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# Detection of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* and their susceptibility rates to antibiotics in University of Benin Teaching Hospital, Benin City, Nigeria.

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**ABSTRACT**

Extended spectrum β-lactamase (ESBL) producing organisms, are now being recognized as one of the major threats to effective management of patients in medical institutions, especially in the less developed nations. This study aimed to determine the prevalence and antibiotic susceptibility patterns of extended-spectrum β-lactamase producing Klebsiella pneumoniae isolated from clinical specimens in University of Benin Teaching Hospital, Benin City, Nigeria from January to June, 2010. A total of 183 clinical isolates of Klebsiella pneumoniae were isolated from wound (23), Blood (60), Urine (36), Ear (13) and High Vaginal Swab/Endocervical Swab (HVS, ECS) (40). Sensitivity to antibiotics were carried out using the disc diffusion method by Kirby-Bauer and phenotypic characterization of ESBL was carried out using double disc synergy test (DDST). The result of the study revealed that 67 (36.6%) isolates were positive for ESBL. 5(7.5%) isolates were from wound, 41 (61.2%) from Blood, 11 (16.41%) from Urine, 3(4.5%) from Ear and 7(10.5) from vagina/Endocervix respectively. All ESBL producing isolates were found to be resistant to the following antibiotics Ceftazidime (67.2%), Cefotaxime (72.1%), Ceftriaxone (75.3%), ciprofloxacin (27.3%), Fusidic acid (86.3), Erythromycin (88.5), sparfloxacin (67.8%), Tetracycline (90.7), Trimethoprim (77.6%), Gentamycin (91.8%), Ampicillin (84.2%) and Sulphamethoxazole (96.2%). ESBL producing Klebsiella were present in University of Benin Teaching Hospital and were resistant to 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.

**Keywords:** Klebsiella pneumoniae, Susceptibility, antibiotics, Extended spectrum beta-lactamases.

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## INTRODUCTION

Extended spectrum β-lactamase (ESBL) producing organisms, are now being recognized as one of the major threats to effective management of patients in medical institutions, especially in the less developed countries [1]. *Klebsiella* species and *Escherichia coli* have been reported by a number of workers to habour ESBL enzyme [2, 3]. ESBL producing organisms are inhibited by β-lactam - β-lactamase inhibitors but are not with extended spectrum-Cephalosporins [4]. ESBL are encoded by genes on plasmids which results in easy transfer of ESBL enzymes to other bacteria species [5]. Originally, ESBL enzymes were derived from the widespread TEM and SHV β-lactamase family, however today, over 110 derivatives of TEM β-lactamase and more than 63 derivatives of SHV β-lactamases are known [6].

Detection of ESBL enzymes using routine laboratory susceptibility tests is often difficult and consequently, ESBL producing *Klebsiella* species and *E. coli* may falsely appear to be susceptible to new Cephalosporins [7]. The National Committee for Clinical Laboratories Standards (NCCLS) recommends that microbiology laboratories should report ESBL-isolates of *E. coli* and *Klebsiella* spp as resistant to all penicillin's and Cephalosporins including cafepime and aztreonam irrespective of their individual in vitro test results [8]. This study aimed to determine the antibiotic susceptibility patterns of ESBL producing *K pneumoniae* from different clinical specimens received in the Medical Microbiology Laboratory of the University of Benin Teaching Hospital, Benin City, Nigeria.

## MATERIALS AND METHODS

183 clinical isolates of *Klebsiella pneumoniae* were isolated from five different clinical specimen namely, wound (23), Blood )60), Urine (36), Ear (13) and High Vaginal Swab/Endocervical Swab (40) obtained from the Medical Microbiology Laboratory of University of Benin Teaching Hospital, Benin City, Southern Nigeria from January to June, 2010. These organisms were identified and characterized based on colony morphology and biochemical reactions [9].

### Antimicrobial Susceptibility Testing

The sensitivity studies were conducted using the Kirby and Bauer method of sensitivity determination [8]. Sterile Petri-dishes of Muller Hinton agar were prepared according to manufacturer's specification. 0.1ml of *Klebsiella pneumoniae* equivalent to 0.5 McFarland Standard was seeded into each of the Petri-dishes containing Muller-Hinton agar. These were allowed to stand for 45 minutes to enable the inoculated organisms to pre-diffusion. The following antibiotics discs were aseptically placed on the surfaces of the sensitivity agar plates; Gentamycin (10 µg), Ampicillin (5µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Ciprofloxacin (25 µg), erythromycin (5 µg), tetracycline (30 µg), Sulphamethoxazole (25mg), fusidic acid (10 µg), Sparfloxacin (25 µg) and Trimethoprim (5 µg).

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**Detection of ESBL Production Using Double Disc Synergy Test (DDST)**

In DDST, synergy was determined between a disc Augmentin (20 µg amoxicillin + 10 µg Clavulanic acid) and 30 µg of disc of Cefotaxime and Ceftazidime antibiotics placed at a distance of 15mm apart from the centre disc on the surface of culture of the resistant isolate under test on Muller 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 183 clinical isolates of *Klebsiella pneumoniae* were screened for the presence of ESBL enzymes, 67(36.6%) positively expressed ESBL enzymes. ESBL enzymes were isolated more frequently from Blood 41(61.2%) followed by Urine 11(16.4%), HVS/ECS 7(10.5%), Wound Swab 5(7.5%) and Ear Swab 3(4.5%) Table1.

All ESBL producing isolates were found to be resistant to the following antibiotics Ceftazidime (67.2%), Cefotaxime (72.1%), Ceftriaxone (75.3%), ciprofloxacin (27.3%), Fusidic acid (86.3), Erythromycin (88.5), sparfloxacin (67.8%), Tetracycline (90.7), Trimethoprim (77.6%), Gentamycin (91.8%), Ampicillin (84.2%) and Sulphamethoxazole (96.2%) Table2.

In general the prevalence of ESBL producing *Klebsiella pneumoniae* from the five clinical samples received in University of Benin Teaching Hospital were high 67 (36.6%) and were resistant to a wide range of antibiotics.

**Table I: Prevalence of ESBL producing Klebsiella pneumonia in University of Benin Teaching Hospital**

| | Isolate Collected | No. of ESBL Positive Isolates (%) |
|---|---|---|
| Wound | 23 | 5(7.5) |
| Blood | 60 | 41(61.2) |
| Urine | 36 | 11(16.4) |
| Ear | 13 | 3(4.5) |
| HVS/ECS | 40 | 7(10.5) |
| | 183 | 67(36.6) |

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**Table 2: Antibiotic Susceptibility Pattern of Klebsiella pneumonia producing ESBL to antibiotics**

| ESBL – Positive Klebsiella Pneumoniae | | | |
|---|---|---|---|
| **S/No.** | **Antimicrobial Agent** | **% of Susceptible** | **% of Resistant** |
| 1. | Ceftazidime | 60(32.8) | 133(69.2) |
| 2. | Cefotaxime | 51(27.9) | 132(72.1) |
| 3. | Ceftriaxone | 47(25.7) | 136(75.3) |
| 4. | Ciprofloxacin | 133(72.7) | 50(27.3) |
| 5. | Fusidic acid | 25(13.7) | 158(86.3) |
| 6. | Erythromycin | 21(11.5) | 162(88.5) |
| 7. | Sparfloxacin | 59(32.2) | 124(67.8) |
| 8. | Tetracycline | 17(9.3) | 83(90.7) |
| 9. | Trimethoprim | 41(22.4) | 142(77.6) |
| 10. | Gentamicin | 15(8.2) | 168(91.8) |
| 11. | Ampicillin | 29(15.8) | 154(84.2) |
| 12. | Sulphamethoxazole | 7(3.8) | 174(96.2) |

## 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 infections. The detection rate of ESBL producing Klebsiella isolates from clinical samples differ from each other. In our study 41(61.2%) were isolated from blood, 11(16.41%) from urine, 7(10.5%) from HVS/ECS, 5(7.5%) from wound and 3(4.5%) from Ear. Studies have shown that ESBL prevalence is more from blood and urine [10]. These isolates were found to be resistant to Ceftazidime (67.2%), Cefotaxime (72.1%) and Ceftriaxone (75.3%). Since all the isolates showed multi-drug resistance, the therapeutic strategies to control infections due to *Klebsiella* spp have to be carefully formulated. The constant use of third generation Cephalosporins in the treatment of infections in Nigeria is probably the reason for the current spread of ESBL organisms in our environment. The therapeutic use of all third generation Cephalosporins should be avoided against *Klebsiella* spp that appear susceptible to any such compound.

During the past decade, ESBL producing *K. pneumoniae* have emerged as one of the major multi-drug resistant organisms [11]. The incidence of ESBL-producing *Klebsiella* isolates in the United States has been reported to be 5%. In France and England 14 to 16% ESBL producers among clinical *Klebsiella* isolates has been reported [12]. 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 [8] while 44.6% was recorded in Enugu [14]. Information as regards ESBL are very uncommon in our environment and as a result, most clinicians probably don't know when to test for ESBL or any preventive measures to adopt that will help to control its spread. This level of ignorance could culminate to devastating consequences.

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. Etrapenem antibiotic has been shown by some workers to have activity against ESBL from *Klebsiella* spp [15]. It is vital to note, that some of these apparently efficacious drugs against ESBL producers are not readily available in Nigeria and where available, may be beyond the reach of the common man. The need to avert the further spread of this enzyme is thus emphasized.

In conclusion ESBL producing *Klebsiella pneumoniae* is present in our study area; 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.

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