

Prevalence of Extended Spectrum Beta Lactamase Producing *Escherichia coli* and *Pseudomonas aeruginosa* Isolated from Clinical Samples

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Abstract: There has been an increasing attention globally over the rising treatment failures caused by Extended Spectrum Beta-Lactamase (ESBL) producing organisms when new generation antibiotics are used. This study was carried out to investigate the prevalence of ESBL producing *Escherichia coli* and *Pseudomonas aeruginosa* isolates from the Federal Medical Centre (FMC) Owerri, Imo State, Nigeria. Two hundred and fifty clinical isolates comprising, *E. coli* (136) and *P. aeruginosa* (114) was used for the study. Antimicrobial susceptibility testing of isolates was determined using the disc diffusion method. ESBL phenotypes were further determined by the double disc synergy test using Ceftazidime, Cefotaxime, Ceftriazone and Amoxicillin clavulanic acid. Out of the 250 isolates tested, 114 (45.6%) were positive for ESBL production comprising 66(26.4%) *E. coli* and 48(19.2%) *P. aeruginosa*. The antimicrobial sensitivity testing showed that the highest resistance of 100% was recorded with Cephalaxine while the least of 0% was recorded with the aminoglycosides and quinolones, giving a clear indication that the aminoglycosides and quinolones could be recommended for the treatment of ESBL infections caused by these organisms. The result of the present study showed that there is apparently high prevalence of ESBL producing *E. coli* and *P. aeruginosa* in the Federal Medical Centre (FMC) Owerri, Imo State, Nigeria.

Keywords: *Escherichia coli*, ESBL, Prevalence, Antimicrobial susceptibility

Introduction

Extended-Spectrum Beta-lactamases (ESBL) are β -lactamases capable of conferring bacterial resistance to the penicillin, and third generation cephalosporins, and aztreonam, but not the cephamycins or carbapenems (Paterson and Bonomo, 2005) and are usually encoded on plasmids which frequently carry genes encoding resistance to other classes of antibiotics. Extended Spectrum beta lactamases (ESBL) were first described in the 1980s and they have been detected in *Klebsiella* species, and later in *Escherichia coli*, *Pseudomonas aeruginosa* and *Serratia marcescens* and other gram-negative bacilli (Kiratisin *et al.*, 2008; Nwosu *et al.*, 2014; Yushau *et al.*, 2010; Ullah *et al.*, 2009). A good number of enteric gram-negative bacteria have been shown to possess naturally occurring chromosomally mediated genes that confer resistance on them to β -lactam antibiotics (Nwosu *et al.*, 2014; Yushau *et al.*, 2010; Ullah *et al.*, 2009; Sabrina *et al.*, 2010; Haque and

Salam, 2010; Bhusal *et al.*, 2011; Sibhghatulla *et al.*, 2015; Madja *et al.*, 2013; Nasa *et al.*, 2012; Sankars *et al.*, 2012; Shakti *et al.*, 2014; Rupinda *et al.*, 2013). ESBL strains are inhibited by β -lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam (Bradford, 2001). ESBLs are an increasingly important cause of transferable multidrug resistance in gram-negative bacteria throughout the world. These bacteria have spread rapidly and have become a serious threat to human health worldwide. The gram-negative bacilli especially *Pseudomonas* species and members of the family enterobacteriaceae are common causes of infections of many parts of the body. They account for more than 50% of all isolates in nosocomial infections (Talaro and Talaro, 1996). Among the most prevalent bacteria pathogens capable of showing resistance to common antibiotics is *Escherichia coli* which is one of the most common causes of urinary tract infections and other opportunistic infections such as wound

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Published at: <http://www.ijsciences.com/pub/issue/2018-02/>

DOI: 10.18483/ijSci.1556; Online ISSN: 2305-3925; Print ISSN: 2410-4477



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abscess which can have serious clinical implication (Iroha *et al.*, 2009). Originally, ESBL enzymes were derived from the wide spread TEM and SHV β -lactamase family, however today, over 110 derivatives of TEM β -lactamase and more than 63 derivatives of SHV β -lactamases are known. ESBLs are undergoing continuous mutation, causing the development of new enzymes showing expanded substrate profile. At present, there are more than 300 different ESBL variants and these have been clustered into nine different structural and evolutionary families based on amino acid sequence. TEM (Temoniera) and Sulphydril variable SHV were the major types. However, CTX-M (Cefotaxime-Munich) is more common in some countries (Paterson *et al.*, 2003). The ESBL enzymes are most commonly produced by *Klebsiella* species and *Escherichia coli*, but may also occur in other gram-negative bacteria including *Salmonella*, *Proteus*, *Citrobacter*, *Morganella*, *Serratia*, and *Shigella* species. That ESBLs are encoded on plasmids and are therefore easily transmissible from one organism to another is a therapeutic challenge for physicians as resistance genes for other antimicrobials such as aminoglycosides, tetracycline, and trimethoprim/sulfamethoxazole are often present on the plasmid (Jones, 2001) thereby contributing further to the narrowing of choices of antibiotics.

This study reports on the prevalence and antimicrobial sensitivity pattern of ESBL producing isolates of *E. coli* and *P. aeruginosa* from specimens such as urine, wound swab and HVS. The antimicrobial susceptibility pattern of ESBL producing *E. coli* and *P. aeruginosa* isolates was also determined.

Materials and Methods

Collection of Clinical Isolates

Two hundred and fifty clinical bacterial isolates from the Microbiology Laboratory unit of the Federal Medical Centre (FMC), Owerri, Nigeria were used for the study. The isolates were obtained from the clinical specimen, urine, wound swab and HVS. All isolates were identified on the basis of colonial appearances, gram staining reactions and standard biochemical test (Cheesbrough, 2005)

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was carried out on all the isolates by the Kirby bauer disc diffusion method as recommended by the CLSI (2010). Sterile petri-dishes of Mueller Hinton agar were prepared according to manufacturer's specification. Colonies of an overnight culture were suspended in normal saline and the turbidity adjusted to 0.5 McFarland turbidity standards. A sterile cotton wool swab was inserted into each test tube containing the standardized inoculum suspension, rotated with firm pressure on the side wall of the test tube to remove excess fluid and used to inoculate the entire surface of the Mueller Hinton agar plate.

The antibiotics used in the testing include Ceftazidime 30 μ g, Cefotaxime 30 μ g, Ceftriazone 30 μ g, Cephalexine 30 μ g, Amikacin 30 μ g, Gentamicin 30 μ g, Tetracycline 30 μ g, ofloxacin 5 μ g, Amoxicilin clavulanic acid 30 μ g (oxoid, UK). All plates were incubated at 37 °C for 24 hrs and the diameter of zones of inhibition was measured to the nearest millimetre using a transparent ruler.

Detection of ESBL Production

All suspected colonies were inoculated in peptone water at 37 °C for 6 hrs and their turbidity adjusted to 0.5 McFarland turbidity standards. Several plates of Mueller Hinton agar were prepared and 30 μ g disc of Ceftazime, Cefotaxime, and Ceftriazone, were placed 55 mm centre to centre from the amoxicillin clavulanic acid disc (20 : 10 μ g). The Standardized inoculum was inoculated into Mueller Hinton agar plate and incubated at 37°C over night. Enhanced zones of inhibition between any of the beta -lactam discs and the centre disc were recorded as ESBL producers according to the (CLSI, 2010) criteria. Control strain used for the study was *E. coli* ATCC 25922.

Results

Antibacterial susceptibility testing by disc diffusion method showed that the highest resistance of 100% was recorded with cephalexin while the lowest of 0% resistance was observed with the quinolones and aminoglycosides (Table 1). Among the third generation cephalosporins tested, the highest resistance of 94.9% was observed with cefotaxime while ceftazidime had the lowest resistance rate of 3.5%.

Table 1: Resistance pattern of *E. coli* and *P. aeruginosa* isolates

Antimicrobial agents (µg)	<i>E. coli</i> Resistance (%)	<i>P. aeruginosa</i> Resistance (%)
Ceftazidime (30)	112(82.4)	4(3.5)
Cefotaxime (30)	129(94.9)	103(90.4)
Ceftriazone (30)	114(83.8)	103(90.4)
Cephalexine (30)	136(100)	114(100)
Ofloxacin (5)	0(0)	0(0)
Perfloxacin (5)	0(0)	0(0)
Gentamicin (30)	136(100)	0(0)
Tetracycline (30)	136(100)	0(0)
Ciprofloxacin(5)	0(0)	0(0)
Amikacin (30)	0(0)	0(0)

Table 2: Distribution of ESBL producers from different specimen.

Clinical Samples	<i>E. coli</i>	<i>P. aeruginosa</i>	Total
Urine	52(20.8%)	17(6.8%)	69(27.6%)
Wound swab	0(0%)	28(11.2%)	28(11.2%)
HVS	17(6.8%)	0(0%)	17(6.8%)
Total	69(27.6%)	45(18%)	114(45.6%)

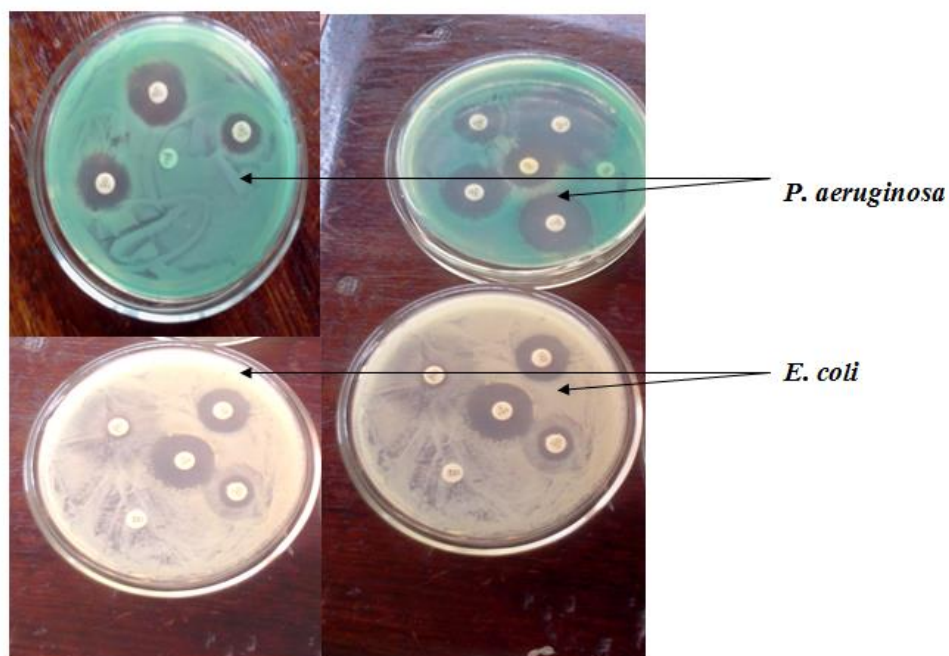


PLATE 1: *E. coli* and *P. aeruginosa* plates showing zones of inhibition using Kirby-Bauer method.

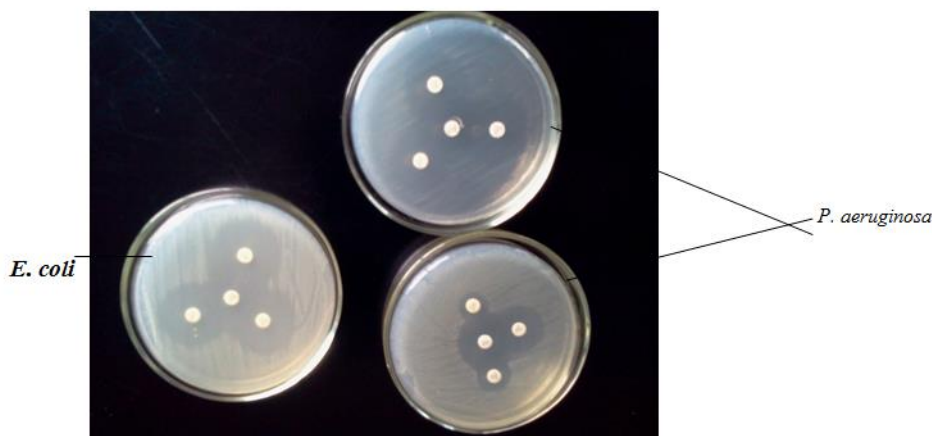


PLATE 2: Double Disk Synergy Test showing *E. coli* and *P. aeruginosa* plates with enhanced inhibition zones.

The result shows that out of the 250 clinical isolates (*Escherichia coli* and *Pseudomonas aeruginosa*) tested, 114 (45.6%) were positive for ESBL production (Table 2) and Plate 1 shows plates of *E. coli* and *P. aeruginosa* plates with zones of inhibition. The susceptibility study shows that for both isolates, the highest resistance of 100% was recorded with cefalexine while the least of 0% was recorded with the quinolones ofloxacin and pefloxacin and aminoglycosides Amikacin and ciprofloxacin (Table 1) and Plate 2 which shows *E. coli* and *P. aeruginosa* with enhanced zones of inhibition.

Discussion

The present study has demonstrated the existence of ESBL producing *E. coli* and *P. aeruginosa* isolates here in the Federal Medical Centre (FMC) Owerri, Imo State, Nigeria. Out of the 250 isolates comprising *E. coli* (136) and *P. aeruginosa* (114) only, only (45.6%) were confirmed positive for ESBL production as judged by the CLSI definition using the double disc synergy test. Out of the 114(45.6%) ESBL positive isolates, 66(26.4%) were *E. coli* and 48(19.2%) were *P. aeruginosa*.

Among the third generation Cephalosporins recommended by CLSI used in the study, Cefotaxime showed the highest resistance rate of 94.9% and 90.4% while Ceftazidime has the least resistance rate of 82.4% and 3.5% for *E. coli* and *P. aeruginosa* respectively. The result showed that *E. coli* was resistant to all the third generation antibiotics with 82.4%, 96.9% and 83.8% respectively. This is in accordance with the 85%, 84% and 75% recorded in the works of Sasirekha *et al.* (2010).

A study carried out in a tertiary hospital in South India recorded a multidrug resistance case in which Cefotaxime was among the drugs with the highest resistance rate of 100% (Padmini *et al.*, 2008). No resistance was recorded with the fluoroquinolones Ciprofloxacin, ofloxacin and Pefloxacin. The aminoglycosides Amikacin and Pefloxacin also showed a 0% resistance. Some studies have also shown that fluoroquinolones and aminoglycosides have antimicrobial activity against ESBL organisms than other non-beta lactam drugs (Jean *et al.*, 2002; Quale *et al.*, 2002). It is interesting to note that the high susceptibility of the fluoroquinolones and the aminoglycosides among other non-beta lactam drugs gave a good indication that they could be a drug of choice in treating infections caused by ESBL producing organisms' particularly *P. aeruginosa* and *E. coli* in the environment.

Consequently, the data highlights a high prevalence of ESBL in Owerri, Nigeria. The 45.6% ESBL recorded in this study is in accordance with the 52.4% high prevalence of ESBL recorded between 2003 – 2007 in Southern and Eastern Nigeria (Aibinu *et al.*, 2003). It is however higher than the 7.5% of ESBL producers recorded in Ogun State (Ruth *et al.*, 2011) and the 16% ESBL producers reported in South Eastern Nigeria (Akujobi and Ewuru, 2010).

In conclusion, this study has revealed the presence of ESBL producing *E. coli* and *P. aeruginosa* in Imo, State Nigeria. The study recommends collaboration between physicians and laboratory scientist before administering any cephalosporin to patients. In addition, molecular typing is *sine qua non* in determining type of ESBL presents in each isolate which is essential for a reliable epidemiology of antimicrobial resistance.

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