Detection, Inhibition and Molecular Analysis of Multidrug Resistant Aerobic Gram-negative Clinical Isolates from a Tertiary Hospital in Nigeria

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ABSTRACT
The challenge of combating the ever emerging multi-drug resistant (MDR) clinical isolates in the face of a slow rate of discovery of new classes of antibiotics is a problem in antibiotic chemotherapy. This study was aimed at (i) linking phenotypic antibiotic drug-resistance characteristics detected in randomly-sampled clinical isolates with detectable genetic markers. (ii) screening a suspected efflux pump inhibitor (EPI) [1-(3-(trifluoromethyl)benzyl)-piperazine (TFMBP)], which could be helpful in combating this challenge. Fifty-one isolates; 28 Klebsiella pneumonia, 3 E. Coli, 1 Enterobacter cloacae, 1 E. aerogenes, 5 Proteus mirabilis, 4 Providencia rettgeri, 1 P. stuartii, 1 Serratia liquefaciens, 6 S. odorifera, and 1 Acinetobacter baumannii obtained from infections of urinary tract, upper respiratory tract, gastrointestinal tract, ear swab, eye swab, and blood cultured were screened for (i) antibiotic-susceptibility over a range of 11 classes of antibiotics, (ii) β-lactamase production, (iii) ESBL production and (iv) Efflux pump activity (EPA) in the presence and absence of 1-[3-(trifluoromethyl) benzyl]-piperazine (TFMBP). Molecular analysis was done using DNA extraction by boiling and the randomly-amplified polymorphic DNA (RAPD) polymerase chain reaction (PCR) procedure with 2% agarose gel electrophoresis stained with ethidium bromide at 10 µg/ml and visualized by UV trans-illumination. AmpC β-lactamase (4%) and K1 β-lactamase (5.8%) were detected with no carbapenemase producers. AcrA and AcrB marker genes were detected in 12% of the isolates while blaCTX-M (8%) and blaTEM (4%) were also detected. Antibiotic resistance due to EPA can be combated with a suitable EPI as demonstrated by TFMBP when combined with specific antibiotics.

Keywords: TFMBP, Efflux Pump Activity, ESBL, MDR, Carbapenemase

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Received: August, 2017; Accepted: November, 2017

Abstracted by: Bioline International, African Journals online (AJOL), Index Copernicus, African Index Medicus (WHO), Excerpta medica (EMBASE), CAB Abstracts, SCOPUS, Global Health Abstracts, Asian Science Index, Index Veterinarius

INTRODUCTION
With the global emergence of various forms of ESBLs and MDR bacteria at a rate much faster than the rate at which new drug molecules are being developed, a molecular analysis of the drug resistance characteristics of randomly-sampled bacterial isolates, from patients attending Lagos University Teaching Hospital (LUTH) is imperative. The methods of PCR and DNA sequencing offer the promise of increased sensitivity, specificity and speed in the detection of specific known resistance genes (Perreten et al., 2005.). The level and extent of involvement of EPA in the multidrug resistance patterns observed among the isolates was demonstrated by use of trfluoro-methyl benzyl piperazine TFMBP as the efflux pump inhibitor (EPI). Molecular analysis of ESBL and efflux pump types present in the isolates was carried out by determination of genetic markers. This study gives an insight into possible solution to the challenge of emergent EPA among MDR strains from LUTH through the incorporation of suitable EPIs into antibiotic formulations for treatment of infected patients.

MATERIALS AND METHODS
The 51 isolates used in this study were collected from the Medical microbiology laboratories of LUTH. These include
28 Klebsiella pneumonia, three Escherichia Coli, one Enterobacter cloacae, one E. aerogenes, five Proteus mirabilis, four Providencia rettgeri, one P. stuartii, one Serratia liquefaciens, six S. odorifera, and one Acinetobacter baumannii isolates.

The isolates were identified with the analytical profile index (API) and the analytical profile index web (APIWeb), E. coli (ATCC 25922) and K. pneumoniae- β-lactamase-producing (ATCC 700603) were included at every stage for quality control. All isolates were identified with the analytical profile index (API) and the analytical profile index web (APIWeb). The isolates were included at every stage for quality control. All isolates were identified with the analytical profile index (API) and the analytical profile index web (APIWeb). The isolates were included at every stage for quality control. All isolates were identified with the analytical profile index (API) and the analytical profile index web (APIWeb).

The antibiotic discs used included ampicillin-10 µg (Amp-10), penicillinG-10 µg (P-10), ceftriaxone-30 µg (CRO-30), ciprofloxacin-5 µg (CIP-5), tetracycline-30 µg (Te-30), cotrimoxazole-1.25 µg+23.75 µg(SXT-1.25/23.75), neomycin-30 µg (N-30), erythromycin-15 µg (E – 15), polymyxin-B-300 i.u. (PB-300), lincomycin-2 µg (L-2), chloramphenicol-30 µg (C-30) and bacitracin-10 µg (B-10). Zones of inhibition obtained were compared and interpreted as recommended (CLSI document M100-S20, 2010). All strains found to be either resistant or intermediate to CRO-30 were regarded as potential ESBL producers, while all strains resistant to more than three classes of antibiotics were classified as MDR. These were picked for further study.

**β-lactamase Assay:** Fifty-one strains found showing some level of resistance to the β-lactam antibiotics were further tested for the production of β-lactamase enzyme by a nitrocefin (NF) assay (Livermore and Brown, 2001). The tests were performed as follows. In the blank assay, 0.25 ml sterile MHB and 0.25 ml of NF assay solution was added. A colour change from yellow to red in the reaction solution within 10 min was regarded as potential ESBL producers, while all strains resistant to more than three classes of antibiotics were classified as MDR. These were picked for further study.

Strains showing the colour change after 10 min were considered as inducible β-lactamase producers as suggested by Livermore and Brown (Livermore and Brown, 2001).

**A Modified Double Disc Synergy Test Method:** A ten-disc procedure was carried out on the suspected ESBL producers in sterile normal saline (equiv. to 0.5 McFarland standard) from overnight MHB cultures, as recommended by the Centre for Disease Control (CDC) and CLSI (CLSI) (CLSI document M100-S20-U, 2010). The disks used were CRO-30, cefotaxime-30 µg/clavulanate-10 µg (CTX-30/10), cefotaxime-30 µg (CTX-30), ceftazidime-30 µg/clavulanate-10 µg (CAZ/CLA-30/10), ceftazidime-30 µg (CAZ-30), cefepime-30 µg (FEP-30), cefoxitin-30 µg (FOX-30), aztreonam-30 µg (ATM-30), eterpenem-10 µg (ERT-10) and imipenem-10 µg (IMP-10).

The zones of inhibition obtained after incubation were interpreted as recommended (CLSI document M100-S20-U, 2010) for possible detection of ESBLs, AmpC β-lactamases, K1 β-lactamases and carbapenemases. An expanded zone of inhibition with a confluent zone between disks such as CAZ/CLA-30/10 and CAZ-30 is as a result of the synergy between the Clavulanic acid of CAZ/CLA-30/10 and CAZ-30. This is referred to as the ‘clavulanic effect’ or the ‘key hole’ configuration which is exhibited by ESBL-producing bacteria (CLSI document M100-S20-U, 2010).

**Efflux Pump Activity (Epa) Test:** The MDR isolates were selected for the EPA Test. MICs of the test drugs were determined in the presence and in the absence of an EPI, TFMBP. A reduction in MIC in the presence of the EPI indicated resistance due to EPA (Bohnert and Kern, 2005). Nine of the isolates tested from the MDDST were picked for this test. The four drugs used in this test were amoxicillin, cefuroxime, ciprofloxacin and tetracycline. These represent four classes of antibiotics to which the MDR organisms were commonly resistant. The distribution of the organisms and the drugs to which they were previously resistant were amoxicillin – 9 strains, cefuroxime - 7 strains, ciprofloxacin - 6 strains, and tetracycline - 9 strains, a total of 31 tests performed.

### Table 1:
Preparation of microtiter wells for mic determination in the presence of 200µg/ml of TFMBP

<table>
<thead>
<tr>
<th>MHB µl</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug µl</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>TFMBP stock 1179 µg/ml</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>34</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Make up water/buffer µl</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>84</td>
<td>89</td>
</tr>
<tr>
<td>Broth culture µl</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total Volume µl</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Final drug concentrations µg/ml</td>
<td>125</td>
<td>62.5</td>
<td>31.25</td>
<td>15.625</td>
<td>7.813</td>
<td>3.9065</td>
<td>1.95325</td>
<td>0.97663</td>
<td>0.49</td>
<td>0.24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Drug Stock solution µg/ml</td>
<td>500</td>
<td>250</td>
<td>125</td>
<td>62.5</td>
<td>31.25</td>
<td>15.625</td>
<td>7.813</td>
<td>3.9065</td>
<td>1.95325</td>
<td>0.97663</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Growth control* *Sterility control*
Broth Macro-Dilution: The MDR strains were subjected to another round of MIC tests with the micro-dilution assay method. The 96-well micro-titre plates were used to perform the MIC tests at different concentrations of the antibiotics against the bacterial strains based on the initial results of the macro-dilution assay.

Before the MIC tests in micro-titre plates, serial double-dilutions of each antibiotic in either sterile phosphate buffer, pH 6.0, for amoxicillin and cefuroxime or sterile water for tetracycline and ciprofloxacin were carried out in a twelve-channel basin to get the desired working concentrations. The serial dilution was performed in a twelve channel basin as discussed below.

The diluent (either sterile phosphate buffer, pH 6.0 for amoxicillin and cefuroxime or sterile water for tetracycline and ciprofloxacin), 1.5 ml, was placed in channels 2 to 12. With Tetracycline as an example, 3 ml of the stock drug solution (2000 µg/ml) was placed in channel no. 1. From this, 1.5 ml was moved into channel 2, mixed and the same volume was moved from Channel 2 to 3 up to channel 12 to give a final concentration of 0.97663 µg/ml. This procedure was done for all the other antibiotics used in this study. One micro-titre plate was used to test two, different drugs at a time, at ten different concentration levels. The eleventh column of wells (A11-H11) was used as a growth control of the test strain, while the twelfth column of wells (A12-H12) was for sterility control of the process (Table 1).

Preparation of Micro-dilution in Micro-titre Plates: The EPA test for each drug was done in duplicate on the micro-titre plate. For example, rows A and B were for amoxicillin with TFMBP, C and D for amoxicillin without TFMBP, E and F for cefuroxime with TFMBP, and G and H for cefuroxime without TFMBP. This was done for all of the other drugs used.

With the aid of the 12-channel multipipettor fitted with appropriate sterile tips, test materials for each of the 96 wells in a plate including the growth controls and the sterility controls were drawn into the corresponding row of wells (Table 1).

For determination of MICs in the absence of the EPI, 34 µl of make-up volume of sterile phosphate buffer or sterile water (for water soluble antibiotics) was then added to each of the 96 wells including growth and sterile controls, while 34 µl of the EPI solution was added to all the wells excluding the growth and sterile control wells in tests in the presence of TFMBP.

More of the buffer or water was then added to the growth control wells (34+50 µl) and sterile control wells (34+55 µl) to compensate for the drug and the bacterial culture not added respectively. Finally, 5 µl of the 1:103 dilution in normal saline solution of the overnight MHB culture of the test strain was now added with the 12-channel multipipettor fitted with 11 sterile tips for all the wells excluding those for sterility.
control (Table 2). EPA tests carried out were 37. The plates were then covered and incubated at 35°C for 18 hr. The optical density (OD) of each well in the plates, were read at 650 nm in a micro-plate reader, Spectramax 384-plus.

**Molecular analysis:** The molecular analysis of the MDR organisms by randomly-amplified polymorphic DNA (RAPD)-PCR and agarose gel electrophoresis was done on DNA-extracted by boiling as described in literature (Pagani et al., 2003; Villegas et al., 2004). DNA quantification and check for purity was also done as described in literature (Pagani et al., 2003; Villegas et al., 2004). For each chromosomal DNA sample, a PCR mix of 25 µl containing magnesium chloride (6%; 1.5 µl), forward and reverse primers (0.8%; 0.2 µl each) was used. Nucleotide sequence of primers used is on Table 2.

The PCR was conducted by an initial denaturation at 95°C for 5 min. followed by 30 cycles of denaturation at 95°C for 30 sec. The annealing step was done for 60 sec. at various temperatures to suit each primer pair followed by elongation at 72°C for 60 sec. The final elongation step was done at 72°C for 10 min.

**RESULTS**

**Antimicrobial susceptibility test result:** The clinical isolates tested were mostly resistant to ampicillin but mostly susceptible to neomycin and ciprofloxacin (Table 3).

**MDR isolates found, β-lactamase test and Modified Double Disc Synergy Test (MDDST) results:** From the isolates tested, nine were found to be MDR strains, 20 (39.2%) isolates were found to be inducible β-lactamase producers, while seven (13.8%) turned out to be intrinsic β-lactamase producers. (Fig. 1).

**Table 3:**

Antimicrobial Susceptibility Test Results of the Isolates Tested

<table>
<thead>
<tr>
<th>Antimicrobial agent used</th>
<th>Number of isolates tested</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of isolates</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
</tr>
<tr>
<td>Ampicillin 30 µg</td>
<td>51</td>
<td>7</td>
<td>13.73</td>
<td>0</td>
</tr>
<tr>
<td>Ceftriaxone 30 µg</td>
<td>51</td>
<td>42</td>
<td>82.35</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin 5 µg</td>
<td>51</td>
<td>45</td>
<td>88.24</td>
<td>1</td>
</tr>
<tr>
<td>Tetracycline 30 µg</td>
<td>51</td>
<td>15</td>
<td>29.41</td>
<td>8</td>
</tr>
<tr>
<td>SXT-1.25/23.75</td>
<td>51</td>
<td>26</td>
<td>50.98</td>
<td>0</td>
</tr>
<tr>
<td>Neomycin 30 µg</td>
<td>49</td>
<td>47</td>
<td>95.92</td>
<td>0</td>
</tr>
<tr>
<td>PolymyxinB 300iu</td>
<td>49</td>
<td>40</td>
<td>81.63</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol 30 µg</td>
<td>49</td>
<td>31</td>
<td>63.27</td>
<td>3</td>
</tr>
</tbody>
</table>

KEY: SXT-1.25/23.75 - cotrimoxazole-1.25 µg+23.75 µg

**Table 4:**

Summary of EPA Findings on MDR Isolates

<table>
<thead>
<tr>
<th>Organism code</th>
<th>Isolate</th>
<th>Source</th>
<th>Efflux Pump Activity (Fold Reduction in MIC)</th>
<th>Number of antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TTC</td>
<td>CPF</td>
</tr>
<tr>
<td>OG 1(4)</td>
<td>K. pneumonia</td>
<td>Semen</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>N 19</td>
<td>K. pneumonia</td>
<td>Skin</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>N 24</td>
<td>K. pneumonia</td>
<td>Skin</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Med. 5(1)</td>
<td>K. pneumonia</td>
<td>HVS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Med. 1(2)</td>
<td>K. pneumonia</td>
<td>HVS</td>
<td>NS</td>
<td>32</td>
</tr>
<tr>
<td>N 3</td>
<td>K. pneumonia</td>
<td>Skin</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>OG 1(3)</td>
<td>K. pneumonia</td>
<td>Semen</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>N 14</td>
<td>Ent. aerogenes</td>
<td>Blood</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>N 21(2)</td>
<td>E. coli</td>
<td>Semen</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>N 5</td>
<td></td>
<td></td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

KEY: NS - Not significant
HVS – High vaginal swab
TTC- Tetracycline; CPF- Ciprofloxacin; AMC- Amoxicillin; CFX – Cefuroxime
Inhibition and molecular analysis of multidrug resistance with Efflux pump activity

Figure 1:
Types of resistance traits exhibited by isolates found.

Figure 2:
Agarose gel electrophoresis of AcrA, AcrB, TEM and CTX amplicons. AcrA amplicons on lanes i to vi (with samples 1, 2, 4, 6, 10 & 11 respectively). Only sample 11 gave the band at 107 bp for acrA. AcrB amplicons on lanes viii to xiii (with samples 1, 2, 4, 6, 10 & 11 respectively). Samples 1, 2, 4, 6 and 10 gave the bands at 107 bp for acrB. TEM amplicons on lanes xv & xvi (with samples 3 & 7) and CTX on lanes xvii to xxi (with samples 3, 7, 8 & 10) gave the appropriate bands for TEM at 931 bp for samples 3 and 7 while samples 3, 7, 8 and 10 gave the bands for CTX at 415 bp. M on lanes vii and xvii is molecular size marker (100 bp DNA ladder).

Efflux Pump Activity (EPA) of MDR Isolates: Out of the nine MDR isolates tested, six (31.6%) isolates gave evidence of EPA. One K. pneumoniae strain showed efflux pump activity to up to three drugs tested while one other gave as much as a 32-fold reduction in MIC for ciprofloxacin in the presence of the EPI (Table 4).

Molecular analysis on MDR Isolates: AcrA and AcrB genes were detected in six (12%) of the 51 isolates tested. This confirms the EPA detected in the four K. pneumoniae strains, one Enterobacter aerogenes and one E. coli (Figs. 2, 3). blaCTX-M was detected as the gene conferring ESBL characteristics in four isolates (8%) including three K. pneumoniae strains and one E. aerogenes, (Figs. 2,3). blaTEM was detected as the gene conferring ESBL characteristics in two (4%) K. pneumoniae isolates (Figs. 2,3).

The agarose gel electrophoresis of acrA and acrB (for EPA) for some of the other isolates, blaSHV, blaOxa (for ESBLs), Mox-M, Cit-M and Dham (for AmpC) and gyrA (for Quinolone), dham, mec, gyr Primers, aacA-aphD and TolC Primers did not produce any amplicons from the isolates tested.

Figure 3:
Distribution of types of resistance genetic markers on MDR isolates tested.
DISCUSSION

All of the CRO-30-resistant strains studied were also found to be MDR, a known characteristic of ESBL producers. About half of these were also either resistant or intermediate to ciprofloxacin, thus corroborating the statement on increasing correlation between ESBL-producing and fluoroquinolone-resistant strains (Dalhoff, 2012).

Some isolates were found to produce β-lactamase enzyme intrinsically. However, there were others that produced the NF colour change much later than 10 minutes. These were considered as either inducible β-lactamase or secondary β-lactamase activity of penicillin binding proteins forming unstable acyl complexes (Livermore and Brown, 2001). Those isolates that did not give any NF colour reaction even beyond 10 minutes, recorded 100% sensitivity to ceftriaxone. Among them were K. pneumoniae strains sensitive to ampicillin, thus showing no β-lactamase production. The seven isolates that gave immediate NF colour change were among the eight strains initially suspected to be ESBL producers from their resistance to CRO-30. The prevalence level obtained for the ESBLs among these isolates, is higher than that found in Hong Kong, Korea, Taiwan, Japan, Australia and Netherland but less than that found in places like the USA, France, Singapore, Asia Pacific and Latin America (Ghafourian et al., 2014). These results are within the range reported in Lagos (Aibinu et al., 2003).

One K. pneumoniae isolate was found to be sensitive to ceftazidime but resistant to aztreonam and was thus diagnosed to produce the chromosomally-borne K1-β-lactamase (CLSI document M100-S20-U, 2010). However cephalosporinase overproduction may be the reason for the detection of ESBL in this strain (Gottlieb and Wofson, 2000).

Typical of plasmid-borne AmpC β-lactamase producer, a Klebsiella isolate was shown to be resistant to cefoxitin and sensitive to cefepime. It did not give the clavulanic effect typical of ESBL-producers in the MDDST, and was sensitive to ceftazidime and the monobactam aztreonam. This isolate proved to be an inducible ESBL-producer. Induced and stable over-production of AmpC cephalosporinase can coexist with acquired ESBL production as was found in one E. aerogenes isolate that was resistant to cefoxitin, intermediate to aztreonam and cefepime but gave the clavulanic effect typical of ESBLs with cefotaxime and ceftazidime (Miro et al., 2013). The level of occurrence of AmpC in this study is similar to levels reported for E. coli and Klebsiella respectively by other researchers (Ding et al., 2008).

The EPI used in this study, TFMBP, with an intrinsic MIC >400 µg/ml, and used at 200 µg/ml, is unlikely to have any appreciable antibacterial activity of its own. This is in line with other reports (Sternitz et al., 2002) that compounds exhibiting MICs of more than 200 µg/ml are generally considered as weak antibacterial agents and potential EPA inhibitors. The MICs given by these organisms in the absence of TFMBP are obviously much higher than clinically-useful in vivo concentrations. With active efflux pumps, even normal clinical doses of an antibiotic will be present as a sub-clinical dose in vivo which will encourage emergence of resistant strains (Xian-Zhi and Hiroshi; 2009).

The TFMBP used in this work was found to appreciably lower MICs of up to two or three drugs in some of the MDR isolates tested. This agrees with reports (Sun et al., 2014) that a single pump is able to confer resistance to multiple compounds. Results showing efflux pump inhibition on organisms from clinical sources such as semen, high vaginal swab (HVS), catheter, skin, blood, eye and ear swabs among others, further underscores the suggestion that the use of such inhibitors, in association with substrate antibiotics, may be useful in increasing both the activity and the range of species for which the drugs may be effective (Webber and Piddock 2003).

The threat of resistance mechanisms due to EPA in these MDR strains further underscores the need for more research into EPIs. This is in consonance with the assertion (Fiamengos et al., 2011) that one plausible antimicrobial alternative to MDR could be the combination of conventional antibiotics with efflux pump inhibitors. Appreciably lowering of MICs obtained from this study reveals that design of newer drugs or modification of existing drug molecules with the inclusion of EPIs may lead to the development of more potent compounds for tackling resistant organisms.

The use of EPIs could facilitate the re-introduction of therapeutically-ineffective antibiotics back into clinical use and even suppress the emergence of MDR strains (Kourteti et al., 2013). The most prevalent ESBL gene in this study was found to be blaCTX-M. From studies on enterobacteriaceae, blaCTX-M have been found as highly prevalent including very high prevalence rates in E. coli and K. Pneumoniae from hospital- acquired infections in various parts of Europe and Asia. A preference for the hydrolysis of cefotaxime over ceftazidime was found in this study; differences in zone diameters of about 3 mm to 7 mm were noticed for Klebsiella samples and one E. aerogenes that showed blaCTX-M genes as detected from other works (Choi et al., 2015).

Half of the strains bearing the blaCTX-M gene tested also carried the blaTEM gene. The blaTEM-24 was reported (Mammi et al., 2001) as an epidemic clone established in the hospital ecology of Amiens Teaching Hospital in France in 1996. In this same period another study (De Gheldre et al., 2001) in Belgium reported that half of their E. aerogenes were carrying a similar blaTEM-24, thus supporting the theory of international dissemination of resistance genes. Cefepime, which was thought to have excellent in vitro activity against Enterobacter spp. (Magiorakos et al., 2012), gave an intermediate result for the E. aerogenes strain and four of the K. pneumoniae strains. This adds to worldwide concerns about strains of Enterobacteriaceae, especially with cases of K. pneumoniae and E. coli harbouring the variety of ESBL-producing blaTEM and blaSHV; the expression of these genes give high MICs for cefepime a fourth generation cephalosporin. The detection of acrA and acrB efflux pump genes in some isolates confirmed EPA as the origin of the MDR.

In conclusion, the result of this work buttresses the theory of international dissemination of various forms of MDR pathogenic bacteria found in this study and as found elsewhere in the world. It also gives hope that the emergent EPA among MDR strains can be tackled with discovery of useful EPIs which can be incorporated into antibiotic formulations to
bring about the lowering of MICs of antibiotics to clinically useful levels.

Acknowledgement
Thanks to the Misher College of Arts and Sciences, University of the Sciences, Philadelphia, USA for the laboratory support and Professor Adeboye Adejare, then Professor, Dept. of Pharmaceutical Sciences, for facilitating Author’s trip to the University of the Sciences in Philadelphia.

REFERENCES


