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BLA$_{\text{CTX-M}}$ AND *BLA*$_{\text{SHV}}$ GENES ENCODING *ESCHERICHIA COLI* FROM THE ENVIRONMENT AND CLINICS OF SECONDARY AND TERTIARY LEVEL HOSPITALS

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Abstract

Escherichia coli from environmental and clinical sources encoding Extended-Spectrum *β*-lactamase (ESBL) genes in Abeokuta, Nigeria were investigated. *Escherichia coli* strains from clinical sources and hospital waste water (HWW) were isolated from tertiary and secondary level hospitals in Abeokuta and swimming pool water (SPW) from hotels in Abeokuta. 103 *Escherichia coli* isolates were identified using standard methods. Antimicrobial susceptibility was by Kirby--Bauer method. ESBL-producers were confirmed by double disk test. *bla*$_{\text{CTX-M}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ in ESBL-producing *Escherichia coli* were detected with PCR. *Escherichia coli* were 45.6%, 43.6% and 9.7% of all isolates from HWW, clinical and SPW respectively. ESBL was expressed in 41 (39.8%) isolates. Isolates from HWW were more resistant to antibiotics than others. Sixty percent of the isolates harbored the ESBL *bla*$_{\text{SHV}}$ gene, while fifty percent had *bla*$_{\text{CTX-M}}$. One isolate had both *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$. This is of grave health implications in the clinics and environment.

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Introduction

Antimicrobial resistance is an emerging problem throughout the world including Nigeria. Particularly resistance associated with Gram-negative bacteria has increased globally (FROST et al. 2019). Serious hospital acquired and community onset bacterial infections in humans including urinary tract infection are commonly caused by *E. coli* (PATERSON 2006). In addition to the prospect of untreatable infections, antimicrobial resistance results in higher economic costs due to longer stay in hospital by infected patients, the requirement for additional diagnostics and more expensive drugs. *β*-lactam antibiotics are important drugs in treating infections caused by *E. coli*, however, *β*-lactamase are the bacterial resistanct enzymes which hydrolyse and make these antibiotics inactive (LIVERMORE and WOODFORD 2006). Resistant genes can be transferred between bacteria via mobile genetic elements (DROGE et al. 1998). One of the mobile elements, integrons that mediate integration of resistance genes may also be involved (PARTRIDGE et al. 2009). Resulting in the development of multidrug-resistant bacteria. Most studies surveying antimicrobial resistance have focused on clinical isolates, while the survival and prevalence of antibiotic-resistant bacteria in the environment has not been well investigated. Furthermore, while hospitals have been regarded as a major source of dissemination of antibiotic-resistant bacteria, very little is known about the contribution of other sources in the spread of these strains. Hence, this study focused on determination of means of community spread of ESBL producing multidrug resistant *Escherichia coli* genes with reference to swimming pools, hospital settings and environments in Abeokuta, Southwest, Nigeria.

Materials and Methods

Sample Collection and Preparation

Clinical isolate of *E. coli* were collected from Sacred Heart Hospital Lantoro, Federal Medical Centre Idiaba, Abeokuta and Ijaye State Hospital. Swimming pool water was collected from 2-star, 3-star and 4-star hotels in Abeokuta in sterile containers. Samples were collected from a depth of 0.5–1 meter. While hospital waste water were collected from effluent of Federal Medical Centre Idiaba, Sacred heart hospital Lantoro and Ijaye State Hospital all in Abeokuta. The samples were transported to the laboratory in ice chests.

Isolation and Identification of *E. coli*

Isolation and identification of *E. coli* was done using bacterial culture media and different biochemical tests. Each samples were cultured on MacConkey agar and incubated at 37°C for 24 hours. Three to five colonies presumptive *E. coli* were randomly selected and identified by subculturing on Eosin Methylene Blue plate (EMB) Representative of pure colonies were picked based on morphology. The isolated colonies were identified as *E. coli* biochemically following standard protocols (OMBARAK et al. 2016).

Antimicrobial Susceptibility Profile of *Escherichia coli* Isolates Using Agar Diffusion Test

Isolates were tested to evaluate the pattern of antimicrobial susceptibilities by Kirby-Bauer disk diffusion method following CLSI guidelines. The following antimicrobial agents were used Nitrofurantoin (300 µg), Ciprofloxacin (5 µg), Ceftazidine (30 µg), Cefuroxime (5 µg), Gentamicin (10 µg), Ofloxacin (5 µg), Cefotaxime (30 µg), Cefixime (5 µg), Cefotaxime (30 µg) (Oxoid UK). After preparation of bacterial suspension, the turbidity of each of them was adjusted to 0.5 McFarland standard equivalent and then inoculated on Mueller-Hinton agar. After overnight incubation at 37°C, diameters of inhibition zones were measured and the results were interpreted as susceptible, intermediate, and resistant (CLSI, 2010).

Screening Test for ESBL Producing *E. coli*

Double Disk Synergy Test (DDST) was done by using cefotaxime (30 mg) and ceftazidime (30 mg) with and without clavulanic acid (10 mg) disks on Mueller-Hinton agar (Oxoid, UK) with 25 mm apart from each other. An increase of equal or more than 5 mm in zone diameter for either antimicrobial agent tested with clavulanic acid versus its zone when tested without clavulanic acid indicated the presence of ESBL (CLSI, 2010).

Genotypic Analysis of the Isolates for ESBL Production DNA Extraction From *E. coli* Isolates

DNA extraction from isolates was performed as described by BAZZAN et al. (2016). Where a few colonies were suspended in 300 µL sterile distilled water and heated at 95°C for 10 minutes. Afterward, they were placed on ice for 5 minutes and then centrifuged at 13 000 rpm for 10 minutes and the supernatant was used as the DNA template.

PCR of *bla*$_{TEM}$, *bla*$_{SHV}$ *and bla*$_{CTX-M}$ *genes*

E. coli isolates were screened by PCR method and using specific oligonucleotide primers to determine *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla* $_{CTX-M}$ genes (SIMA et al. 2016). Primer sequences and their size were used for the detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla* $_{CTX-M}$ genes (Table 1). The PCR reactions for detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla* $_{CTX-M}$ genes were done in a total volume of 25 mL by using Master Mix Red, Taq DNA polymerase with $MgCl_2$. Amplicons were separated by agarose gel electrophoresis at 80 V for 2 h. After which, fragments were stained and visualized using ultraviolet light. Polymerase chain reaction products were sequenced. Then, nucleotide sequences were compared with sequences in the GenBank and European Molecular Biology Laboratory databases by using the BLAST.

Table 1

Primer used for polymerase chain reaction

Gene	Nucleotide sequence fragment	Length (bp)	Reference
bla $_{TEM}$	5-TCGGGGAAATGTGCGCG-3 5-TGCTTAATC AGTGAGGCACC-3	972	SIMA et al. (2016)
bla $_{SHV}$	5-GGGTTATTCTTATTTGTCGC-3 5-TTAGCGTTGCCAGTGCTC-3	615	SIMA et al. (2016)
bla $_{CTX-M}$	5-ACGCTGTTGTTAGGAAGTG-3 5-TTGAGGCTGGGTGAAGT-3	450	SIMA et al. (2016)

Data Analysis

Data were analyzed using SPSS version 17 statistical package and the association between ESBL producing organism's comparison was determined using Chi square test.

Results and Discussion

Distribution of *Escherichia coli* Isolates According to Sampled Areas

In this study a total of 103 samples were randomly collected from the three sampled areas clinic, hospital waste water and swimming pool water (Table 2). The highest percentage occurrence of *Escherichia coli* were obtained from hospital waste water (HWW) (45.6%), followed by clinical (43.6%) while swimming pool water (SPW) (9.7%) had the least. This result is in agreement with the report of DIWAN et al. (2010) and KORZENIEWSKA et al.

(2013). Both reported higher occurrence of this important member of the *Enterobacteriaceae* in clinical and waste water effluent. Isolation of lower percentage of these coliforms in swimming pool water might be linked to low pH.

Table 2

Escherichia coli from clinical, hospital waste water and swimming pool water

S/N	Sample source	*E. coli* [%]
Clinical		
1	FMC	20(44.4)
2	LANT	12(26.7)
3	IJAYE	13(28.9)
Total		45(43.6)
Waste water		
4	FMC	24(50.0)
5	LANT	10(21.3)
6	IJAYE	13(27.7)
Total		47(45.6)
Swimming pool		
7	IDB	3(30)
8	MTG	5(50)
9	LYR	3(20)
Total		11(9.7)
Grand total [%]		103(100)

Explanations: FMC – Federal Medical Center Idi Aba; LANT – Lantoro Catholic Hospital; IJAYE – State Hospital Ijaye; IBD, MTG and LYR are two-star-, three-star, and four-star hotels within Abeokuta Metropolis

Also, it may be as a result of low effectiveness of the chlorine used in the swimming pool water. However the detection of *E. coli* in swimming pool water indicate fecal contamination of the water (ABD EL-SALAM 2012) According to sample type, waste water had more *E. coli* isolates than swimming pool water. This result is in line with the findings of DIWAN et al. (2010) and JORGENSEN et al. (2017). Both of them reported high prevalence of *E. coli* from waste water sources.

Based on sample source the occurrence of *E. coli* were more from waste water sample collected from FMC (44.4%) followed by those from Ijaye (28.2%) then Lantoro (26.2%) (Table 2 and Table 3). A study by ALIPOUFARD and NILII (2010) reported most of the ESBL-producing isolates were from the medical wards, followed by the out-patient's clinic. Adequate Hospital planning and surveillance can be a powerful tool to improve and decrease the burden of communicable diseases (NEIDERUD 2015).

Table 3

Escherichia coli isolates based on sample source

S/N	Sample sources	Sites of collection of *E. coli* (n = 103)		
		FMC	LANT	IJAYE
1	clinic	20	12	13
2	waste water	24	10	13
3	swimming pools	DBI	MTG	LYR
		3	5	3

Explanations: FMC – Federal Medical Center Idi Aba; LANT – Lantoro Catholic Hospital; IJAYE – State Hospital Ijaye; IDB, MTG and LYR are swimming pools located in hotels within Abeokuta; n – number of samples

Among the isolates evaluated (Table 4), ESBL was expressed phenotypically in 39.8%. The percentage of ESBL recorded in this study concurred to report by IROHA et al. (2009) and can be compared to the report of AKANBI et al. 2013. The variation in ESBLs prevalence rates reported between geographical areas and institutions may be attributed to the complex epidemiology of ESBLs, specific type of bacteria involved and methods used for ESBL detection among other factors (AL-JASSER et al. 2006, KAUR et al. 2013).

Table 4

ESBL producing *Escherichia coli* isolates from various sources

S/N	Sample sources	Sites of collection of *E. coli* (n = 41)			Total
		FMC	LANT	IJAYE	
1	clinic	2	6	7	15(36.6)
2	waste water	11	5	5	21(51.2)
3	swimming pools	DBI	MTG	LYR	
		1	2	2	5(12.2)
Grand total					41(39.8)

Explanations: FMC – Federal Medical Center, Idi Aba; LANT – Lantoro Catholic Hospital; IJAYE – State Hospital Ijaye; DBI, MTG and LYR are swimming pools located within within Abeokuta, n – number of samples

On comparing the distribution of ESBL based on sample source, isolates from Hospital waste water had the highest ESBL occurrence of 51.2%, followed by 36.6% from Clinic and then 12.2% from swimming pool water (Table 4). The findings reported in this study is in line with the report of JORGENSEN et al. (2017) who reported high occurrence of ESBL among isolates from hospital waste water, compared to clinical sample. This justify the possibility of ESBL to be transferred between humans and animals, but may also spread in aquatic environments and potentially contaminate and infect exposed individuals (CONTROL ECFDPA 2015).

Resistance Profile of ESBL Producing Isolates From Clinics and Environments

Table 5 showed antibiotics resistant profile of *E. coli* based on sample collection source. Generally, isolates from hospital waste water recorded the highest resistance of 85.1% while the least resistance of 35.6% was from clinical isolates. This is in agreement with the findings of IBRAHIM et al. (2012), who reported high resistance rates of MDR *E. coli* isolates to the first-line oral antimicrobial agents. Generally, most isolates were susceptible to Cefotaxime, because of the low resistance (35.6%) recorded compared to other antibiotics. Among clinical isolates, 80% were resistant to Ceftazidime while most of them were susceptible to Cefotaxime. In HWW, most of the isolates were resistant to Ciprofloxacin (85%) and susceptible to Ofloxacin.

Table 5

Antibiotics resistance profile of ESBL producing *E. coli* based on sample source

S/N	Antibiotics	*E. coli* resistant profile		
		clinic (n = 45) [%]	hospital waste water (n = 47) [%]	swimming pool water (n = 10) [%]
1	CRX	29 (64.4)	33(70.2)	6(60.0)
2	GEN	27 (60.0)	19(40.4)	5(50.0)
3	CXM	34 (75.6)	37(78.7)	6(60.0)
4	OFL	24(53.3)	16(34.0)	8(80.0)
5	AUG	24(53.3)	35(74.5)	4(40.0)
6	NIT	26(57.8)	32(68.1)	5(50.0)
7	CPR	22(48.9)	40(85.1)	7(70.0)
8	CAZ	36(80)	37(78.7)	8(80.0)
9	CTX	16(35.6)	23(48.9)	4(40.0)

Explanations: n – number of isolates CRX (cefuroxime); GEN – gentamycin; CXM – cefixime; OFL – ofloxacin; AUG – augmentin; NIT – nitroflaxacin; CPR – ciprofloxacin; CAZ – Ceftazidine; CTX – cefotaxime

Escherichia coli isolates from SPW showed susceptibility to Augmentin and Cefotaxime. This could be attributed to insufficient decontamination in waste water treatment plants, which induces the risk of artificial selection of extended-spectrum β-lactamase production among *Escherichia coli* isolates (GUNDOGDU et al. 2017). High level resistance to these drugs has also been reported by UGWU et al. (2017). This might be linked to recent misuse of antibiotics in hospital settings and low dose of first-line therapeutic drugs (SAHUQUILLO-ARCE et al. 2011). In the clinic this study observed that the best antibiotics which could be recommended for the treatment of *E. coli*

associated infection is Cefotaxime followed by Ciprofloxacin. In Sewage Ofloxacin, Gentamicin and Amoxicillin-Clavulanic could be recommended, while in swimming pool Amoxicillin-Clavulanic could be the drug of choice. The selective efficacy of these drugs could be associated to lower selective pressure due to their restricted use (SAHUQUILLO-ARCE et al. 2011). Gentamycin, and Ceftazidine were observed to be more resistant in isolates from clinic followed by those from the swimming pool and waste water while to Ciprofloxacin and Cefotaxime isolates from the waste were more resistant than those from the Swimming pool and Clinic, respectively.

PCR Detection of the Presence of *bla*$_{TEM}$ Genes in ESBL Producing *E. coli* strains From Swimming Pools, Hospital Waste Water and Clinics

The result showed that none of the *E. coli* isolates encoded the *bla*$_{TEM}$ genes (Fig 1).

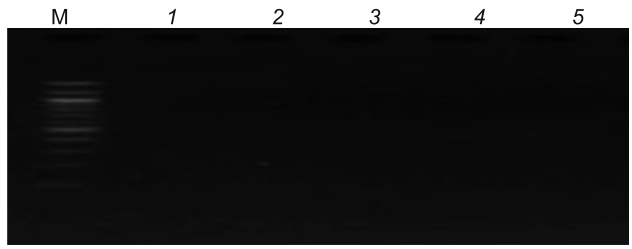

Fig 1. Gel electrophoresis of PCR assay for the detection of *bla*$_{TEM}$ gene from ESBL producing *E. coli* from clinics, hospital waste water and swimming pool water

PCR detection of *bla*$_{CTX\text{-}M}$ genes among ESBL producing *E. coli* strains isolated from clinical samples, hospital waste water and swimming pool water.

The results from Figure 2 showed amplifications which indicate the presence of the *bla*$_{CTX\text{-}M}$ genes.

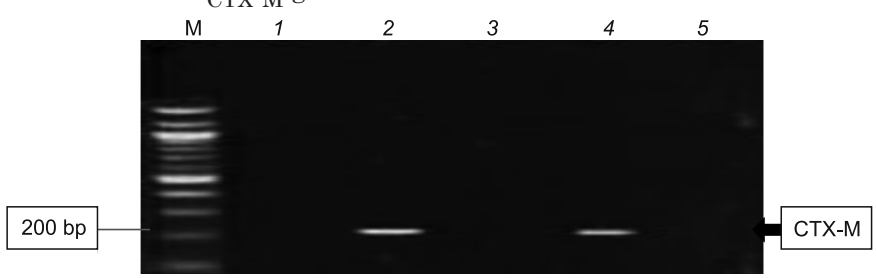

Fig 2. Gel electrophoresis of PCR assay for the detection of *bla*$_{CTX\text{-}M}$ genes from ESBL producing *E. coli* strains from clinics, hospital waste water and swimming pool water. Explanations: lane M – Ladder; *2* – *E. coli* (Clinic FMC); *4* – *E. coli* (Clinic Ijaye)

PCR detection of *bla*$_{\text{SHV}}$ genes among *E. coli* isolated from hospital waste water, clinics and swimming pool water.

Previous studies have noted that most prevalent types of ESBLs are *bla*$_{\text{SHV,}}$ *bla*$_{\text{TEM}}$ and *bla*$_{\text{CTX-M}}$ which can arise because of mutation in the *β*-lactam genes. *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$ were the *β*-lactams genes observed among the *E. coli* isolates (Fig. 3). Even though *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ are the most common type of the ESBLs genes in the past decade, recently *bla*$_{\text{CTX-M}}$ have been found more prevalent than *bla*$_{\text{SHV}}$ and *bla*$_{\text{TEM}}$ genotypes

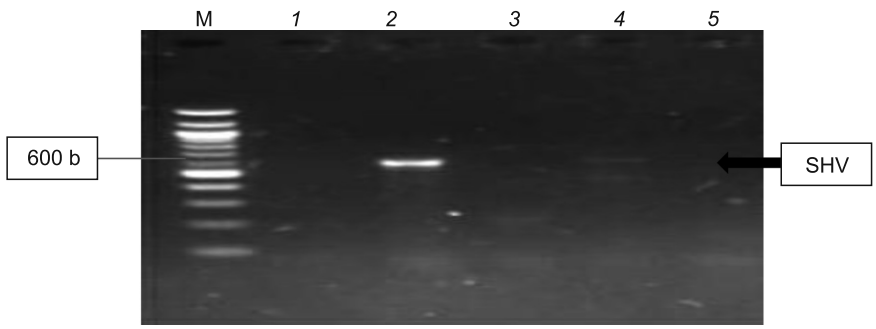

Fig. 3. Gel electrophoresis of PCR assay for detection of ESBL genes for *bla*$_{\text{SHV}}$ among *E. coli* from hospital waste samples, clinics and swimming pool water.
Explanations: lane M – Ladder; *1 – E. coli* (Clinic FMC); *2 – E. coli* (Clinic FMC); *3 – E. coli* (Clinic Lantoro); *4 – E. coli* (Clinic, Ijaye); *5 – E. coli* (Clinic, Lantoro)

(BARGUIGUA et al. 2011, GAUTAM et al. 2019). In our study *bla*$_{\text{CTX-M}}$ was more prevalent (40%) followed by *bla*$_{\text{SHV}}$ (20%) and none of the isolate encode the genes for *bla*$_{\text{TEM.}}$ The high prevalence in our study concurred with earlier report by SEPUTEINCE et al. (2009). This is in line with the report of HASSAN et al. (2014) in Saudi Arabia and KIRATISIN et al. (2014) in Thailand. These workers recorded low prevalence of *bla*$_{\text{SHV}}$ compared to *bla*$_{\text{CTX}}$ in their countries, respectively.

Conclusion

This study showed the presence of MDR ESBL producing *Escherichia coli* encoding *β*-lactamase genes *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX-M}}$ from waste water and clinical samples. ESBL were more predominant in waste water sample followed by those from the clinic. This report is of public health importance because if these isolates find their way to surface or groundwater. It can transfer the resistant genes to other strains and other pathogenic microorganisms. Thereby acting as reservoir for further dissemination of the *bla*$_{\text{SHV}}$ and *bla*$_{\text{CTX}}$ ESBL resistant genes.

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