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# Carbapenem Resistance among Extended Spectrum Beta-Lactamases Producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from Patents with Urinary Tract Infections in Port-Harcourt, Nigeria.

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## ABSTRACT

Extended Spectrum Beta-Lactamases (ESBLs) producing urinary bacteria with reduced susceptibility to carbapenems are a serious public health problem that is making the treatment of urinary tract infections (UTIs) a difficult task. Thus, this study investigated the prevalence of carbapenem resistance among ESBLs producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from UTIs patients in Port Harcourt, Nigeria. Three hundred non-duplicated urine samples of UTIs patients deposited at the medical laboratory of University of Port Harcourt Teaching Hospital were collected, cultured and screened for the presence of *Escherichia coli* and *Klebsiella pneumoniae* isolates using standard microbiological techniques. The confirmed isolates were screened for phenotypic ESBLs expression and antibiotic susceptibility testing was carried out on the ESBLs positive isolates using Kirby-Bauer disc diffusion method. A total of 64 *E. coli* and 108 *Klebsiella pneumoniae* isolates were recovered and a total 81 (47.1%) of all the isolates expressed ESBLs production. The *E. coli* isolates were found to significantly expressed higher proportion of ESBLs enzymes than the *Klebsiella pneumoniae* (Odds ratio = 1.993, *P* = 0.030). *E. coli* and *Klebsiella pneumoniae* isolates exhibited very high resistance (73-100%) to most of the tested antibiotics and moderate resistance (35-48%) to ertapenem. The prevalence of multi-drug resistance (MDR) among the isolates was 100%. The high prevalence of carbapenem resistance in this study calls for very stringent measures on the prudent use of antibiotics and infection control program through the promotion of hand hygiene in order to reduce the spread of MDR pathogens in our society.

**Keywords**: MDR, *E. coli*, *Klebsiella pneumoniae*, carbapenem resistance, UTIs, Nigeria.

## INTRODUCTION

The increasing emergence of antibiotic resistance in UTIs is a major public health problem that is associated with increasing morbidity, delay discharge from hospital and expensive cost of treatment (Kola *et al.,* 2007; Spellberg *et al.,* 2008). This phenomenon especially in Africa and other developing countries has been attributed to factors such as poverty, unhygienic and overcrowding living conditions with large proportion of uncontrolled antibiotic use in hospitals, veterinary practice and agriculture (McEwen, 2006; Patrick and Hutchinson, 2009). These factors favour the development and transmission of antibiotic resistant organisms by selective antibiotic pressure through the bacterial mechanisms of resistance which include the prevention of access to drug targets, altering the antibiotic targets and the production of antibiotics inactivating enzymes such as beta lactamases and extended spectrum beta lactamases (ESBLs) (Blair *et al.,* 2015). Extended spectrum beta-lactamases are a rapidly evolving group of β-lactamases that are able to hydrolyze third-generation cephalosporins and aztreonam and they are commonly produced by *Klebsiella pneumoniae, Escherichia coli* and other members of the *Enterobacteriaceae* (Turner, 2005; Briongos-Figuero *et al.,* 2012). The ESBLs producing bacteria besides being resistant to the beta-Lactam antibiotics also possess cross-resistance to many important antibiotic groups like fluoroquinolones and aminoglycosides since their resistance determinants are often located on the same transferable plasmids thus, resulting in an extremely limited range of effective agents against these organisms (Paterson and Bonomo, 2005; Canton and Ruiz-Garbajosa, 2011). Thus, Infections due to ESBLs producing bacteria can pose a major threat to life since they are often difficult and expensive to treat; and can invariably lead to delay discharge from hospital (Kola *et al.,* 2007; Melzer and Petersen, 2007). The ESBLs producing *E. coli* are

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commonly implicated in urinary tract infections and are usually resistant to most or all the antibiotics commonly used in the treatment of patients with urinary tract infections; such as trimethoprim, ciprofloxacin, amoxicillin/clavulanic acid, and all cephalosporins (Paterson and Bonomo, 2005; Schmiemann *et al.,* 2010). Carbapenems are a potent class of β-lactams that are much more resistant to β-lactamases (including ESBLs and cephalosporinase) and are considered the last line of effective therapy available for the treatment of multi-drug resistant bacterial infections because of their high wide spectrum of activity (Hawkey and Livermore, 2012; McLaughlin *et al.,* 2013). However, the rapid emergence and dissemination of carbapenem-resistant Gram negative bacteria due to the production of carbapenem-hydrolysing beta-lactamases is most worrisome and has become a worldwide major public health issue since invasive infections with these bacteria are associated with high rates of morbidity and mortality as a result of the reduced clinical therapeutic choices and frequent treatment failure (Papp-Wallace *et al.,* 2011; Nordmann, 2014). Hence, careful detection of these multi-drug resistant bacteria provides a fundamental basis for infection control measures and antimicrobial surveillance systems. Thus, this study investigated carbapenem resistance among the ESBLs producing *Escherichia coli* and *Klebsiella pneumoniae* from UTIs patients in Port Harcourt, Nigeria as a means of providing antimicrobial surveillance information from this region of the country.

## MATERIALS AND METHODS

### Study Design

A cross sectional study was carried out among the patients with urinary tract infections, who deposited their urine samples at the Medical Laboratory Department of the University of Port Harcourt Teaching Hospital (UPTH), Rivers State. The teaching Hospital is a tertiary hospital of 500 bed spaces located at $4^{o}53'58''$N longitude and $6^{o}55'43''$E latitude of Rivers state which renders services to different classes of humans with various kinds of diseases and infections in Rivers State and its neighbouring states. The objective of this study was to determine the antibiotic resistance profile of phenotypically screened ESBLs producing *Escherichia coli* and *Klebsiella pneumoniae* isolates from the urine samples.

### Settings and Ethical Approval

The study involved the collection of 300 aliquot amounts of different urine samples submitted to the Hospital's Laboratory from April to August 2015, with the patients' biographic data like age and gender (excluding their names and addresses) from the Laboratory staff. The study was approved by the ethical committees of Niger Delta University and that of the hospital prior to the commencement of the study.

### Collection of Samples and Bacteriology

Portions of the 300 urine samples deposited by UTIs patients at the Hospital's Laboratory were collected into sterile, dry, universal urine containers in a cold ice packs carrier and transported to the Department of Pharmaceutical Microbiology and Biotechnology laboratory of Niger Delta University, Bayelsa State within three hours of collection for microbial culture. A loopful of each urine sample was streaked for discrete colonies on Cysteine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar (Oxoid, UK) and Eosin Methylene Blue (EMB) agar plates before being incubated at 37 ℃ for 24 h for bacterial growth. The characteristic discrete colonies on the plates were identified using microbiological techniques which include colony morphology on selective media, Gram's stain reaction and biochemical characteristics for *Escherichia coli* and *Klebsiella pneumoniae*.

### Detection of Extended Spectrum Beta-Lactamases (ESBLs) Producing Organisms

All the pure *Escherichia coli* and *Klebsiella pneumoniae* isolates from the urine samples were screened phenotypically for ESBLs production using third generation cephalosporins (Cefotaxime (30 µg) and Ceftazidime (30 µg) as specified by Clinical Laboratory Standard Institute (CLSI, 2014). The standard suspension of each isolate was prepared using its overnight colony culture and used to swab the surface of a dried Mueller Hinton (MH) agar (MAST, UK) plate and the single discs of Cefotaxime (30 µg) and Ceftazidime (30 µg) were placed on the MH agar plate. Resistance of each of the isolates to any of these cephalosporins was determined by the measurement and interpretation of the observed zone diameter of inhibition after incubation at 37 ℃ for 24 hours (CLSI, 2014). The isolates of these bacteria that were resistant to the test cephalosporins were then subjected to combination discs of Cefotaxime/Clavulanic acid, Ceftazidime/Clavulanic acid, single disc of each Cefotaxime and Ceftazidime (MAST, UK) on the same Mueller Hinton agar plates and incubated at 37 ℃ for 24 hours for the detection of ESBLs enzymes. The zone diameter around each disc was measured and if the diameter around Cefotaxime/Clavulanic acid or Ceftazidime/Clavulanic acid was 5 mm or

more greater than the zone diameter around the single disc of Cefotaxime or Ceftazidime respectively, the bacterial isolate is said to be an ESBLs producing organism (CLSI, 2014).

**Antibiotic Susceptibility Testing**

Antibiotic susceptibility tests of all the ESBLs producing *Escherichia coli* and *Klebsiella pneumoniae* isolates were performed with 9 antibiotic discs from MAST, UK, using the modified Kirby-Bauer disc diffusion technique (CLSI, 2014). The freshly prepared standard suspension of each isolate (adjusted to the turbidity of 0.5 McFarland Standard) was used to swab the surface of a dried Mueller Hinton (MH) agar plate and the following Antibiotics discs were placed on the MH agar (in duplicates) after 20 min of inoculation: Ampicillin (AMP 10 µg), Co-trimoxazole (SXT 25 µg), Ciprofloxacin (CIP 5 µg), Levofloxaxin (LEV 5 µg), Gentamicin (CN 10 µg), Ceftazidime (CAZ 30 µg), Cefotaxime (CTX 30 µg), Nitrofurantoin (F 300 µg) and Ertapenem (ETP 10 µg). The inoculated plates with the discs were allowed to stand for at least 30 min before incubated at 37 ºC for 24 hours. The zone diameter of inhibition around each antimicrobial disc was measured and interpreted using the CLSI chart.

**Statistical analysis**

The groups differences were tested using the Chi-square test (or Fisher's exact test when expected frequencies were too low), with the assumed level of statistical significance at a *p*-value of $< 0.05$ while the strength and direction association or relationship was measured using the Phi Coefficient/Cramer's V tests where applicable. Data analysis was performed with SPSS version 15.0 for Windows (SPSS Inc, USA)

**RESULTS**

**Study Population**

A total of 64 *E. coli* and 108 *Klebsiella pneumoniae* isolates were recovered from the 300 urine samples of UTIs patients at the University Teaching Hospital. The isolates were distributed among the patients of 1 - 90 years age group with mean age of 30 years and 65.1% of the isolates were from female.

In total, 81 (47.1%) ESBLs producing isolates were recovered from all the *E. coli* and *Klebsiella pneumoniae* isolates. The *E. coli* isolates were found to significantly expressed higher proportion of ESBLs enzymes than the *Klebsiella pneumoniae* (Odds ratio = 1.993: $\chi^2 = 4.701$; $P = 0.030$) (Table 1).

Table 1: The frequency of ESBLs producers among the UTI enterobacteria

| Urinary Bacteria | No. of Isolates | No. (%) of ESBLs producers | Chi-Square | P -Value |
|---|---|---|---|---|
| *Escherichia coli* | 64 | 37 (57.8) | 4.701 | 0.030* |
| *Klebsiella pneumoniae* | 108 | 44 (40.7) | | |
| TOTAL | 172 | 81 (47.1) | | |

*Statistically significant ($P < 0.05$)

**Antibiotic susceptibility testing of ESBLs producing *E. coli* and *Klebsiella pneumoniae* isolates**

The ESBLs producing isolates of these bacteria exhibited very high resistance to ampicillin, co-trimoxazole, the tested third generation cephalosporins and the fluoroquinolones (73 – 100%) but *E. coli* isolates were moderately resistance to gentamicin and nitrofurantoin (46 – 54%). However, the bacterial resistance to the tested carbapenem antibiotic (ertapenem) was 35 - 48%, hence, ertapenem was the most effective antibiotic (Table 2). The level of carbapenem resistance among the ESBLs producing *E. coli* isolates (35.1%) was lower than that of *Klebsiella pneumoniae* (47.7%) but the difference is not significant. All the isolates were 100% multi-drug resistant (MDR) since they were all resistant to at least one agent in three or more classes of antibiotics tested, as described by Magiorakos *et al*. (2012).

**DISCUSSION**

Our study revealed a prevalence of 37 (57.8%) ESBLs producing *E. coli* of all the *E. coli* isolates screened and 44 (40.7%) ESBLs producing *K. pneumoniae* of all the *Klebsiella pneumoniae* isolates screened. The ESBLs enzymes was significantly expressed among the *E. coli* than the *K. pneumoniae* isolates ($P = 0.03$) with Odds ratio of 1.993 which signifies that the *E. coli* in this study was prone to expressing the ESBLs enzymes twice as much as the *K. pneumoniae*. This tends to show the higher rate of resistant genes acquisition capacity of *E. coli* over *K. pneumoniae* (Nataro and Kaper, 1998).

Table 2: Antibiotic resistance pattern of ESBLs Producing *E. coli* and *Klebsiella pneumoniae* isolates

| Antibiotics | Number of Resistant Isolates (%) | |
|---|---|---|
| | *E. coli* N = 37 | *Klebsiella pneumoniae* N = 44 |
| Ampicillin | 37 (100) | 44 (100) |
| Cefotaxime | 33 (89.2) | 44 (100) |
| Ceftazidime | 30 (81.1) | 38 (86.4) |
| Co-trimoxazole | 36 (97.3) | 44 (100) |
| Gentamicin | 20 (54.1) | 39 (88.6) |
| Nitrofurantoin | 17 (45.9) | 43 (97.7) |
| Ciprofloxacin | 27 (73.0) | 37 (84.1) |
| Levofloxacin | 27 (73.0) | 33 (75.0) |
| Ertapenem | 13 (35.1) | 21 (47.7) |

This observation however, disagrees with the findings of previous studies which reported higher prevalence of ESBLs production among *K. pneumoniae* isolates (Chander and Shrestha, 2013).

The phenotypic expression of ESBLs enzymes by *E. coli* isolates (57.8%) in this study is higher than previous similar studies of Ogbolu *et al.* (2011) in Osogbo, South-West Nigeria (25%), Yusuf *et al.* (2013) in Kano, North-West Nigeria (19.3%), Akanbi *et al.* (2013) in Abuja, North-Central Nigeria (31.3%), Anago *et al.* (2015) in Cotonou of Benin Republic (25%) and Yadav and Prakash (2017) in Nepal (35%). However, our finding is similar to the reports of Ogefere *et al.* (2015) in Benin, South-South Nigeria (44.4%), Muhammad and Swedan (2015) in Jordan (54%), Perez *et al.* (2007) in Turkey (54.7–61%) and Hawkey, (2008) in India (66.7%). The observed differences might be due to regional attitudinal behaviour towards consumption of antibiotics especially the cephalosporins in both hospital and community settings. However, the results in the various regions of the world tend support the fact that ESBLs producing *E. coli* in urinary tract infections is a worldwide problem which varies by countries and regions within a country (Coque *et al.*, 2008). There have been varied reports of prevalence of ESBLs producing *K. pneumoniae* in clinical specimens worldwide. In Nigeria, prevalence of 7.6% *K. pneumoniae* was reported by Ejikeugwu *et al.* (2012) in Awka (East), 27% by Faari *et al.* (2015) in Ilorin (West) and 25% by Yusha *et al.* (2007) in Kano (North) which are lower than our findings (40.7%) in Port-Harcourt (South South). Other similar studies on ESBLs producing *K. pneumoniae* isolates reports in South Africa (51%) Habte *et al.* (2009), Pakistan (58.7%) Chlebicki *et al.* (2004) and India (53%) Rodrigues *et al.* (2004) are higher than our finding. However, lower prevalences were reported by Chong *et al.* (2011) in Japan (4.9%), Kumar *et al.* (2006) in India (19.8%), Romero *et al.* (2007) in Spain (20.8%), Ahmed *et al.* (2013) in Egypt (21%) and El *Bouamri et al.* (2015) in Morocco (25.5%). The observed differences reflect the burden of antibiotic use in different geographical regions across the globe at different periods of time since the microbial selective pressure to massive prescription and misuse of broad spectrum antibiotics in both hospital and community settings usually result in the rapidly rising prevalence of ESBLs production among *Enterobacteriaceae*. Thus, there is a high tendency of experiencing an increasing rapid change of this prevalence overtime due the increasing spread of these multi-drug resistant strains except strict urgent measures are instituted especially in developing countries to control the inappropriate massive use of antibiotics.

The ESBLs producing *E. coli* and *K. pneumoniae* in this study had very high resistance (73 -100%) to ampicillin, ceftazidime, cefotaxime, co-trimoxazole, ciprofloxacin and levofloxaxin. The *E. coli* isolates had moderate resistance to gentamicin (54.1%), nitrofurantoin (45.9%) and ertapenem (35.1%) while the resistance exhibited by the *K. pneumoniae* isolates to gentamicin, nitrofurantoin and ertapenem was 88.6%, 97.7% and 47.7% respectively. The very high resistance (81-100%) of these ESBLs producing uropathogens to ampicillin and the tested cephalosporins observed in this study is widely reported in Nigeria and other parts of the world as expected since ESBLs production in Gram negative bacteria is a key factor that confer resistance to beta lactam antibiotics except cephamycins and carbapenems (Ejikeugwu *et al.*, 2012; Igbinoba and Osazuwa, 2012; Okesola and Fowotade, 2012; Briongos-Figuero *et al.*, 2012; Ahmed *et al.*, 2013; El Bouamri *et al.*, 2015; Chander and Shrestha, 2013). The almost 100% resistance exhibited by these bacteria to co-trimoxazole which is also a common

report by similar studies might be due to its high frequent use for the treatment of community acquired UTI being an old antibacterial used as a first-line treatment of uncomplicated UTI. This study's observation therefore suggests that co-trimoxazole should no longer be considered for the treatment of uncomplicated UTI (El Bouamri *et al.,* 2015).

Furthermore, ESBLs producing bacteria have also been reported to exhibit cross resistances to many other commonly used antibiotics which is being reflected in our study (Canton and Ruiz-Garbajosa, 2011). The high resistance (45-73%) of the ESBLs producing *E. coli* and (75-97.8%) of ESBLs producing *Klebsiella pneumoniae* to gentamicin, ciprofloxacin and nitrofurantoin in this study pose a significant clinical failure in the treatment of urinary tract infections with these commonly used antibiotics which tends to further narrow the choice of antimicrobial agents in the effective treatment of UTIs caused by ESBLs producing bacteria (Paterson *et al.,* 2001). This observed resistance of these ESBLs producing uropathogens is similar to resistance rate reported in Nigeria and other developing countries of the world where antibiotics purchase and use are not restricted (Ejikeugwu *et al.,* 2012; Ahmed *et al.,* 2013; El *Bouamri et al.,* 2015; Chander and Shrestha, 2013; Zorgani *et al.,* 2017). The prevalence of multidrug resistance among the ESBLs producing uropathogens in this study is 100%. This observed result is similar to the findings of Eshetie *et al.* (2015) in Ethiopia who reported MDR in 87.4% of *K. pneumoniae* and *E. coli* UTIs isolates, Chander and Shrestha (2013) in Nepal who reported MDR of 91.66% and 87.5% among ESBLs producing *E. coli* and *Klebsiella pneumoniae* urinary isolates respectively and Wemambu *et al.* (2016) reported 81.2% of ESBLs multidrug resistant uropathogenic *E. coli* in Ota, South Western Nigeria. The report of Giwa *et al.* (2018) of MDR in UTIs *E. coli* (50%) and *K. pneumoniae* (40%) in Zaria, Northwestern Nigeria is however in variance with our findings. The implication of these findings tend to leave the clinicians with an extremely limited range of effective agents against these organisms thereby posing a serious therapeutic challenge to both clinicians and clinical microbiologists (Paterson and Bonomo, 2005; Canton and Ruiz-Garbajosa, 2011). However, ertapenem was the most effective antibiotics against the ESBLs producing *E. coli* and *Klebsiella pneumoniae* isolates in this study, though with an alarming high proportions of resistance of 35% and 47.7% to *E. coli* and *Klebsiella pneumoniae* isolates respectively. These resistance rates of these bacteria is alarming high because this agent is the last resort in the treatment of multidrug resistant ESBLs producing bacteria and increasing resistance to this agent might lead us to a post antibiotic era where infections that were easily treated in the past will now be causing the death of infected patients (Overturf, 2010; Ejikeugwu *et al.,* 2012). In the past few years, Ejikeugwu *et al.* (2012) and Igbinoba and Osazuwa (2012) in Nigeria reported total susceptibility of ESBLs producing *E. coli* and *Klebsiella pneumoniae* isolates respectively to imipenem and meropenem. However, carbapenem resistance has been reported widely as an increasing public health problem limiting the treatment of life threatening infectious diseases caused by ESBLs producing bacteria (Garbati et al., 2016; Buehrle *et al.,* 2017; Pang *et al.,* 2018; ECDPC, 2018). The reports of Ahmed *et al.* (2013) in Egypt and El *Bouamri et al.* (2015) in Morocco showed ESBLs producing *Klebsiella pneumoniae* to exhibit 33.3% and 7% resistance to ertapenem respectively while those of Muhammad and Swedan (2015) in Jordan and Zorgani *et al.* (2017) in Libya showed ESBLs producing *E. coli* to exhibit 13% and 2.8% resistance to ertapenem respectively. The higher prevalence of carbapenem resistant ESBLs producing uropathogens observed in this study might be due to the increasing rate at which these organisms acquire resistance to available antibiotics due to high volume of antibiotic use in both human and agricultural activities and this call for more frequent surveillance of MDR pathogens especially the molecular epidemiological studies of their resistance genes which will help to provide details on the bacterial clones circulating in our hospitals and community in order to frequently adopt strategies for their control and spread in our society.

## CONCLUSION

This study reports high phenotypically expressed ESBLs enzymes among the UTIs' most prominent bacteria which exhibited total multidrug resistance to commonly used antibiotics. These bacteria also exhibited high ertapenem resistance, a condition that predicts the treatment of UTIs with very limited options of antibiotics. Thus, this situation demands very stringent measures on the prudent use of antibiotics in both community and hospital settings, infection control policies and prevention program through the promotion of regular hand hygiene in order to reduce the spread of these MDR pathogens in our society.

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