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*Full Length Research Paper*

# Imipenem and meropenem resistance amongst ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates

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**Abstract**

**This study was undertaken to evaluate the occurrence of imipenem and meropenem resistance amongst *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates producing extended spectrum β-lactamase enzymes. Seventy nine isolates of *E. coli* (n=40) and *K. pneumoniae* (n=39) were analyzed and identified by standard microbiology techniques. Antibiogram was evaluated by the disk diffusion method as per clinical laboratory standard institute guidelines. Imipenem and meropenem, followed by amoxicillin-clavulanic acid were the most potent antibiotics against the isolates. Extended spectrum β-lactamase production was determined by double disk synergy test in 3.8% *E. coli* and 7.6% *K. pneumoniae* strains. All *E. coli* strains expressing extended spectrum β-lactamase were entirely susceptible to imipenem and meropenem. Also, four out of the 6 *K. pneumoniae* strains that expressed extended spectrum β-lactamase were entirely susceptible to imipenem and meropenem but 2 strains remained completely resistant, and were confirmed to produce metallo-β-lactamase enzymes. Our study shows occurrence of *E. coli* and *K. pneumoniae* isolates expressing extended spectrum β-lactamase, and complete resistance of 2 *K. pneumoniae* strains producing extended spectrum β-lactamase to imipenem and meropenem. We recommend prompt and accurate detection of carbapenem resistant bacteria from clinical specimens in order to contain antibiotic resistance in our environment.**

**Keywords:** Carbapenems, *Enterobacteriaceae*, Antimicrobial Susceptibility, Gram negative bacteria, Nigeria.

## INTRODUCTION

Carbapenems are a group of fused-β-lactam antibiotics (with wide spectrum antibacterial activity) that are used for the treatment of infections caused by multidrug resistant (MDR) Gram negative bacteria including those that produce extended spectrum β-lactamase (ESBL) enzymes (Walsh *et al*., 2005; Chakraborty *et al*., 2010). Gram negative bacteria that are resistant to the carbapenems due to their inherent production of carbapenemases (carbapenem-hydrolyzing enzymes) have been increasingly reported in some parts of the world (Chakraborty *et al*., 2010; Walsh *et al*., 2005; Bashir *et al*., 2011; Saderi *et al*., 2008). Typical examples of carbapenems are imipenem, meropenem, ertapenem and doripenem. They are the most potent agents for the treatment of ESBL infections and other MDR Gram negative infections (Overturf, 2010; Igbinoba *et al*., 2012). Nonetheless, the prevalence of bacterial resistance to the carbapenems is gradually on the rise (Chakraborty *et al*., 2010; Yoshichika *et al*., 2000; Bashir *et al*., 2011; Ghibu *et al*., 2011). This phenomenon puts to threat the clinical effectiveness of these agents in treating MDR infections including those caused by ESBL producing bacteria if nothing is done to curtail it. Resistance gene can be acquired by a pathogen via horizontal gene transfer from one organism to another (e.g. conjugation) or through

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mutation, and a bacterium becomes multiply resistant when it undergoes mutation or acquires multiple resistance genes; though some organisms can be inherently resistant to some class of antibiotics (Dzidic *et al*., 2008; Chroma *et al*., 2010). However, the healthcare system has encountered plethora of bacterial resistance to some frontline antimicrobial agents (e.g. penicillins and co-trimoxazole) in recent times, and these organisms can spread in health institution from one patient to another and even to the community. ESBLs are plasmid-mediated β-lactamase enzymes capable of hydrolyzing many β-lactam antibiotics including 3rd generation cephalosporins and monobactams but are yet inhibited by clavulanic acid (Bonnet, 2004). Organisms producing ESBLs were first reported in the early 1980s, but they are now found worldwide especially amongst *Enterobacteriaceae* (e.g. *E. coli*, *K. pneumoniae* and *K. oxytoca*) in both the community and hospital settings (Jacoby *et al*., 1996). Risk factors for the acquisition of ESBL producing bacteria include long hospitalization, prolonged antibiotic usage (especially 3rd generation cephalosporins), use of central venous catheterization, surgical experience and exposure to nosocomial isolates. Over 150 different ESBLs have been so far characterized, and they are found worldwide with increasing morbidity and mortality rates (Bradford, 2001; Jacoby *et al*., 1996). The introduction of carbapenems into clinical practice was largely heralded as a major breakthrough in the fight against ESBL infections and MDR bacterial infections owing to the fact that this class of antibiotics is stable to ESBLs and other forms of beta-lactamase enzymes produced by MDR Gram negative bacilli even Gram positive bacteria. But the good qualities of these agents are now being threatened following the production of enzymes (carbapenemases) that hydrolyze the carbapenems (Franklin *et al*., 2006; Walsh *et al*., 2005 and Lo, 2011). The indiscriminate use of antimicrobial agents amongst many other factors is the number one reason there is for the emergence and spread of antibiotic resistant pathogens in the community and hospital environment (Chroma *et* al., 2010; Pitout *et al*., 2008 and Jacoby *et al*., 1996). In view of the invaluable place of the carbapenems in treating ESBL infections and reported resistance of pathogens to these agents, the present day study was undertaken to determine the occurrence of imipenem and meropenem resistance amongst ESBL positive *E. coli* and *K. pneumoniae* strains in Enugu, Nigeria so as to proffer appropriate measures for their sustained and rational usage in Nigeria's health institutions.

## MATERIALS AND METHODS

### Microorganisms

Seventy nine (79) non-duplicate clinical isolates comprising of *E. coli* (n=40) and *K. pneumoniae* (n=39) were isolated from urine specimens of both in-patients and out-patients of a Nigerian hospital from November 2011 to July 2012. All isolates were identified by standard microbiology identification techniques (Cheesbrough, 2000).

### Susceptibility studies

Antibiogram was evaluated on all the test isolates using the Kirby-Bauer disk diffusion method as per the clinical laboratory standard institute, CLSI guidelines (CLSI, 2010). The antibiotics used were sulphamethoxazole-trimethoprim (SXT-25 µg), ciprofloxacin (CIP-5 µg), ofloxacin (OFX- 5 µg), cefotaxime (CTX- 30 µg), ceftazidime (CAZ- 30 µg), amoxicillin-clavulanic acid (AMC- 30 µg), imipenem (IPM- 10 µg), meropenem (MEM- 10 µg), gentamicin (CN- 10 µg), amikacin (AK- 30 µg) and cefepime (FEP- 30 µg) (Oxoid, UK).

### Detection of extended spectrum-β-lactamase (ESBL) enzymes

ESBL expression was detected phenotypically by the double disk synergy test (DDST) method (Ullah *et al*., 2009). Isolates that showed reduced susceptibilities to any of the cephalosporins (CTX and CAZ) were confirmed for ESBL production by the DDST method. Amoxicillin-clavulanic acid (30 µg) disk was centrally placed on a Mueller-Hinton (MH) agar plate that has been swabbed with the test isolate(s). Disks of CTX and CAZ were each placed 15 mm apart from the central disk, and the plates were incubated at 37°C overnight. A difference of ≥5 mm in zone diameter between CAZ and CTX when tested alone and in combination with AMC confirms ESBL production phenotypically (Ullah *et al*., 2009).

### Detection of metallo-β-lactamase

Metallo-β-lactamase (MBL) enzyme was detected phenotypically according to a previously described method (Bashir *et al*., 2011) in only strains that showed reduced susceptibility to any of the carbapenems (imipenem and meropenem). Overnight cultures of the test strains (adjusted to 0.5 McFarland turbidity standards) were inoculated on MH agar plates. One imipenem and one ceftazidime disks with and without ethylene diamine tetra-acetic acid (EDTA) were each placed 25mm apart on the MH agar plates and the plates were incubated at 37°C for 24hrs. An increase of ≥7mm in the zone size of imipenem or ceftazidime disks compared to imipenem-EDTA disk or ceftazidime-EDTA disk was confirmed as a metallo-β-lactamase positive organism.

**Control organisms**

*Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 (Oxoid, UK) were used as positive control strains for antimicrobial susceptibility studies.

## RESULTS

All 79 isolates were screened for susceptibility to a range of frontline antibiotics including sulphamethoxazole-trimethoprim, ciprofloxacin, ofloxacin, gentamicin, ceftazidime, cefotaxime, cefepime, amoxicillin-clavulanic acid, imipenem, meropenem and amikacin. Our result shows that sulphamethoxazole-trimethoprim had the lowest antibacterial activity with a susceptibility rate of 2.5% and 10.3% for *E. coli* and *K. pneumoniae* respectively (Table 1). However, ciprofloxacin, cefepime, ceftazidime, ofloxacin, and amikacin also had a low activity against *E. coli* strains (35%, 42.5%, 42.5%, 35% and 45% respectively) and *K. pneumoniae* strains (41%, 23.1%, 48.7%, 43.6% and 28.2% respectively).

Imipenem and meropenem were the most effective antibiotics in terms of activity against *E. coli* (92.5% and 95% respectively) and *K. pneumoniae* (87.2% and 87.2 % respectively). This was followed by gentamicin (*E. coli* 72.5% and *K. pneumoniae* 69.2%), amoxicillin-clavulanic acid (*E. coli* 70% and *K. pneumoniae* 84.6%) and cefotaxime (*E. coli* 50% and *K. pneumoniae* 46.2%) (Table 1).

ESBL production was phenotypically evaluated by double disk synergy test (DDST) and the result is as shown in Table 2. Overall, ESBL was expressed in 11.4% strains. 37 (46.8%) *E. coli* and 33 (41.8%) *K. pneumoniae* isolates were confirmed as non-ESBL producers (Table 2). All the ESBL positive *E. coli* isolates were completely susceptible to imipenem and meropenem (Table 3). Only 4 out of the 6 ESBL positive *K. pneumoniae* strains were completely susceptible to imipenem and meropenem (Table 4). The two *K. pneumoniae* strains that were resistant to imipenem and meropenem were confirmed to produce metallo-β-lactamase (MBL) enzymes phenotypically.

## DISCUSSION

The worrisome trend in the emergence and spread of multidrug resistant bacteria in both the community and hospital settings is disturbing, and it is a global threat which is gradually reducing the efficacy of available antibiotics used for the treatment of infectious diseases. ESBL production is a key factor which is responsible for the resistance of pathogenic bacteria to extended spectrum antibiotics (especially the 3$^{rd}$ generation cephalosporins), and they pose tremendous problems to affected patients while creating real challenge for both the physicians and clinical microbiologists who should prescribe drugs appropriately and accurately detect these enzymes respectively. In this age of decreased susceptibilities of pathogenic microorganisms to readily available drugs as have been widely reported (Bonnet, 2004; Varghese *et al*., 2010; Jyothsna *et al*., 2011; Eze, 2012; Ullah *et al*., 2009; Ghibu., 2011), coupled with the use of antibiotics for non-human purposes (e.g. in poultry and animal husbandry) and the slow pace in the development of new antimicrobials, there is need for proper utilization of available antibiotics and accurate detection and reporting of antibiotic resistance in order to keep multidrug resistant bacteria (MDR) under control and consolidate on the gains of antimicrobial agents in the treatment of infectious diseases since their discovery in the 1920's. Table 1 showed the antimicrobial susceptibility profile of all the test bacteria. Our study shows a high frequency of resistance in the *E. coli* and *K. pneumoniae* isolates to some of the tested antibiotics (amikacin, ofloxacin, ceftazidime, cefotaxime, cefepime, ciprofloxacin and sulphamethoxazole-trimethoprim) (Table 1). Interestingly, a very high rate of resistance was recorded for sulphamethoxazole-trimethoprim against our *E. coli* (97.5%) and *K. pneumoniae* (89.7%) isolates, and this was followed by cefepime (*E. coli* 57.5 and *K. pneumoniae* 76.9%), amikacin (*E. coli* 55% and *K. pneumoniae* 71.8%), ciprofloxacin (*E. coli* 65% and *K. pneumoniae* 59%), ofloxacin (*E. coli* 65% and *K. pneumoniae* 56.4%), cefotaxime (*E. coli* 50% and *K. pneumoniae* 53.8%), and ceftazidime (*E. coli* 57.5 and *K. pneumoniae* 51.3%). Previous studies by Ullah *et al*. (2009), Eze (2012) and Sanjay *et al*. (2010) also reported high prevalence of resistance of *E. coli* and *K. pneumoniae* strains to sulphamethoxazole-trimethoprim, ciprofloxacin, cefepime, cefotaxime, ceftazidime, and amikacin. Nevertheless, the carbapenems (imipenem and meropenem) were the most potent and effective antibiotics against the *E. coli* and *K. pneumoniae* strains used in our study (Table 1). This was followed by amoxicillin-clavulanic acid and gentamicin which showed moderate activity (Table 1). Different studies (Spanu *et al*., 2002; Jyothsna *et al*., 2011 and Eze, 2012) have also reported the good antimicrobial activity of the carbapenems on Gram negative bacteria (as envisaged in our study). ESBL was detected in 3 (3.8%) *E. coli* and 6 (7.6%) *K. pneumoniae* strains (Table 2) and this is notably substantial – owing to the clinical significance of bacterial organisms harbouring ESBLs. ESBL producing bacteria is at the moment considered to be a health risk amongst hospitalized patients worldwide (Bradford, 2001) due to their exceptional ability to hydrolyze and cause resistance to the 3$^{rd}$ generation cephalosporins, and their prevalence is increasing gradually in both hospital and community settings (Jacoby *et al*., 1996; Bradford, 2001 and Bonnet, 2004). In Pakistan and Singapore, ESBL production was reported to be 58.7% and 44% in *K. pneumoniae* isolates respectively (Chlebicki *et al*., 2004

**Table 1.** Antimicrobial susceptibility pattern of test isolates

| Antibiotics (µg) | % susceptibility *Escherichia coli* (n=40) | | % susceptibility *Klebsiella pneumoniae* (n=39) | |
|---|---|---|---|---|
| | Resistant | Susceptible | Resistant | Susceptible |
| SXT (25) | 39 (97.5) | 1 (2.5) | 35 (89.7) | 4 (10.3) |
| CIP (5) | 26 (65) | 14 (35) | 23 (59) | 16 (41) |
| FEP (30) | 23 (57.5) | 17 (42.5) | 30 (76.9) | 9 (23.1) |
| CTX (30) | 20 (50) | 20 (50) | 21 (53.8) | 18 (46.2) |
| CAZ (30) | 23 (57.5) | 17 (42.5) | 20 (51.3) | 19 (48.7) |
| AMC(30) | 12 (30) | 28 (70) | 6 (15.4) | 33 (84.6) |
| OFX (5) | 26 (65) | 14 (35) | 22 (56.4) | 17 (43.6) |
| AK (30) | 22 (55) | 18 (45) | 28 (71.8) | 11 (28.2) |
| IPM (10) | 3 (7.5) | 37 (92.5) | 5 (12.8) | 34 (87.2) |
| MEM (10) | 2 (5) | 38 (95) | 5 (12.8) | 34 (87.2) |
| CN (10) | 11 (27.5) | 29 (72.5) | 12 (30.8) | 27 (69.2) |

**Key:** SXT=sulphamethoxazole-trimethoprim, CIP=ciprofloxacin, FEP=cefepime, CTX=cefotaxime, CAZ=ceftazidime, AMC=amoxicillin-clavulanic acid, OFX=ofloxacin, AK=amikacin, IPM=imipenem, MEM=meropenem, CN=gentamicin

**Table 2**. Frequency of ESBL production

| Bacteria | ESBL | Non-ESBL |
|---|---|---|
| *Escherichia coli* (n=40) | 3 (3.8%) | 37 (46.8%) |
| *Klebsiella pneumoniae* (n=39) | 6 (7.6%) | 33 (41.8%) |
| Total | 9 (11.4%) | 70 (88.6%) |

**Key:** ESBL=extended spectrum beta-lactamase

**Table 3.** Zone of inhibition of ESBL positive *E. coli* to imipenem (10 µg) and meropenem (10 µg)

| Isolate No | Imipenem (10 µg) Inhibition zone | Meropenem (10 µg) diameter (mm) |
|---|---|---|
| E44 | 26 | 25 |
| E60 | 30 | 31 |
| E69 | 30 | 30 |

and Ullah *et al*., 2009), a result higher than ours (Table 2). Iroha *et al.* (2010) here in Nigeria also reported higher prevalence of ESBL production in *E. coli* isolates (56.6%). Nevertheless, our result of ESBL production in E. *coli* (3.8%) and K. *pneumoniae* (7.6%) strains is consistent with that of a similar work conducted in Italy (Spanu *et al*., 2002). Antibiotic resistance mediated by ESBLs causes increased morbidity, prolonged illness, a greater risk of complications in affected patients and higher mortality rates (Pitout *et al*., 2008 and Bradford, 2001). The susceptibility of our ESBL positive *E. coli* and *K. pneumoniae* strains to imipenem and meropenem was evaluated in the current study. Our results showed that all the ESBL positive *E. coli* strains were remarkably susceptible to imipenem and meropenem (Table 3). This result however, confirm the claims that the carbapenems (e.g. imipenem and meropenem) are the best treatment options for multidrug resistant (MDR) bacterial infections, and also the drugs of last resort for ESBL producing organisms which are increasingly resistant to cephalosporins, sulphamethoxazole-trimethoprim, the fluoroquinolones, and the aminoglycosides (Overturf, 2010; Walsh *et al*., 2005; Franklin *et al*., 2006). On the other hand, the complete susceptibility of ESBL positive *E. coli* strains to imipenem and meropenem (as is envisaged in our study: Table 3) is also in line with a recent study conducted in Lagos, Nigeria which showed 80% and 100% activities of ESBL positive *E. coli* strains to imipenem and meropenem respectively (Igbinoba *et al*., 2012; Okesola *et al*., 2012). Table 4 shows the

**Table 4.** Zone of inhibition of ESBL positive *K. pneumoniae* to imipenem (10 µg) and meropenem (10 µg)

| Isolate No | Imipenem (10 µg) Inhibition zone | Meropenem (10 µg) diameter (mm) |
|---|---|---|
| K28 | 27 | 28 |
| K57 | 28 | 19 |
| K58 | 25 | 28 |
| K47 | 26 | 23 |
| K51 | — | — |
| K52 | — | — |

—= No zone of inhibition

inhibition zone diameter of ESBL positive *K. pneumoniae* strains to imipenem and meropenem. Out of the 6 ESBL positive *K. pneumoniae* strains, only 4 strains showed complete susceptibility to both imipenem and meropenem. Two ESBL positive *K. pneumoniae* strains (with isolate Nos: K51 and K52) showed no zone of inhibition to imipenem and meropenem (Table 4), and these were confirmed to produce metallo-β-lactamase (MBL) enzymes phenotypically. However, this result is in contrast to a similar work conducted in Abuja, Nigeria which showed a 100% susceptibility of ESBL positive *K. pneumoniae* strains to imipenem and ertapenem (Igbinoba *et al*., 2012). However, our result of no zones of inhibition of ESBL positive *K. pneumoniae* strains to imipenem and meropenem (Table 4) is in line with similar works done in India and Iran (Varaiya *et al*., 2008; Saderi *et al*., 2008) where incidences of carbapenem-hydrolyzing enzymes amongst *Enterobacteriaceae* has been reported. Carbapenems are used to treat life threatening infections caused by MDR bacterial pathogens, and antibiotics in this class represent the last line of therapy in treatment options against very serious infections such as those caused by ESBLs. However, carbapenem-resistance has also been reported elsewhere as an increasing public health problem that should be dealt with holistically (Walsh *et al*., 2005; Chakraborty *et al*., 2010; Bashir *et* al., 2011 and Saderi *et al*., 2008). The resistance of ESBL producing *Enterobacteriaceae* (including *E. coli* and *K. pneumoniae*) to carbapenems (as envisaged in our study) is worrisome and of clinical and microbiology importance because such pathogens are usually resistant to a host of beta-lactam antibiotics and they may also carry genes that confer on them co-resistance to non-beta-lactam antibiotics as well. This leaves very little or no options for treating MDR infections including those caused by ESBL producing pathogens. Walsh *et al*., (2005) also opined that Gram negative bacteria have plethora of resistance mechanisms that they use to evade the actions of carbapenems and other beta-lactam antibiotics. Antimicrobial resistance (especially to expanded spectrum antibiotics) is without doubt one of the most lethal problems faced by the health sectors since some available antibiotics are no longer effective in the treatment of infectious diseases. Owing to the fact that the carbapenems are actually the last line of defense against drug resistant infections (Ullah *et al*., 2009; Varghese *et al*., 2010; Ghibu *et al*., 2011), it is vital that the detection and reporting of MDR bacterial infections be given utmost attention in our tertiary hospitals so as to bring this scourge under control.

## CONCLUSION

Our study has shown high rate of resistance of *E. coli* and *K. pneumoniae* isolates in our environment to some front line antibiotics. In addition, our results also showed zero zones of inhibition of 2 ESBL positive *K. pneumoniae* strains to imipenem and meropenem, and the production of metallo-β-lactamase (MBL) enzymes by these strains. Owing to the relevance of imipenem and meropenem in the treatment of ESBL infections and other multidrug resistant Gram negative infections, it is crucial to monitor closely the changes in susceptibility patterns of *Enterobacteriaceae* through detection and surveillance in both the community and hospital settings. Stringent measures including prompt and accurate detection methods for ESBLs and MBLs, adequate infection control measures and a review of antibiotic guidelines should be introduced in Nigerian hospitals so as to assess the burden of antibiotic resistance and contain their possible emergence and spread. Our study further buttresses the need for the establishment of an “antimicrobial resistance detection and monitoring reference laboratory” across Nigeria so that antibiotic resistance cases can be properly detected, reported and contained.

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