Prevalence of Extended-Spectrum Beta-lactamases (ESBLs) and Plasmid status of *Eschericha coli* and *Klebsiella pneumoniae* isolates from clinical sources in UPTH, Port-Harcourt, Nigeria.

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Abstract: The aim of this study is to determine the ESBL and plasmid status of *Eschericha coli* and *Klebsiella pneumoniae* isolates from clinical sources in UPTH, Port-Harcourt, Nigeria. A total of 200 specimens, ranging from stool, urine and ear swabs were collected. Data obtained included age, sex and ward/clinic attended. All samples were analysed using standard methods. The results showed that the prevalence of ESBL in this study population was 41.7% (n=25) and *E. coli* (43.9%) and *K. pneumoniae* (36.8%). These isolates were subjected to the Double Disc Synergy Test to test for the presence of ESBL producing enzymes. An enhanced zone of inhibition between anyone of the β-lactam discs and the beta-lactamase inhibitor combination disc was interpreted as evidence for the presence of an ESBL. Clinical isolates of *K. pneumoniae* and *E. coli* that were positive for ESBL production by the double disk synergy test were subjected to Acridine orange. Plasmid curing was done in order to study some characters of the organism that may have been conferred upon them by the presence of these plasmids. P-values <0.05 were accepted as significant. Among the clinical samples tested, urine yielded the most ESBL producers [13(41.9%)], followed by stool samples [9(45.0%)]. Ear swab yielded the least ESBL producers [3(33.3%)]. Of the 25 ESBL producing organisms, *E. coli* [18(72.0%)] was the major ESBL producer and *K pneumoniae* 7(28.0%). Patients in the 11 - 20 years of age group had the highest number of ESBL-Producing isolates accounting for (20.0%), followed by the age group 21-30 years, 31-40 years and 51-60 years which had 4(16.0%). Each patients in the age groups 61-70 years did not harbouring ESBL producing organisms. Females [16(64%)] were detected with the highest rate of ESBL-producing organism and males accounted for 9(36%). ESBL-producing organisms were most frequently accounted in the GOPD [7(28%)]. This was closely followed by the Paediatric ward [6(24%)], while the Accident, Emergency and Surgical ward recorded 6(16%), respectively. From the results, 40.0% of the ESBL producing isolates lost their ESBL enzymes after plasmid curing while 60.0% were resistant. *E. coli* were more susceptible to plasmid curing (44.4%) than *K. pneumoniae* isolates (28.6%) whereas on the other hand *K. pneumoniae* isolates were more resistant to plasmid curing (71.4%) than *E. coli* (55.6%). There was a positive synergy (an increase in the zone of inhibition between the Amoxycillin-Clavulanic acid disc and Ceftazidine which is one the 3rd generation Cephalosporins when compared to the respective zones of inhibition). While no synergy appeared in the negative plate. The finding from this study showed that there is a high prevalence of ESBL-producing organisms in University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria with *E.coli* producing more ESBL enzymes than *K. pneumoniae*. The presence of ESBL producers could further compound the problem of the patients by exhibiting co-resistance to other classes of antimicrobials. It is pertinent to note that health authorities as a matter of urgency should include molecular epidemiology in its infection control policy. This would help to detect changing patterns and emergence of new resistance genes before wide spreads dissemination occurs.
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1. Introduction

Extended-Spectrum Beta-lactamases (ESBLs) are a group of diverse, complex and plasmid-mediated, rapidly evolving enzymes that pose a major therapeutic challenge in the treatment of patients. They are beta-lactamases that are able to hydrolyse a broader spectrum of beta-lactam antibiotics than the simple parent beta-lactamases from which they were derived, hence, the term extended spectrum. They are able to inactivate beta-lactam antibiotics containing an oxyimino-group such as oxyimino-cephalosporins (e.g. Ceftriaxone, Cefotaxime, Ceftazidime), as well as oxyimino-monobactam, Aztreonam (Bradford, 2001; Paterson and Bonomo, 2005) and also Penicillins (Nathisuwan, et al., 2001).

Infection and colonization with ESBL-producing organisms are usually hospital-acquired especially in intensive care units (Babini and Livermore, 2000;

Lautenbach *et al*., 2001). Other hospital units that are at increased risk include surgical wards, paediatrics and neonatology, rehabilitation units and oncology wards (Bermudes, 1997; Martinez-Aguilar, 2001). Community clinics and nursing homes have also been identified as potential reservoirs (Wiener, 1999). Recent studies have demonstrated the danger of ESBL producers in livestock (Winokur, 2000).

A wide range of gram-negative rods have been found to be ESBL producers with the vast majority belonging to the family Enterobacteriaceae (Bradford, 2001). ESBLs are most commonly produced by *E.coli, Klebsiella* species, with *Klebsiella pneumonia* seemingly the major ESBL producer (Al-Jasser, 2006).

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 nosocomial infections (Shah *et al*., 2004). Failure to detect ESBL-mediated resistance has led to therapeutic failure and facilitated the spread of ESBL-producing organisms (Paterson and Bonomo, 2005). For cost-effective isolation and effective medical treatments, there is a need to screen patients for ESBL production (Siegel *et al.*, 2007).

In Nigeria, there have been reports of carbapenemase-producing clinical isolates of enteric bacteria particularly among *E. coli* and *Klebsiella spp* (Motayo *et al., 2012).* In addition, regular reports of various infections of ESBL organisms have been recorded in our environment; there has also been laboratory evidence of co-acquisition of multi-resistant plasmids in clinical isolates of *E. coli* and other enteric organisms from Abeokuta (Akinduti *et al., 2011;* Motayo *et al., 2012).* This has most undoubtedly put Abeokuta on the global antibiotic multi-drug resistant map. Previous reports have indicated the usefulness of plasmid profiling in categorising and determining the extent of antibiotic multi-drug resistance and risk of epidemic spread (Akindui *et al.,* 2011; Schawaber *et al., 2005;* Adenipekun *et al.,* 2009).

These reports have shown that community colonisation and other reservoir sources such as food animals are capable of conjugative spread of these R-plasmids with ESBL and carbapenemase activity within the hospital community to cause nosocomial outbreaks (Motayo *et al., 2012*), the implication of this on the community and National public health system can be enormous because of the cost of treating affected patients and managing outbreaks. Therefore the essence of maintaining a log of Epidemiological record of multi-resistant isolates to include their possible plasmid profiles, conjugative activity and molecular epidemiology is essential in our study setting.

ESBLs are group of enzymes occasionally present in *Klebsiella* species and *Escherichia coli* that confer upon the bacteria the additional ability to hydrolyze the ß-lactam rings of oxyimino- third and second generation cephalosporins (ceftaxime, ceftazidime and ceftriaxone) and/or aztreonam. These ß-lactamases generally belong to Ambler's molecular class A 2 and Bush's functional class 2be. They are susceptible to ß-lactamase inhibitors such as clavulanic acid but do not affect cefamycins as cefoxitin, cefotetan and cefmetazole and cerbepenems as imipenem and meropenem.

The ESBL enzymes are carried on plasmids which also frequently carry genes encoding resistance to other drug classes such as Fluoroquinolones, Aminoglycosides, Trimethoprim, Sulphonamides, and Tetracycline. This results in very limited treatment alternatives, posing a major therapeutic challenge. This lack of effective treatment leads to longer hospital stays, rising healthcare costs and high mortality in patients with serious infections. This study seeks to determine the presence and prevalence of ESBL producers in the hospital, the types and also their antibiogram. This will help the management improve measures for screening for these organisms, practice institutional controls and preventive measures and finally to serve as a guide in empirical therapy.

With the spread of ESBL-producing strains in hospitals all over the world, it is necessary to *assess* the prevalence of ESBL positive strains in UPTH, Port-Harcourt so as to formulate a policy of empirical therapy in high risk units where these organisms are present. The detection of ESBL-producing organisms is critical for the appropriate management of patients, infection prevention and control efforts. Laboratory information on any isolate from a patient is essential to avoid misuse of extended-spectrum Cephalosporins which would further increase the development of resistance. Knowledge of the resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious use of antimicrobial agents. Information on the presence of ESBL producers will also help in determining the right antimicrobial to use for empirical treatment as those that are effective *in-vitro* may not be effective *in-vivo*.

The high prevalence rates and the consequences of infection with an ESBL-producing organism (treatment failures, longer hospital stays, increased resistance, higher mortality rates, emergence of new strains in the community and the fact that these organisms are not routinely tested for nor detected by routine susceptibility testing) justify the need for this study. The aim of this study is to determine the ESBL and plasmid status of *Escherichia coli* and *Klebsiella*

pneumoniae isolates from clinical sources in UPTH, Port-Harcourt, Nigeria.

2. Materials And Methods

2.1. Study Area: This study was carried out in the University of Port-Harcourt Teaching Hospital (UPTH), being a referral and tertiary healthcare facility located in Port-Harcourt, the capital of Rivers State, Niger Delta region of Nigeria.

2.2. Study Population: The study group consisted of patients attending clinics as out-patients and in-patients. Patients from Surgical, Paediatric, General outpatient Department (GOPD), Medical and Antenatal clinic attendees, Accident and Emergency, Gynaecology, and staff clinics hospital were also considered. A total of 200 specimens, ranging from stool, urine and ear swabs were collected. Approval for collection of samples as obtained from the Ethical Review Board of the University of Port-Harcourt Teaching Hospital (UPTH). Written consent was obtained from adult patients and parents of children who participated in this.

2.3. Nutrients Media used for Sample Processing: Different types of media were utilized for this study include: Eosin Methylene Blue agar (oxoiduk) for isolation and purification of *Escherichia coli;* Cysteine Lactose Electrolyte-Deficient agar CLED (LAB M, UK) for the isolation of Klebsiella and E.coli species; MacConkey agar (Biomark, India) for Lactose fermenting organisms; Mueller-Hinton Agar (LAB M, UK) for antibiotic sensitivity tests; Nutrient Agar (Fluka, Spain) for the preparation of slants; Deoxycholate Agar (Merck, Germany) for Isolation and Purification; and Acridine orange (Merck, Germany) for Plasmid curing.

2.4. Standard Organisms Used: The following standard organisms (*Escherichia coli* ATCC25922 and *Klebsiella pneumonia*e ATCC70603), obtained from the National Veterinary Research Institute Jos, were use as controls in this study.

2.5. Sample collection and processing: All urine and stool samples were collected from patients using sterile, dry, leak-proof containers at UPTH and ear swab samples were collected by qualified medical personnel of the hospital aseptically. Each specimen was clearly labelled. Data obtained included age, sex and ward/clinic attended.

2.6. Laboratory Analysis

2.6.1. Culturing and Isolation: This was carried out as described by Cheesbrough (2006). All urine samples were cultured on CLED (LAB M, UK), Eosin Methylene Blue agar (Oxoid, UK) and MacConkey Agar (Biomark, India) plates. The stool samples were inoculated directly on MacConkey agar plates while ear swab samples were inoculated directly on MacConkey and Deoxycholate agar plates. These plates were incubated at 37ºc for 24 - 48hours. Discrete colonies of isolates were sub-cultured on Nutrient agar to obtain pure culture for characterization and series of biochemical tests were done for proper characterization and identification.

2.6.2. Identification of isolates: Suspected colonies are confirmed with conventional biochemical techniques. Biochemical testing includes Grams staining, motility test, Citrate Utilization Test, Indole production test, Methyl-Red test, Voges-Proskauer test, reaction on triple sugar iron agar. The bacterial isolates were also identified by comparing their characteristics with those of known taxa, as described by Jolt *et al.* (1994) and Cheesbrough (2006).

2.7. Antimicrobial susceptibility test: The sensitivity and resistance of the isolates to antibiotics was observed using the Kirby Bauer (1966) disc diffusion technique (CLSI, 2013). Four to five colonies of 24 hrs pure culture isolates were inoculated into 3ml sterile physiological saline using a sterile wire loop and the turbidity adjusted to match a 0.5 McFarland standard. Bacterial suspensions were inoculated on Mueller Hinton agar (HiMedia) by streaking the swab over the entire sterile agar surface. The procedure is repeated by streaking two more times, rotating the plate approximately 60˚ each time to ensure an even distribution of inoculum. The antibiotic discs were placed over the lawn of 150 mm plate incubated at 37˚C for 18-48 hours until moderate growth was seen on the agar plates. The clear zone around each antibiotic disc was measured in millimeter. The diameter zone inhibition is classified as being resistant, intermediate or sensitive. The following commonly in use antibiotics were used; ciprofloxacin (5μg), cefotaxime (30μg), gentamicin (10μg), Aztreonam (30μg), cefpodoxime (10μg), ceftazidime (30μg), Tetracycline (10μg), ertapenem (10μg), Imipenem (10μg), and trimethoprim-sulfamethoxazole (1.25/23.75μg) (Oxoid, UK). The zone sizes were interpreted to ensure the ranges were within the limits published by the CLSI M100-S23 Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Third Informational Supplement (CLSI, 2013) guidelines interpretative chart. *Escherichia coli* American Type Culture Collection (ATCC) 25922 reference strain was used as control to test the performance of the method.

2.8. Detection of ESBL Producing Strains: Isolates from the susceptibility testing with zone diameters found to be resistant to the Cephalosporins such as Cefpodoxime (≤mm), Cefotaxime (≤27mm), Ceftazidime (≤22mm) and Aztreonam (≤27mm) were said to be ESBL Positive (Beta lactamase positive isolates).

2.9: Double Disc Synergy Test for ESBL: Isolates found to be resistant to Ceftazidime, Cefotaxime were subjected to the Double Disc Synergy Test to test for

the presence of ESBL producing enzymes. Isolates were streaked on the surface of Muller-Hinton agar plates and disc containing the standard concentration of Azteronam, Ceftazidime, Cefpodoxime, Cefotaxime were placed 15mm from Amoxicillin/Clavulanic acid combination disc. The inoculated media were incubated overnight at 35ºC. An enhanced zone of inhibition between anyone of the β-lactam discs and the beta-lactamase inhibitor combination disc was interpreted as evidence for the presence of an ESBL.

2.10. Plasmid curing of ESBL isolate: Clinical isolates of *K. pneumoniae*, and *E. coli* that were positive for ESBL production by the double disk synergy test were subjected to Acridine orange (Merck, Germany). Mediated plasmid elimination using the method described by (Iroha *et al.*, 2010) was adapted. Each of the isolates positive for ESBL production were grown in 5ml of double strength Mueller Hinton broth supplemented with 0.1 mg/ml of Acridine orange and incubated at 37^0C for 18-24 hrs. After incubation, the test organisms were re-tested for ESBL production using the Double Disk Synergy Test. For quality control, an ESBL negative isolates was tested as with the test organisms, also an ESBL positive isolate was grown in the double strength Mueller Hinton broth without Acridine orange and tested for ESBL Production.

2.10.1. Plasmid curing with acridine orange: Plasmid curing is a term used to describe the removal of plasmids from microorganisms that possess them. This is usually done in order to study some characters of the orgs that may have been conferred upon them by the presence of these plasmids. This will help to determine whether such characters are chromosomal or plasmid borne. It can be effected with the use of some dyes like acridine orange. The method of plasmid curing used in this study is that of Iroha et al. (2010).

2.11. Quality Control: *Escherichia coli* ATCC 25922 reference strain was used as control for antimicrobial susceptibility testing. *Escherichia coli* ATCC 25922 (β-lactamse negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing) were used the reference strains for quality control for the DDST test. Sterility of all media was ascertained by pouring the autoclaved media into Petri dishes without inoculating them and then incubating at 37ºc for 18-24 hours.

2.12. Data analysis: Data generated from this study were subjected to statistical analysis using ANOVA significance test and regression analysis. P-values <0.05 were accepted as significant.

3. Results

A total of 60 isolates comprising *E. coli* and *K. pneumoniae* were recovered from 187 clinical samples. Stool samples (n=51) yielded 20 isolates, Urine samples (n=112) yielded 31 isolates while 9 isolates were recovered from ear swab samples (n=24). *E.coli* was the most frequently isolated organism isolated [41(68.3%)] and *K. pneumoniae* [19(31.7%)] as shown in Table 1. The prevalence of Extended-Spectrum Beta-lactamase organisms isolated from patient samples in UPTH, Port-Harcourt is also presented in Table 1. A total of 60 bacterial isolates were obtained out of which 25 were ESBL- producing organisms giving a prevalence rate of 41.7%. Among the clinical samples tested, urine yielded the most ESBL producers 13(41.9%), followed by stool samples 9(45.0%). Ear swab yielded the least ESBL producers, 3(33.3%) as shown in Table 1.

Table 1: Prevalence of clinical isolates and extended spectrum Beta-Lactamase producing isolates from patient samples in UPTH Port-Harcourt, Nigeria using the DDST Technique

Sample	No. Tested	No. isolates (%)	No. ESBL Producers (%)	Non-ESBL Producers (%)
Stool	51(27.3)	20(33.3)	9 (45.0)	11(55.0)
Urine	112(59.9)	31(51.7)	13 (41.9)	18(58.1)
Ear swab	24(12.8)	9(15.0)	3 (33.3)	6(66.7)
Total	**187(100.0)**	**60(100.0)**	**25 (41.7)**	**35(58.3)**

The results of the Antimicrobial Susceptibility testing is shown in Table 2. Table 2 shows that 25 strains of *Escherichia coli* showed 100% resistance to Ciprofloxacin. Nineteen strains also show 76% resistance to chloramphenicol. It further shows resistance for 18 strains at 72% Augmentin, while 17 strains showed 68% resistance to Gentamycin. Sixteen strains also shows 64% resistance to Ampicillin, 13 strains show resistance at 52% to Tetracycline, 11 strains showed 44% to ciprofloxacin and finally or shows that 9 strains are 36% resistant to Ciprofloxacin.

Table 3 also the susceptibility pattern of *E. coli* and *K. pneumoniae* isolates from patients in UPTH, Port-Harcourt, Nigeria. Imipenem was the most effective antimicrobial agent with 100% activity against all the isolates. Ertapenem was sensitive to 92.7% of *E. coli* and 78.9% for *K. pneumoniae*. Tetracycline and Trimethoprim-Sulfame Thoxazole were the least effective antimicrobials to *E. coli* with susceptibility rates of 14.6% and 9.8% respectively and 31.6% and 26.3% for *K. pneumoniae* (Table 3).

Table 2: Multi Disc Antibiotics Susceptibility Study on *Escherichia coli* in urine (Gram negative microorganisms)

Antibiotics	No. tested	No. Susceptible (%)	No. Resistant (%)	No. Intermediate (%)
Tetracycline	25	9(36.0)	13(52.0)	3(12.0)
Chloramphenicol	25	5(20.0)	19(76.0)	1(4.0)
Ciprofloxacin	25	14(56.0)	11(44.0)	0(0.0)
Ofloxacin	25	12(48.0)	9(36.0)	4(16.0)
Augumentin	25	7(28.0)	18(72.0)	0(0.0)
Gentamycin	25	5(20.0)	17(68.0)	3(12.0)
Peflacin	25	0(0.0)	25(100.0)	0(0.0)
Ampicillin	25	9(36.0)	16(64.0)	0(0.0)

Table 3: Antimicrobial Sensitivity Pattern of *E. coli* and *K. pneumoniae* isolates from Patients Samples in UPTH, Port Harcourt, Nigeria

Antimicrobial	***E. coli* (n=41)**		***K. pneumoniae* (n=19)**	
	Susceptible (%)	**Resistant (%)**	**Susceptible (%)**	**Resistant (%)**
Imipenem (10µg)	41 (100)	0 (0)	19 (100)	0 (0)
Ceftazidime(30µg)	24 (58.5)	17 (41.5)	9 (47.4)	10 (52.6)
Aztreonam(30µg)	26 (63.4)	15 (36.6)	7 (36.8)	12 (63.2)
Cefotaxime(30µg)	26 (63.4)	15 (36.6)	7 (36.8)	12 (63.2)
Ertapenem(10µg)	38 (92.7)	3 (7.3)	15 (78.9)	4 (21.1)
Cefpoxime(10µg	26 (63.4)	15 (36.6)	8 (42.1)	11 (57.9)
Gentamycin(10µg)	32 (78.0)	9 (22.0)	12 (63.2)	7 (36.8)
Ciprofloxacin(5µg)	34 (82.9)	7 (17.1)	10 (52.0)	9 (47.4)
Tetracycline (30µg)	6 (14.6)	35 (85.3)	6 (31.6)	13 (68.4)
Trimethoprim-Sulfame-thoxazole (1.25/23.73µg)	4 (9.8)	37 (90.2)	5 (26.3)	14 (73.7)

The distribution of clinical isolates producing ESBL enzymes is shown in Table 4. Of the 25 ESBL producing organisms, *E. coli* [18(72.0%)] was the major ESBL producer and *K pneumoniae* 7(28.0%).

Table 4: Distribution of clinical isolates producing ESBL on UPTH, Port-Harcourt, Nigeria

Clinical samples	***ESBL Producers* (%)**	***E. coli* (%)**	***K. pneumoniae* (%)**
Stool	9 (45.0)	6(66.7)	3(33.3)
Urine	13 (41.9)	10(76.9)	3(23.1)
Ear swab	3 (33.3)	2(66.7)	1(33.3)
Total	**25 (100.0)**	18(72.0)	7(28.0)

The age distribution of patients harbouring ESBL producing isolates from clinical samples in UPTH Port-Harcourt, Nigeria is shown in Table 5. From the table, patients in the 11 - 20 years of age group had the highest number of ESBL-Producing isolates accounting for (20.0%), followed by the age group 21-30 years, 31-40 years and 51-60 years which had 4(16.0%). Each patients in the age groups 61-70 years did not harbouring ESBL producing organisms (Table 5).

Table 5: Age Distribution of patients having ESBL-producing isolates from clinical samples in UPTH, Port-Harcourt, Nigeria

Age Group	**No. isolates**	**No of ESBL organisms (%)**		
		E. coli	***K. pneumoniae***	**Total**
<1	18(30.0)	2(66.7)	1(33.3)	3 (12.0)
1-10	4(6.7)	1(50.0)	1(50.0)	2 (8.0)
11-20	5(8.3)	3(60.0)	2(40.0)	5 (20.0)
21-30	6(10.0)	4(100.0)	0(0.0)	4 (16.0)
31-40	8(13.3)	3(75.0)	1(25.0)	4 (16.0)
41-50	9(15.0)	1(33.3)	2(66.7)	3 (12.0)
51-60	7(11.7)	4(100.0)	0(0.0)	4 (16.0)
61-70	3(5.0)	0(0.0)	0(0.0)	0 (0.0)
Total	**60(100.0)**	**18(72.0)**	**7(28.0)**	**25 (100.0)**

The gender distribution of patients with ESBL-producing isolates from clinical samples in UPTH, Port Harcourt is shown in Table 6. Females [16(64.0%)] were detected with the highest rate of ESBL-producing organism and males accounted for 9(36.0%).

Table 6: Gender Distribution of Patients with ESBL Producing Organisms Isolated from Clinical Samples in UPTH, Port-Harcourt, Nigeria

Organism	No. ESBL Isolates (%)	Males (%)	Females (%)
E. coli	18(72.0)	7 (38.9)	11 (61.1)
K. pneumoniae	7(28.0)	2 (28.6)	5 (71.4)
Total	**25(100.0)**	**9 (36.0)**	**16 (64.0)**

The distribution of ESBL-producer according to Ward/Clinic in UPTH is shown in Table 7. ESBL-producing organisms were most frequently accounted in the GOPD [7(28.0%)]. This was closely followed by the Paediatric ward [6(24.0%)], while the Accident, Emergency and Surgical ward recorded 6(16.0%), respectively (Table 7).

Table 7: Distribution of ESBL-Producing Organisms according to Ward/Clinic in UPTH, Port Harcourt, Nigeria

Location	No. Isolates (%)	No. of ESBL Organisms (%)		Total (%)
		E. coli	*K. pneumoniae*	
A & E (Accident and Emergency)	5(8.3)	3(75.0)	1(25.0)	4 (16.0)
Gynae (Gynaecology ward)	1(1.7)	0(0.0)	1(100.0)	1 (4.0)
Paediatric ward	15(25.0)	4(66.7)	2(33.3)	6 (24.0)
GOPD (General Outpatients Dept)	18(30.0)	5(71.4)	2(28.6)	7 (28.0)
Surgical ward	11(18.3)	3(75.0)	1(25.0)	4 (16.0)
Staff Clinic	2(3.3)	1(100.0)	0(0.0)	1 (4.0)
Medical ward	8(13.3)	2(100.0)	0(0.0)	2 (8.0)
Total	**60(100.0)**	**18(72.0)**	**7(28.0)**	**25 (100.0)**

The total number of *E. coli* positive for Extended Spectrum Beta-Lactamase production is 43.9% and the total number of *E. coli* negative for ESBL is 56.1% as shown in Table 8. The total number of *K. pneumoniae* positive for ESBL is 36.8% and the total negative is 63.2% as shown in Table 8.

Table 8:_Number of *E. coli* and *Klebsiella pneumoniae* Positive for Extended Spectrum Beta-Lactamase production

Sample	**No. of Isolates (%)**	***E. coli***	**ESBL Positives (%)**	***Klebsiella pneumoniae (%)***	ESBL Positives (%)
Urine	34(56.7)	25(73.5)	8 (32)	9(26.5)	3(33.3)
Stool	17(28.3)	11(64.7)	6 (55)	6(35.3)	2(33.3)
Swab	9(15.0)	5(55.5)	4 (80)	4(44.5)	2(50.0)
Total	**60(100.0)**	**41(68.3)**	**18 (43.9)**	**19(31.7)**	**7(36.8)**

The rate of plasmid curing of ESBL isolates using Acridine Orange mediated technique is shown in Table 9. From the results, 40.0% of the ESBL producing isolates lost their ESBL enzymes after plasmid curing while 60.0% were resistant. *E. coli* were more susceptible to plasmid curing (44.4%) than *K. pneumoniae* isolates (28.6%) whereas on the other hand *K. pneumoniae* isolates were more resistant to plasmid curing (71.4%) than *E. coli* (55.6%) as shown in Table 9.

Table 9: Plasmid curing rates for ESBL – Positive *Escherichia coli* and *Klebsiella pneumoniae*

Isolates	**No. (%)**	**ESBL-Positive isolates (%)**	**Non-ESBL isolates after curing (%)**	**No. Resistant to plasmid curing (%)**
K. pneumoniae	19(31.7)	7(36.8)	2(28.6)	5(71.4)
E. coli	41(68.3)	18(43.9)	8(44.4)	10(55.6)
Total	60(100.0)	25(41.7)	10(40.0)	15(60.0)

Plate I & II showed a positive and negative ESBL test, respectively using the Double Disc Synergy Test (DDST). In plate I, there was a positive synergy (an increase in the zone of inhibition between the Amoxycillin-Clavulanic acid disc and Ceftazidine which is one the 3rd generation Cephalosporins when compared to the respective zones of inhibition). While no synergy appeared in the negative plate (plate II).

Plate I: A Positive ESBL Test showing synergy

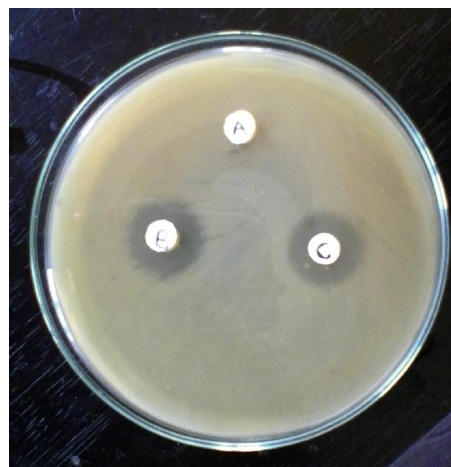

Plate II: Negative ESBL test showing no synergy

Keys: A = Azitreonam; B = Amoxycillin-Clavulanic acid; C = Ceftazidime

4. Discussion

The procedure of ESBL-producing organisms has been increasing rapidly worldwide. This situation is alarming because the prevalence of ESBL, type of enzyme, gender and age group of persons affected varies in different geographical areas. In addition ESBL producers exhibit co-resistance to many other classes of antibiotics resulting in limited therapeutic options.

In this study, the overall prevalence of ESBL producers among the clinical isolates was 41.7%. This is comparable with the findings of Iroha *et al*. (2009) who reported 39.8% in Enugu, Nigeria. Akanbi et al. (2013) reported overall prevalence rate of 33.6% in Abuja, Nigeria. Adeyankinnu *et al*. (2014) in Ogun State, Nigeria recorded a prevalence rate of 26.4% while Ejikeugwa *et al.* (2013), reported a prevalence rate of 26% in Enugu State, Nigeria. Higher prevalence rates of 58.6% (Enugu); 76.9% (Ibadan), 80.0% (Ebonyi) have been reported in studies by Iroha *et al*. (2010), Okesola and Fowotade (2012) and Nwakaze *et al.* (2013), respectively. Global reports show 29.45% (Riaz *et al.,* 2012) in Pakistan; 59.6% in Sudan (Ahmed *et al*., 2013), and 57.5% in India (Rao *et al.*, 2014). These higher rates may be attributed to the fact that prevalence of ESBL varies worldwide in geographical areas from institution to institution, and from country to country (Kaur *et al.*, 2013), and may also depend on the method used for defection (Al-Jasser, 2006).

Although *E.coli* and *K. pneumoniae* have been identified as the major ESBL producing microorganisms, there has been no consensus on which has a greater capacity to produce ESBL as evident in varying results reported from different studies in Nigeria and around the world. For instance, Adeyankinmu *et al.* (2014) in Ogun state; Alipourfards and Nili (2010) in Bangladesh; and Riaz *et al*. (2012) in Pakistan identified *E.coli* as the major ESBL producer, other reports by Olowe and Aboderin (2010) in Ogun state; Osazuwa (2011) in Ghana; and Ahmed *et al*. (2013) in Sudan show *K. pneumoniae* as the major ESBL producer. In this study, of the 25 ESBL producing, organisms encountered *E.coli* accounted for 18(72.0%) of the isolates while *K. pneumoniae* was 7(28.0%).

In this study, the prevalence of ESBL producing organisms was highest in patients within the age group 11-20 years and within the <1 year age groups (20.0%). This corresponds to reports by Motayo *et al.* (2013) and Adeyankinnu *et a*l. (2014) who found the <1 year group as the highest producers. The findings by Riaz *et al.* (2012) in Pakistan show highest prevalence of ESBL in both the 41-50 years and 51-60 years age groups (19.23%).

Regarding gender classification, females (64.0%) than their male counterparts (36.0%). Similarly, there were higher detection rates of *E. coli* and *K. pneumoniae* from females (61.1% and 71.4%) than from males (38.9% and 28.6%). These results are similar to findings by Riaz *et al.* (2012) in Pakistan who obtained detection rates of 60% and 55% for *E. coli* and *K. pneumoniae* from females and 40% and 45% from males; Akanbi *et al.* (2013), 59.4% and 55.2% from females and 40.6% and 44.8% from males in Abuja, Nigeria.

In this study, it was observed that ESBL producers were most frequently encountered in the General Outpatient Department (GOPD) (28.0%) closely followed by surgical wards and Accident and Emergency (16.0%) respectively, and medical wards (8.0%). A study by Alipourfard and Nili (2009) reported most of the ESBL producing isolates (29.6%) were from the medical wards, followed by Outpatients clinic (24.3%). Among the ESBL producers from the Outpatient Department, *E. coli* accounted for 83.33% of the organisms isolated which is consistent with reports that ESBL producing *E. coli* is predominant in the community (Paterson and Bonomo, 2005; Pitout *et al.,* 2005; Riaz *et al*., 2012).

The possible cause of these high rates of community acquired infections with ESBL producing organisms is not yet clear, but may not be unconnected with the use of antibiotics in foodstuffs and animal rearing, and frequent patient contact with health care facilities (Pitout *et al*., 2005). However, it has been suggested that diabetes mellitus, prior quinolone use, recurrent urinary tract infections, prior hospital admissions, and older age were independent risk factors (Rodriguez – Bano *et al.,* 2004).

As reported by Talbot *et al*. (2006), the Infectious Diseases Society of America has listed *E. coli* and *K. pneumoniae* as two out of six pathogens for which new drugs are urgently needed in order to combat resistance development. Majority of the isolates encountered in this study were multidrug resistant strains (48.3%) showing resistance to at least 5 antimicrobials while 56.7% showed resistance to 4 or more antimicrobials. The resistance was more pronounced among the ESBL producers (77.8%) showing resistance to at least 5 antimicrobials while 88.5% of all the isolates were resistant to 4 or more antimicrobials. The reason for this multidrug resistance could be as a result of gross misuse or over use of antimicrobials in the hospital on non-compliance or adherence of antimicrobials use by patients, while the increased resistance among the ESBL producers would likely be because the plasmid which carried the ESBL enzymes also harboured resistance genes to other classes of antimicrobials.

Findings from this study show that imipenem was the most effective antibiotic to all *E.coli* and *K. pneumoniae* isolates (100% susceptibility). This is in line with studies by Ejikeugwu *et al.* (2012), Iroha *et al*. (2012) and Ejikeugwu *et al.* (2013). This was followed by Ertapenem 92.7 for *E. coli* and 78.9 for *K. pneumoniae*. Tetracycline and trimethoprim/ sulfamethoxazole were the least effective antibiotics with susceptibility rates of 14.6% and 9.8% for *E. coli* and 31.6% and 26.3% each for *K. pneumoniae*. This again could be attributed to gross misuse and abuse of these antimicrobials which are readily available as over-the-counter (OTC) drugs and can be purchased without doctor's prescription.

Consequently, this poses problems for treatment of infections caused by ESBL producers because cheap and commonly used antimicrobials like tetracycline, trimethoprim-sulfamethoxazole and ciprofloxacin are ruled out as therapy options. Imipenem which is the most effective drug against ESBL producing organisms is very expensive and being a broad spectrum antimicrobials, its prolonged use could result in the alteration of patient's microbiota. Also, its indiscriminate use could result in the development of imipenen-resistant strains leaving no therapeutic alternative in the future.

ESBL-producing organisms have been known to exhibit co-resistance to many classes of antibiotic (Khalaf et al., 2009) including resistance to the aminoglocogenes coding for ESBL are often located on large plasmids that also encode genes for resistance to other antibiotics. Such co-resistance must have occurred in this study. For instance, when comparing ESBL producers to non-producers, ciprofloxacin susceptibility dropped 60.9% in non-producer to 38.9% among the ESBL producers for *E. coli* while for *K. pnuemoniae*, susceptibility dropped from 75.0% to 28.6% respectively. *E. coli* susceptibility to Gentamycin dropped from 65.2% in non-producers to 27.8% among the ESBL producers, while for *K. pneumoniae* the susceptibility dropped from 83.3% in non-producers to 28.6% among the ESBL producers. All *K. pneumoniae* ESBL producers were resistant to sulfamethoxazole-Trimethopixian. Aibinu *et al.* (2003) and Kizilca *et al.* (2012) have also reported similar co-resistant patterns at various degrees of susceptibilities. In this study, both ESBL producers and non-producers were all susceptible to imipenem (100%), as reported by Ahmed *et al.* (2013) and Adeyankimu *et al*. (2014), but there was a drop in the susceptibility to Ertapenem from 100% (non-ESBL) to 83.3% (ESBL). The high resistance to these antibiotics by the ESBL producing strains poses a threat of treatment failure by these drugs and it also limits the therapeutic choice to the carbapenems, dependence on which can led to resistant strains like imipenem resistant *P. aeruginosa* and *Acinetobacter spp* (Rupp and Fey, 2003).

Studies have indicated that genes encoding for ESBL resistance reside within Plasmids (Paterson and Bonomo, 2005; Rawat and Nair, 2010). This means that an ESBL-producing isolate on losing its plasmid should no longer express resistance. In this study, after plasmids curing was carried out, 40% of the ESBL-producing isolates were ESBL negative while 60% remained ESBL positive. In a similar study by Adeyankinmu *et al*. (2014), 7(13.5%) out of 52 positive ESBL isolates were cured while 45(13.5%)

were not cured. This differs from a study by Iroha *et al*. (2012) in which all 58(100%) ESBL-positive isolates could not express ESBL enzymes after plasmids curing. The result from this study may be unconnected with the fact that plasmid curing is effective only against some plasmids and that their likely response to curing can vary unpredictable. Also, the efficiency of curing can vary widely depending on the plasmids and the particular bacterial host carrying it. The result observed in this study of different plasmid curing rates may also suggest that the ESBL enzymes in the uncured isolates may be chromosomally mediated as suggested by Iroha *et al.* (2012) and Adeyankinmu *et al*. (2014) in their reports.

5. Conclusion

The major findings from the study show that the overall prevalence of ESBL was 41.7%. ESBL producing *E. coli* (72.0%) and *K. pneumoniae* (28.0%) were isolated from the patient samples with *E. coli* being the major producer of ESBL. The finding from this study showed that there is a high prevalence of ESBL-producing organisms in University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria with *E.coli* producing more ESBL enzymes than *K. pneumoniae*. ESBL producing organisms exhibited a high level of co-resistance to other antibiotics especially the fluoroquinolones (ciprofloxacin) and Aminoglycosides (Gentamycin). Majority of the organisms in this study were multidrug resistant, being resistant to more than 4 antibiotics. Carbapenems remain the drug of choice for ESBL therapy with all isolates being susceptible to imipenem, Ertapenem was also quite effective.

Most of the organisms isolated were multidrug resistant strains and the presence of ESBL producers further compounded the issue by exhibiting co-resistance to other classes of antimicrobials especially the fluoroquinones and aminoglycosides. However, the carbapanems remain the most effective therapeutic option for both ESBL and non-ESBL producing organisms, Imiperem being the most effective followed by Ertapenem.

It is worthy to note that as a matter of urgency, that molecular epidemiology should be included in infection control policy. This would help in early detection of any change in patterns and advent of new resistance genes.

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