Extended Spectrum Beta – Lactamase (ESBL) Mediated Resistance to Antibiotics Among *Klebsiella Pneumoniae* in Enugu Metropolis

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**Abstract**

**Aim.** The objectives of this study were to determine the prevalence and antibiotic susceptibility patterns of extended – spectrum b - Lactamase (ESBL) producing *Klebsiella pneumoniae* isolated from three major diagnostic laboratories in Enugu State metropolis, Nigeria.

**Material and Methods.** Clinical isolates of *Klebsiella pneumoniae* were obtained from urine, sputum and blood samples of patients attended diagnostic laboratories in Enugu metropolis. The isolates were subjected to susceptibility testing using Kirby and Bauer method of determining antimicrobial susceptibility and ESBL production was phenotypically determined using double disc synergy test.

**Results.** A total of 300 clinical isolates of *Klebsiella pneumoniae* were isolated from three major diagnostic laboratories in Enugu namely Ambulin (100 urine samples), Mendex (100 blood sample) and Edisson (100 sputum samples) within a four month period (January – April 2009). ESBL production was determined among 300 isolates of *Klebsiella pneumoniae* and 186 (62%) *Klebsiella pneumoniae* express ESBL. ESBL producing *Klebsiella pneumoniae* were most frequently isolated from blood 76 (40.0%) followed by urine 66 (30.5%) and sputum 44 (23.6%). Resistance patterns of *Klebsiella pneumoniae* revealed that 64% were resistance to sparfloxacin, 92.6% to gentamicin, 90.9% to fusidic acid, 82.3% to erythromycin, 79% to trimethoprim, 96.3% to sulphamethoxazole, 88.5% to tetracycline 31.2% to nitrofurantoin, 31.2% to ciprofloxacin, 69% to ceftazidime, 74% to cefotaxime, 79.6% to ceftriaxone and 0% to imipenem.

**Conclusion.** ESBL producing *Klebsiella pneumoniae* were present in these diagnostic laboratories and were resistant to different classes of antibiotics resulting in limited treatment options. Therefore we suggest that it will be important to perform screening and confirmatory tests for ESBL detection to any organisms resistant to any of the second and third generation cephalosporins in a routine diagnostic laboratory work.

**Introduction**

*Klebsiella pneumoniae* is an opportunistic pathogen that causes various illnesses such as diarrhoea, septicemia, urinary and respiratory tract infections (1). Resistance of *Klebsiella* spp. to the cephalosporins such as oxyimino beta – lactams was first described in 1980 and since then a linear increase in resistance has been recorded. Their resistance is by the production of extended spectrum beta lactamases (ESBLs). ESBLs are encoded by transferable conjugative plasmids which often encode resistant determinants to other classes of antibiotics. The plasmids mediated resistant against cephalosporins can spread among related and unrelated Gram – negative bacteria. ESBLs are mostly
the products of point mutations at the active site of TEM and SHV enzymes (2). Majority of ESBL – producing organisms are *E. coli* and *K. pneumoniae* others includes *Enterobacter* spp., *Salmonella* spp, *Morganella* spp, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas* spp. The major risk factors implicated are long term exposure to antibiotics, prolonged intensive care unit stay, nursing home residency, severe illness, instrumentation or catheterization (3). ESBLs are plasmid mediated and they confer resistance to oxyimino-cephalosporins (cefotaxime, ceftriaxone, ceftazidime) and to monobactam (aztreonam) but are not active against cephamsycins and carbapenems.

ESBLs are more prevalent in *Klebsiella pneumoniae* than in any other enterobacteria species and outbreaks of infections caused by ESBL producing strain have been reported widely. ESBL producing strains are probably more prevalent than currently recognized because they are often undetected by routine susceptibility testing methods. Recent reports have highlighted the emergence of ESBL producing strains endowed with an extremely wide spectrum of antibiotic resistance, including resistance to trimethoprim, amikacin, streptomycin and gentamicin (4).

Due to the extensive spread of multi – drug resistant ESBL producing *Klebsiella pneumoniae*, the present study was designed to examine the prevalence of ESBL producing strains and multi-drug resistant strains of *Klebsiella pneumoniae* isolated from three major diagnostic laboratories in Enugu metropolis.

### Material and Methods

300 clinical isolates of *Klebsiella pneumoniae* were collected from three different major laboratories in Enugu metropolis namely Ambulin laboratory (100 urine samples), Mendix (100 blood samples) and Edisson (100 sputum samples). These organisms were identified and characterized based on colony morphology and biochemical reactions (5).

**Antimicrobial susceptibility testing**

Sensitivity of the isolates to various classes of antibiotics and third generation cephalosporins such as penicillin (10 μg), ceftazidime (30 μg), gentamicin (10 μg), fusidic acid (10 μg) tetracycline (30 mg), imipenem (30 mg), cefotaxime (30 mg) ceftriaxone (30 mg), clindamycin (10 mg), erythromycin (5 mg), trimethoprim (5 mg) sulphamethoxazole (25 mg) nitrofurantoin (25 μg), ciprofloxacin (25 μg) and imipenem (30 μg). (Oxoid Uk) was determined by the disc diffusion methods (6). The results were interpreted as per National Committee for clinical laboratory standards (NCCLS) recommendations (7). Isolates which were resistance or intermediate susceptibility by NCCLS criteria to any of third generation cephalosporins were selected for ESBL detection/screening phenotypically.

**ESBL Detection using Double disc synergy test (DDST)**

In DDST, synergy was determined between a disc Augmentin (20 mg amoxicillin + 10 μg clavulanic acid) and 30 mg of disc of cefotaxime and ceftazidime antibiotics placed at a distance of 15 mm apart from the center disc on the surface of culture of the resistant isolate under test on Mueller Hinton agar (Oxoid Uk). The test organisms were considered to produce ESBL if the zone size around the test antibiotic disc were more than 5mm and above towards the augmentin disc. This increase occurs because the clavulanic acid present in the augmentin disc inactivates ESBL enzymes produced by the test organism.

### Results

All the 300 clinical isolates of *Klebsiella pneumoniae* were screened for the presence of ESBL enzymes, 186 (62%) positively express ESBL enzymes. ESBL enzymes were isolated more frequently from blood 76 (40.0%) followed by urine 66 (30.5%) and sputum 44 (23.6%) (Table 1).

**Table 1: Prevalence of ESBL producing Klebsiella pneumoniae from three major laboratories in Enugu metropolis, Nigeria.**

<table>
<thead>
<tr>
<th>S/No</th>
<th>Laboratory name</th>
<th>Isolated collected</th>
<th>Total ESBL positive isolates</th>
<th>Total ESBL Negative isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ambulin</td>
<td>Blood (100)</td>
<td>62%</td>
<td>38%</td>
</tr>
<tr>
<td>2</td>
<td>Mendix</td>
<td>Urine (100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Edisson</td>
<td>Sputum (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All ESBL producing isolates were found to be resistant to the following antibiotics ceporex 64%, gentamicin 92.6%, fusidic acid 90.0%, erythromycin 82.3%, trimethoprim, Sulphamethoxazole 96.3% tetacycline 88.5%, ciprofloxacin 31.2% nitrofurantoin 31.2%, ceftazidime 69%, cefotaxime 74%, ceftriaxone 79.6% and imipenem 0% (Table 2).
In general the prevalence of ESBL producing *Klebsiella pneumoniae* from three major diagnostic laboratories in Enugu metropolis were high (186 (62%)) and were resistance to a wide range of antibiotics.

### Discussion

The incidence of ESBL – producing strains among clinical isolates of *Klebsiella pneumoniae* has been on steady increase over the past few years and thus accounts for about 17% of all nosocomial urinary tract infections. The detection rate of ESBL producing *Klebsiella* isolates from clinical samples differs from each other. In our study 76 (40.0%) were isolated from blood, 66 (30.5%) from urine and 44 (23.6%) from sputum: studies have shown that ESBL prevalence is more from blood and urine (8). These isolates were found to be resistant to cefotaxime (74%), ceftriaxone (79.6%) and ceftazidime (69%). Since all the isolates showed multi-drug resistance, the therapeutic strategies to control infections due to *Klebsiella* spp have to be carefully formulated. The therapeutic use of all third generation cephalosporins should be avoided against *Klebsiella* spp that appear susceptible to any such compound. Since all the isolates were sensitive to imipenem, it is recommended as the drug of choice for the treatment of infectious due to ESBL producing *K. pneumoniae* strains.

During the past decade, ESBL producing *K. pneumoniae* have emerged as one of the major multi-drug resistant organisms (9). The incidence of ESBL – producing *Klebsiella* isolates in the Unites States has been reported to be 5%. In France and England 14 to 16% ESBL producers among clinical *Klebsiella* isolates has been reported (10). In particular regions or hospital, the incidence can reach 25 to 40% (11), however, the percentage of third generation cephalosporins resistant strains may be much higher because the conventional disc diffusion criteria used in the routine laboratory, under estimate the incidence of these isolates. ESBL producing organisms have been isolated in Western and Eastern part of Nigeria. 25% prevalence was recorded in the West (12) while 44.6% was recorded in Enugu and 6.7% in Ebonyi Eastern Nigeria (13-14).

ESBL – producing organisms have become an important clinical problem due to their resistance to multiple antibiotics. Thus antibiotic options in the treatment of these organisms are extremely limited. Beta lactams are usually used for treatment of lower respiratory tract infections in children where Gram negative bacteria are isolated. Detection of ESBL production is important, because it is recommended that any organism that is confirmed for ESBL production according to CLSI criteria should be reported as resistant to all extended – spectrum beta lactam antibiotics, regardless of their susceptibility test results. These drugs should not be used to treat serious infections caused by ESBL producers. Carbapenems have been recommended as the drugs of choice for serious infections with ESBL producers. Imipenem showed the best in vitro activity to all tested beta lactams. In our study all ESBL producers were susceptible to imipenem, there results agree with those reported previously by other studies (15).

In conclusion ESBL producing *Klebsiella pneumoniae* are present in our diagnostic laboratories, therefore, it is necessary and useful to perform screening and confirmatory tests for phenotypic detection of these organisms in a routine laboratory diagnosis work. All the ESBL producers are resistant to many classes of antibiotic resulting in limited treatment options. Treatment of infections due to these organisms could be difficult and complex. Therefore it is important to control such strains in order to prevent and reduce their spread.

### References


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