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Research article

SCREENING AND DETECTIONS OF EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING *ESCHERICHIA COLI* ISOLATES AMONG OUTPATIENTS IN PORT HARCOURT, RIVERS STATE, NIGERIA

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Abstract

Extended spectrum beta lactamases (ESBL) are a group of enzymes produced by Gram negative bacteria that have the ability to hydrolyse extended spectrum beta lactams such as cephalosporins (ceftazidime, cefotaxime, and ceftriaxone) and monobactams (Aztreonam) in addition to penicillin. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic option. For this reason, the significance of such ESBL–mediated infections has been increasingly reported worldwide. The presence of ESBL in some *E. coli* strains poses an important challenge in clinical practice, since this organism is common cause of serious infections. The aim of the study was to screen and detect ESBL producing *Escherichia coli* from clinical isolates among outpatients in Port Harcourt Rivers State, South South Nigeria. *E. coli* isolates were obtained from urine, aspirates and swabs of outpatients who visited University of Port Harcourt Teaching Hospital (UPTH), Military Hospital Port Harcourt and Braithwaite Memorial Specialist Hospital (BMSH) Port Harcourt. The isolates were purified and preserved and were used for characterization and susceptibility testing. ESBL preliminary screening was performed on all the isolates, those that tested positive were subjected to ESBL confirmatory test. A total of 250 isolates were tested, comprising of 78 isolates from males and 172 isolates from female subjects. Among the isolates tested, 16.40 % (41 of 250) tested positive for ESBL preliminary detection test while 9.60% (24

of 250) were confirmed to be ESBL – producing E. coli strains. A total of 15 of the isolate (6.00%) showed multidrug resistance. There was no significant difference in the presence of the ESBL – producing E. coli between males and females. The study calls for more concerted efforts in the elimination of drug resistant bacterial isolates as it highlights the negative effect of these organisms which have high morbidity and mortality with great potential to frustrate treatment. **Copyright © WJMMS, all rights reserved.**

Keywords: ESBL, *E. coli*, prevalence

1. Introduction

Microbial resistance to antimicrobial is a growing major public health problem and a strong concern in the medical community (Bradfor, 2001). Production of β - lactamase is a major strategy which most Gram – negative bacteria exhibit resistance to β – lactam antibiotics (Emery and Weymouth, 1997). Extended spectrum beta lactamases (ESBL) are a group of enzymes produced by Gram negative bacteria that have the ability to hydrolyse extended spectrum beta lactams such as cephalosporins (ceftazidime, cefotaxime, and ceftriaxone)andmonobactams (Aztreonam) in addition to penicillin. As a result bacteria that produce ESBL become resistant to these β – lactams, thereby reducing treatment options. ESBL has indeed become a source of concern for clinical microbiologists especially considering the fact that it has been detected among many clinical isolates of *Escherichia coli* and *Klebsiella species* (Bush *et al.,* 1995 and Colodner*et al.,* 2004). These organisms already carry genes for TEM – 1 and TEM – 2 and SHV – 1 β – lactamases which are broad spectrum, but not extended β – lactamase, and are capable of hydrolyzing penicillin but not cephalosporin (Colodner*et al.,* 2004).

Extended spectrum beta-lactamases (ESBL) are plasmid mediated, derived enzymes conferring broad resistance to penicillin, cephalosporin and monobactam but not to carbapenem (Mehrgan, *et al*., 2008) The enzyme has one position mutation in the gene at the active site that is believed to be the cause of high beta-lactamase activity. These enzymes are produced by *Enbterobacteriaceae* mainly by *Escherichia coli*, *Klebsiellapneumonia*e and *oxytoca*(Philippon, *et al.,* 1989). They have been detected in other gram-negative bacilli such as *Proteus* species, *Salmonella* species, *Pseudomonas aeruginosa*and other *Enterobacteriaceae*, Most ESBL are encoded on a large plasmid that can be horizontally transferred to different genera of bacteria, which may be involved with both prevention and treatment aspects of infections, (Bradford, 2001). In addition, ESBL-producing *Escherichia coli* have been reported in a community-acquired bacteremic infection (Sorlozano, *et al*., 2004).

The ESBL enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but are inactive against cephamycins and imipenem. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in limitation of therapeutic option. For this reason, the significance of such ESBL–mediated infections has been increasingly reported worldwide (Bradford, 2001) and there is an increased need to detect ESBL-producing gram-negative bacteria in routine microbiological work. Rapid detection of ESBL is important, not only for treatment guidelines but also to facilitate improved prevention of infections (Shah, *et al*., 2004).

The Clinical Laboratory Standards Institute (CLSI) formerly called National Committee for Clinical Laboratory Standards(NCCLS) recommended that Microbiology laboratories should report ESBL-producing isolates of *E. coli* as resistant to all penicillins, cephalosporins (including cefepime), and aztreonam, irrespective of their individual *in vitro* test results. The presence of ESBL in some *E. coli* strains poses an important challenge in clinical practice, since this organism is common cause of serious infections. Imipenem and meropenem are considered the therapy of choice for patients with serious infections due to ESBL producing strains. Many ESBL-producing isolates are not always phenotypically resistant to oximino-cephalosporins. However, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if an extended spectrum of cephalosporins

(ESC) are prescribed. Therefore, it is imperative for the clinical Microbiology laboratory to identify the isolates that possess increased MICs (2μg/mL)to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC 8 μg/mL) (NCCLS, 2000).

The ESBL have serine at their active site and attack the amide bond in the lactam ring of antibiotics causing their hydrolysis. Because of inoculum effect and substrate specificity, their detection is a major challenge. Two indicators of ESBL are eight-fold reductions in MIC and potentiation of the inhibitor zone of third generation cephalosporin in the presence of clavulanic acid (Mehrgan, *et al*., 2008). For this reason, detection of ESBL, using conventional antimicrobial susceptibility methods and delay in there cognition and reporting of ESBL production by Gram negative bacilli is associated with prolonged hospital stay, increased morbidity, motility and health care expenses (Mehrgan, *et al*., 2008). So, it becomes necessary to know the prevalence of these organisms and to formulate the treatment policy. The aim of the study was to screen and detect ESBL producing *Escherichia coli* from clinical isolates among outpatients in Port Harcourt Rivers State, South South Nigeria.

2. Materials and Methods

2.1 Sample Collection

Isolates of *Escherichia coli* that were isolated and purified from various clinical samples (urine, swabs, aspirates) obtained from outpatients in the following hospitals: University of Port Harcourt Teaching Hospital (UPTH), Military Hospital Port Harcourt and Braithwaite Memorial Specialist Hospital (BMSH) Port Harcourt. The isolates were processed in the Microbiology laboratory unit of Braithwaite Memorial Specialist Hospital. This study was done on 250 *Escherichia coli* isolates. Medical and demographic data of the patients were collected using patient's files.

2.2 Sample Processing

Each of the isolate obtained from the culture media suspected to be *E. coli* were processed as follows: A pure colony of the suspected isolate were sub-cultured into a freshly prepared Eosine Methylene Blue Agar (EMB) and MacConkey Agar aseptically and incubated at 37^{o}C for 24 hrs.

2.3 Culture Purification and Preservation

After incubation of the sub-cultured isolate at 37^{o}C for 24hrs, growth was observed and compared between the two cultured media. The *E. coli* colony seen had a diameter of 2-3mm, dark violet cultures with black center, and a green metallic shine on EMB Agar; while it ferments the lactose and turned the media pink on MacConkey Agar. They were then preserved on Nutrient Agar slant prepared in a screw-capped McCartney bottle and incubated for 24hrs at 37^{o}C. The culture slants were further refrigerated to inhibit excessive growth.

2.4 Identification of Isolates

The isolates were identified using colonial morphology, Gram reaction (microscopy) and biochemical reactions which include; motility test, indole, coagulase, catalase, H_2S, Acid and Gas production (Triple Sugar Iron Fermentation) as described by Cheesbrough (2004). The confirmed *E. coli* isolates were then subjected to antimicrobial susceptibility testing.

2.5 Antimicrobial Susceptibility Testing

The Isolates were screened using known method of Sensitivity determinant disc diffusion method. The *E. coli* isolates were inoculated onto a Mueller Hinton medium by a 0.5 McFarland standard to determine the susceptibility of the following Gram negative antimicrobials used: Gentamycin, Streptomycin, Ceporex (Cefalexin), Ciproflox, Ampicilin, Septrin (Co-trimoxazole), Nalidixic acid, and Tarivid (Ofloxacin). The ofloxacin was used as a positive control (Alo, *et al*., 2013). Then, it was incubated in an aerobic environment at 37^0C for 24hours. The results were read based on the acceptable benchmark (5mm enhanced in zone of inhibition diameter) as recommended by the National Committee for Clinical Laboratory Standard (NCCLS, 2000). Isolates which were resistant or had intermediate susceptibility to any of the antimicrobials were selected for ESBL detection/ screening.

2.6 Detection of ESBL

The following procedures were used for ESBL detection, they include: ESBL disc screening and ESBL disc confirmation.

2.7 ESBL Disc Screening (Preliminary Detection)

Disk-diffusion method for ESBL screening was performed using the following discs: Ceftazidime (inhibition zone ≤22mm) and Aztreonam (inhibition zone ≤27mm) as recommended by CLSI guideline (2014). Therefore, we proceeded with ESBL testing (confirmation) when the zone of inhibitions was expressed as indicated above.

2.8 ESBL Disc Confirmation

Isolates suspected to be producers of ESBLs enzymes after they underwent preliminary detection, were subjected to the following confirmation tests: Combination Disc Test (CDT) using Ceftazidime alone, Ceftazidime + Clavulanic Acid; and Double-Disc Synergy Test (DDST) using Ceftazidime, Amoxicilin, Amoxicilin + Clavulanic Acid. The test was positive for ESBL production as zone of inhibition of Cephalosporin + Clavulanic acid increased to ≥5mm (CLSI, 2014). These confirmatory tests permit to evaluate the inhibition of ESBL activity by Clavulanic Acid.

3.Results

A total of 250 isolates of E. coli from various specimens of patients attending the outpatient clinics of selected hospitals in Port Harcourt, Rivers State, Nigeria were screened and detected for ESBL production, among these, 78 *E. coli* isolates were from male outpatients while 172 *E .coli* isolates were from female outpatients. A total of 41 *E. coli* isolates were positive for ESBL production by preliminary screening and were subjected for confirmatory test. Among these, 24 (9.60%) were positive for ESBL production.

Among the isolates tested, a total of 48, 97, 73, 97,97,100, 97 and 97 showed resistance to ofloxacin, ciprofloxacin, gentamicin, streptomycin, ceftriaxone, nalidixic acid, cotrimoxazole and ampicillin respectively. There was an overall increased susceptibility to ofloxacin. Multidrug resistance was found in a total of 15 (6.00%) isolates. Among these, 14 and 1 were from urine and High vaginal swab specimens respectively.

A total of 78% (32 out of 41 *E .coli* isolates) of the resistant *E .coli* isolates were from female outpatients while 22% (9 out of 41 *E .coli* isolates) of the resistant *E .coli* isolates used for ESBLs screening and detection were from male outpatients. However, there was no significant difference in the mean of male (X_2=7.000) and female

(X_1=7.156) out patients from whom resistant*E .coli*isolates were found to the eight (8) antibiotics used for sensitivity testing. There was no significant effect of outpatient's gender on *E .coli* resistance to antibiotics and its detection of ESBL. Though, the higher percentage of female (78%) over the male (22%) only shows that females visit the hospitals more often than their male counterparts.

Table 1: T-Test of Female and Male Outpatients Resistant *E. Coli* Isolates on 8-Antibiotics

Out patient	No of Isolates	Mean ($\bar{X}$)	SD	df	α	t	P(sig) value	Decision
Female	32	7.156	0.884	39	0.05	0.456	0.651	Accept H_0, Not sig. P>0.05
Male	9	7.000	1.000					

Table 2: Chi-square (x^2) Relationship Between Gender and ESBL Detection of Isolates.

Gender	**Positive**	**Negative**	**Total**	**df**	**α**	**x^2-cal**	**x^2–crit**	**Decision**	
Male	6 (4.5)	3 (4.5)	9	1	0.05	1. 84	3. 84	Accept Ho	Not Significant (x^2-cal < x^2 –crit)
Female	18 (16)	14 (16)	32						
Total	24	17	41						

4. Discussion

This study found a prevalence of 9.60% (24 OF 250) for ESBL among the study population and a prevalence of 6.00% for multidrug resistant *E. Coli* isolates. This finding is lower than the prevalence of 46.58% found byAlo and Ugah (2015). The lower prevalence found by this study may be as a result of the difference in the location of both studies. Port Harcourt is an old city and many residents are relatively more wealthy and as a result visit the hospital more often hence they may not be as prone to self-medication as people in other parts of the country where there is higher poverty index. This may be responsible for the lower prevalence found. Also, there has been many studies that have repeatedly highlighted the significance of drug resistance and their methods of prevention. The lower prevalence found in this study may be connected to the increased education of both health practitioners and the general public of this menace and hence the observation made in this study.

E. coli is known to be a common organism that is involved in serious nosocomial and community acquired infections. It is responsible for the outbreak of different kinds of diarrhoea, especially traveler's diarrhoea in developing countries and also it is implicated in urinary tract infections. The increased use of broad spectrum cephalosporins has become one of the major factors responsible for the high rate of selection of extended spectrum beta-lactamase producing micro-organisms (Colodner*et al.*, 2004; Bhat*et al.*, 2012).

The results of ESBL production amongst *E. coli* isolates and subsequent resistance of these organisms to most available conventional drugs is worrisome and calls for concerted national effort (particularly including further molecular studied) to contain the situation. Bacterial organisms producing ESBL enzymes pose a great therapeutic challenge to both clinicians and Medical Laboratory Scientists owing to the multidrug resistance nature of such microbes which compounds antibiotics prescription, thus, limiting treatment options (Alo and Ugah, 2015)

In addition, ESBL-producing organisms frequently carry antibiotic resistance genes which usually encode resistance to other non β-lactams, hence it is of utmost importance to control antibiotic usage. This may be responsible for the prevalence of 6.00% of multidrug resistant E. coli isolates observed in this study.

Hospital acquired ESBL producing organisms have been known to cause high mortality (Ho *et al.*, 2002). The increasing prevalence of ESBL-producing organisms among high risk patients has been noted to mirror a national increase in ESBL production among *Enterobacteriaceae*(NNIS, 2003). *E. coli* strains have been isolated in the highest numbers in bacteremic patients (Xiong*et al.,* 2002; Jain *et al.,* 2003). Hence, an increase in ESBL producing *E. coli* isolates will result in a widespread increase in the mortality rate of infected patients with this resistant bug.

Infections caused by multidrug resistant Gram-negative bacilli that produce extended spectrum beta –lactamase (ESBL) enzymes have been reported with increasing frequency (Gupta *et al.,* 2003).because of resistance to numerous antimicrobial agents, treatment can be challenging.

ESBL producing organisms are now being recognized as one of the major threats to effective management of patients in medical institutions especially in the less developed nations like Nigeria. *Klebsiellapneumoniae, Escherichia coli and Klebsiellaoxytoca* have been reported by a number of workers to harbor ESBL enzyme (Nathisuwen*et al.,* 2001). ESBL producing organisms are inhibited by β –lactamase inhibitors but are not with extended spectrum cephalosporins. ESBL are encoded by genes on plasmids which result in easy transfer of ESBL enzymes to other bacteria species (Alo and Ugah, 2015).

4.1 Conclusion

This study found 16.40% for ESBL – producing *E. coli* by preliminary screening, among these, 9.60% was found by confirmatory test as ESBL – producing *E. coli*. Also, a total of 6.00% prevalence was found for multidrug resistant E. coli. The study was performed among subjects who visited the outpatient clinics of University of Port Harcourt Teaching Hospital, Military Hospital Port Harcourt and Braithwaite Memorial specialist Hospital, Port Harcourt. The study calls for more concerted efforts in the elimination of drug resistant bacterial isolates as it highlights the negative effect of these organisms which have high morbidity and mortality with a great potential to frustrate treatment.

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