producing isolates in the present study.

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