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Research Paper

MULTIDRUG RESISTANCE *ESCHERICHIA COLI* CARRYING EXTENDED-SPECTRUM β-LACTAMASES ENZYMES IN A TERTIARY CARE HOSPITAL IN OSOGBO, SOUTH WESTERN NIGERIA

Olowe O A[1*], Ayilara O A[2], Oladipo G O[3], Makanjuola O A[1,2] and Olaitan J O[4]

*Corresponding Author: **Olowe O A,** ✉ oaolowe@lautech.edu.ng

Objective: Is to investigate the prevalence of extended-spectrum β-lactamases among *Escherichia coli* isolates in a tertiary care hospital in Osogbo. South western Nigeria. Materials and Methods: A total of 255 clinical isolates of *E. coli* (*n* = 255) were recovered from various clinical samples over a period of twelve months from October 2010 to September 2011 and there antimicrobial susceptibility testing was determined to commonly used antibiotics using the modified Kirby-Bauer's disc diffusion method. ESBL detection was done by the screening method of double disc synergy test as recommended by the Clinical Laboratory Standards Institute (CLSI) Results: Out of 255 isolates of *E. coli* screened for ESBL production, 105 were found to be potential ESBL producers. Of these, 14 isolates were confirmed to be ESBL producers. Thus the prevalence of ESBL-producing isolates of *E. coli* was found to be 5.5% (14 out of 255). We also report a high percentage of resistance to Augumentin (84.3.%) while Imipenem has the highest percentage of sensitivity, 249 (97.5%). Conclusion: In conclusion ESBLs were present in the study location and were resistant to most antibiotics applied. This has a significant implication for patients. We therefore suggest that routine and confirmatory tests should be included in routine diagnostic laboratory work.

Keywords: *Escherichia coli*, extended-spectrum β-lactamase, Third-generation cephalosporins

INTRODUCTION

Eschericia coli is one of the most common bacteria that produces β-lactamase and this is one of the most common causes of resistance to β-lactam antibiotics (Jarlier *et al*., 2001). Extended spectrum β-lactamase (ESBLs) are enzymes conferring broad resistance to penicillin, cephalosporin and monobactam but not to carbapenem (Mehrgan *et al*., 2008). Even though ESBL production is known commonly to occur in *E coli* and Klebsiella it have also been found in other members of the Enterobacteriaceae family (Navon-Venezia *et al*., 2003); ESBL-producing bacteria may appear falsely susceptible when

[1] Department of Medical Microbiology and Parasitology, College of Health Sciences, Ladoke Akintola University of Technology (LAUTECH) Oshogbo, P.M.B 4400, Osun state, Nigeria.
[2] Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology Teaching Hospital (LTH) Oshogbo. Osun state. Nigeria.
[3] Department of Medical Microbiology and Parasitology, Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile- Ife, PMB 5538, Ile- Ife, Osun State, Nigeria.
[4] Department of Microbiology, Osun State University, Osogbo, Nigeria.

tested by routine *in vitro* susceptibility methods. (Livermore *et al*., 1995). Even though certain strains may demonstrate *in vitro* susceptibility, there have been instances of clinical failure (Meyer *et al*., 1993). Emergence of resistance to β-lactam antibiotics started even before the first plasmid–mediated β-lactamase TEM-1 was discovered in 1960. ESBL producing bacteria continue to increase thereby leading to different types of infections both in hospitalized patients that is in patients and outpatients. Previous studies from Europe, Indian and Cameron have reported ESBL production varying from 6% to 87% and 22% depending on institute sometimes within a country. Tankhiwale *et al*. (2004) keeping in view the above facts, this study was undertaken to find the prevalence of ESBL producers among *E. coli* isolates in a tertiary care settings. The continuous increase of drug resistance among these organisms has made treatment of infection difficult and this had led to greater use of more expensive broad spectrum antibiotics such as third generation of cephalosporin. Moreover, the aim of this study was also to compare the antibiotic susceptibility pattern of ESBL producers with that of non-ESBL producers and to delineate the magnitude of the problem and to define appropriate therapeutic options in Nigeria.

MATERIALS AND METHODS

A total of 255 consecutive non-repeat culture isolates of *Escherichia coli* were isolated from five different clinical specimen namely, wound , Blood, Urine, Ear and High Vaginal Swab/ Endocervical Swab obtained from the Medical Microbiology of Ladoke Akintola University Teaching Hospital Osogbo, South West Nigeria, over a period of twelve months from October 2010 to September 2011. Ladoke Akintola University Teaching hospital is about 300 bedded hospital. This research is a laboratory based research in which Medical and demographic data of the patients were collected using patients form filled with their samples submitted to the medical microbiology laboratory of the hospital. Specimens were inoculated onto blood and MacConkey agar. All plates were incubated at 37°C for 24 h. Significant isolates were identified at species level using conventional bacteriological methods (Mahon *et al*., 2007).

ANTIMICROBIAL SUSCEPTIBILITY TEST

Antimicrobial susceptibility was determined by Kirby-Bauer disk diffusion method as per CLSI recommendations. Antimicrobial disks used include Gentamycin (GM) (10 μg), Ampicillin (AP) (5 μg), Cefotaxime (CTX) (30 μg), Ceftazidime (CAZ) (30 μg), Cefoxitin (FOX) (30 μg), Ciprofloxacin (CIP) (10 μg), Imipenem (IMI) (10 μg), Tetracycline (TE) (30 μg),Nalidicicacid (NA) (10 μg), Augumentin (AUG) (30 μg), Oflaxacin (OF) (5 μg), Chloraphenicol (CHL) (25 μg), Nitrofuration (N) (30 μg), Oxacillin (OX) (5 μg), Amoxicillin (AX) (25 μg), Norflaxallin (NB) (5 μg).

SCREENING TEST FOR ESBLs

According to the CLSI guidelines, isolates showing inhibition zone size of ≤ 22 mm with Ceftazidime (30 μg), and ≤ 27 mm with Cefotaxime (30 μg) were identified as potential ESBL producers and shortlisted for confirmation of ESBL production.

Phenotypic Confirmatory Test with Combination Disk for ESBLs Phenotypic Confirmatory Test with Combination Disk for ESBLs.

This test requires the use of a third-generation cephalosporin antibiotic disk alone and in combination with clavulanic acid. In this study, a disk of Ceftazidime (30 μg) alone and a disk of Ceftazidime + Clavulanic acid (30 μg/10 μg) were used. Both the disks were placed at least 25 mm apart, center to center, on a lawn culture of the test isolate on Mueller Hinton Agar (MHA) plate and incubated overnight at 37°C. Difference in zone diameters with and without clavulanic acid was measured. When there is an increase of ≥5 mm in inhibition zone diameter around combination disk of Ceftazidime + Clavulanic acid versus the inhibition zone diameter around Ceftazidime disk alone, it confirms ESBL production.

STATISTICAL ANALYSIS

Chi-square test was used with appropriate correction for the observation. Where the cell frequency was less than five, Fisher exact test was applied to see the significance of difference between the resistance levels of various drugs in ESBL producer strains and non-ESBL producer strains using EPI 6 software, $p \leq 0.05$ was considered significant.

RESULTS

A total of 255 isolates of *E. coli* (*n* = 255) were recovered from different clinical specimens submitted for routine microbiological analysis from both inpatients and outpatients of a tertiary care hospital during a twelve-month period. The number of potential ESBL producers shortlisted by screening test was 105 out of the total 255. All of them showed inhibition zone size of ≤22 mm with Ceftazidime during screening test. Confirmatory tests for ESBL production were performed on these 105 isolates. Out of suspected 105 isolates, 14 (5.5%) of the *E. coli* isolates were found to be ESBL producers by phenotypic confirmatory test with combination disk while 241(94.5%) of the isolates were negative for ESBL. Table 1 shows the distribution of ESBL-positive isolates were highest among wound isolates, accounting for 8(8.6%) followed by urine5 (7.2%), stool 3(3.5%), blood and HVS 0(0%) respectively. The Rate of ESBLs among in patients were found to be 78.5% and 21.4% among outpatients. Table 3 shows resistance pattern to various antibiotics used during this study, Imipenem has the highest percentage of sensitivity, 249 (97.5%), followed by Ciprofloxacin,

Table 1: Distribution of ESBL Positive *E. coli* Among the Sources of Sample

Sample	ESBL Positive		ESBL Negative		Total
	Number	Percentage (%)	Number	Percentage (%)	
Urine	5	7.3	50	92.7	55
Stool	3	3.5	83	96.5	86
Wound	8	8.6	64	91.4	70
HVS	0	0	21	100	21
Blood	0	0	23	100	23

Table 2: Prevalence of ESBL Producing Organism Among Outpatient and Inpatients

ESBLs Positive(in Patients)		ESBLs Positive (Outpatients)	
No	%	No	%
11	78.5	3	21.4

Table 3: Antimicrobial Susceptibility Pattern of 255 Bacterial Strains of *E.coli*

Antibiotics(μg/ml)	Sensitive		Resistance	
	Number	Percentage (%)	Number	Percentage (%)
CAZ (30)	211	82.7	30	11.8
CTX (30	172	67.5	56	21.9
IMI(10)	249	97	6	2.3
GM(10)	195	76.2	33	12.9
NA(10)	154	60.4	77	30.2
AP(5)	51	20.0	195	76.5
CIP	219	85.5	31	12.1
OX	15	5.9	227	88.7
FOX	204	79.5	30	11.8
TET	43	16.8	185	72.5
OF	145	56.9	88	33.7
CHL	42	16.5	191	74.6
NB	75	29.4	161	63.1
N	42	16.5	186	72.9
AUG	26	10.6	215	84.3
AX	51	20.0	164	64.3

KEY: Antibiotic disc applied include Gentamycin (GM) (10 μg), Ampicillin (AP) (5 μg), Cefotaxime(CTX) (30 μg), Ceftazidime(CAZ) (30 μg), Cefoxitin (FOX) (30 μg), Ciprofloxacin(CIP) (10 μg), Imipenem (IMI) (10 μg),Tetracycline (TE) (30 μg),Nalidicicacid(NA) (10 μg),Augumentin(AUG) (30 μg), Oflaxacin(OF) (5 μg),Chloraphenicol(CHL) (25 μg),Nitrofuration(N) (30 μg), Oxacillin(OX) (5 μg), Amoxicillin(AX) (25 μg), Norflaxallin(NB) (5 μg).

219(85.9%) Ceftazidime 211(82.7%), Cefoxitin 204(79.2%), Gentimicin 195(76.6%), Cefotaxime 172(67.5%), Nalidicicacid 154(60.4%), Ofloxacin 145(56.9%), Amoxicillin 51(20.0%), Ampicillin 51(20.0%), Tetracycline 43(16.8%), Nitrofuratoin 42 (16.5%), Chloraphenicol(16.5%), Augmentin 26 (10.6%), Oxacillin 15 (5%). Oxacillin has the highest resistance rate 227(88.7%). ESBL producing isolates were resistant to more antimicrobial agents than non-ESBL producing isolates. Imipenem has the highest susceptibility rate of 100% of among ESBLs producer while

oxacillin, nitrofurantoin and ampicillin has the highest rate of resistance 100% showed in Table 4.

Table 4: Comparison of drug resistance among ESBL producing and non ESBL producing isolates of *E. coli*

Antibiotics	ESBL producer (N=14) Resistance in %	Non ESBL producer (N =241) Resistance in %
CAZ	64.2	9.1
CTZ	64.2	19.9
IMI	0	2.5
GEN	14.2	13.3
NA	85.7	27.4
AP	100	15.2
CIP	35.7	11.2
TET	85.7	72.1
OF	57.1	32.7
CHL	100	73.6
NB	76.9	62.4
N	100	73.9
AX	42.9	65.9
OX	100	88.7
AUG	0	89.2
FOX	0	12.4

KEY: Ceftazidime (CAZ) (30 µg), Cefotaxime (CTX) (30 µg), Imipenem (IMI) (10 µg), Gentamycin (GEN) (10 µg), Nalidicicacid (NA) (10 µg), Ampicillin (AP) (5 µg), Ciprofloxacin (CIP) (10 µg), Tetracycline (TET) (30 µg), Oflaxacin (OF) (5 µg),Chloraphenicol(CHL) (25 µg), Norflaxallin (NB) (5 µg), Nitrofuration (N) (30 µg), Amoxicillin (AX) (25 µg), Oxacillin (OX) (5 µg), Augumentin (AUG) (30 µg), Cefoxitin (FOX) (30 µg),

DISCUSSION

For some time now, ESBLs producing gram negative organism especially *Escherichia coli* had emerged as a very serious pathogens causing problems both in hospitals and in community acquired infections worldwide. ESBLs has spread rapidly and this indicates that effective infection control and continuous monitoring system should be put in placed in our communities. The occurrence of ESBL among clinical isolates vary greatly worldwide and geogra-phically and are rapidly changing over time (Babypadmini *et al*., 2004). Previous studies from Kano Nigeria shows 5% of ESBLs among enterobacteriaceae (Yusha'u *et al.,* 2007). Moland and colleagues have shown that ESBL-producing isolates were found in 75% of 24 medical centers in the United States (Moland *et al.,* 2002**)**. ESBLs have also been documented in Israel, Saudi Arabia, and a variety of North African countries (AitMhand *et al.,* 2002). From China, the figures of ESBL producers vary between 25-40% (Yu *et al.,* 2002). National surveys have indicated (Borer *et al*., 2002 and AitMhand *et al.,* 2002**)** the presence of ESBLs in 5-8% of *E. coli* isolates from Japan, Korea, Malaysia and Singapore but 12-24% of isolates from Thailand, Taiwan, Philippines and Indonesia (Paterson *et al.,* 2005). Emergence of ESBLs may be due to extensive usage of antimicrobial agents and this is posing a serious problems in our health system.

In this study 5.5% of *Escherichia coli* isolates were found to be ESBLs positive. The high rate of resistance noted among the isolates in this study is of serious concern. 100% of ESBLs producing *Escherichia coli* were resistance to ampicilline, nitrofurantoin chloraphenicol and oxacillin respectively while in non ESBLs resistance rate were 15.2,73.9,73.6 and 88.9 respectively. Prevalence of resistance to fluroroquinolones such as ofloxacins and ciprofloaxacin was 57.1% and 35.1% respectively. Imipenem has the highest sensitivity rate among both the ESBLs positive and non ESBLs. The effectiveness of carbapenems against ESBL

producers and their ability for treatment of infections caused by ESBL producing organisms were well established (Bush *et al* 1995). From this study prevalence of ESBLs among inpatient is higher than that of outpatients.

CONCLUSION

In conclusion, the prevalence of ESBL producers at our institute was 5.5% and this is in accordance to the prevalence reported from other hospitals in Nigeria as well as across some part of the world. Multiresistant was higher among the ESBLs produer than the non ESBLs producer. All the ESBL-positive isolates were found to be sensitive to Imipenem, this shows that imipenem is still drug of choice for ESBLs. Because of the presence of ESBLs in our community regular monitoring and judicious usage of antibiotics especially cephalosporin should be controlled, periodic surveillance of antibiotic resistance patterns, and efforts to decrease empirical antibiotic therapy. This would go a long way in addressing some of the problems associated with ESBLs. The control measures should include proper usage of antibiotics, strict hand-hygiene protocols, and implementation of appropriate infection-control measures in the hospital, especially while treating high-risk patients.

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