Prevalence of extended spectrum beta-lactamase (ESBL) production among *Klebsiella* isolates in some parts of South West Nigeria

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ABSTRACT

In this study, the prevalence of Extended spectrum Beta-Lactamase (ESBL) production among *Klebsiella* isolates in some parts of South West, Nigeria was investigated. A total of nine hundred and seventy (970) clinical specimens were collected out of which 544 isolates were recovered. The specimens were collected from four different States namely, Ekiti, Lagos, Ondo and Osun States. Comparing the percentage relative distribution, Lagos State had 72.5% which was the highest while the lowest came from Osun State with 43.6%. The organisms were collected from sources which included urine, blood, sputum, ear swab, semen, cerebro spinal fluid, nasal swab, stool and high vaginal swab. About 100 isolates representative organisms selected based on their antibiotic resistance phenotypes were subjected to double-disc synergy test with ceftriaxone, aztreonam, cefpodoxime and amoxicillin-clavulanic acid to detect ESBL-producing isolates. Two types of ESBLs were discovered, SHV-type ESBL was 53.0% while TEM-type were only 3.0% and 44.0% did not produce ESBL. Ondo state produced the highest SHV-type (18) while Osun state produce only (1) SHV-type. This present study suggested that clinical microbiology laboratories should take into account the changing epidemiology of ESBL producers in order to establish a proper treatment protocol.

Keywords: Extended Spectrum Beta-Lactamase (ESBL), Double-disc method, *Klebsiella*, antibiotics, Prevalence, Isolates.

INTRODUCTION

*Klebsiella* is a genus of non-motile, oxidase-negative, rod-shaped Gram-negative encapsulated bacteria in the family Enterobacteriaceae (Podschun and Ullmann, 1998). It is well known to most clinicians as a cause of community-acquired bacterial pneumonia, occurring particularly in
chronic alcoholics (Carpenter, 1990). The species *Klebsiella* are opportunistic pathogens and primarily attack immuno-compromised individuals who are hospitalized and suffering from one severe underlying disease or the other (Podschum *et al*., 1994). They have been incriminated in nosocomial infections with common sites including the urinary tract, lower respiratory tract, biliary tract and surgical wound sites where they cause destructive changes such as necrosis, inflammation, and haemorrhage. These changes occur within tissues sometimes producing a thick bloody, mucoid sputum described as “currant jelly” sputum (Kobash *et al*., 2000).

Moreover, the extensive use of broad-spectrum antibiotics in hospitalized patients has led to both increased carriage of *Klebsiella* and subsequently, the development of multiple antibiotic-resistant strains that produce extended-spectrum $\beta$-lactamases (ESBL) (Paterson, 2000). ESBLs are plasmid mediated which encodes enzymes that hydrolyze the oxyimino $\beta$-lactams and monobactams (Aztreonam) but have no effect on cephamycins, carbapenems and related compounds (Livermore, 1995). Its production has emerged as an important mechanism of resistance to $\beta$-lactam drugs (Bonnet, *et al*., 1999). Among Enterobacteriaceae, ESBLs have been found mainly in *Klebsiella* spp and *E. coli* but have also been reported in other genera worldwide such as *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Salmonella*, and *Serratia* (Arlet and Philippon, 1991; Ivanova, *et al*., 2008). The prevalence of ESBL-positive enterobacteria varies greatly among different geographical areas, the highest value (44.9%) was reported in Latin America (Winokur, *et al*., 2001). It also differs from country to country, with the highest percentages in Greece (27.4%) and the lowest in the Netherlands (2.0%) (Bouchilon, *et al*., 2004).

Therefore, this study was focused to detect ESBL production among *Klebsiella* isolates from different locations in South West, Nigeria.

**MATERIALS AND METHODS**

**Sources of samples**
Samples were collected from five different hospitals in four Southwest States, Nigeria for the isolation of *Klebsiella*. They include Ekiti (University Teaching Hospital, Ado-Ekiti and Federal Medical Centre, Ido-Ekiti), Lagos (State General Hospital, Broad Street Lagos), Ondo (Federal Medical Centre, Owo) and Osun (Obafemi Awolowo University Teaching Hospital Annex, Ilesa).

**Collection of samples**
Samples collected included urine, high vaginal swabs, blood, ear swab, sputum, pus, cerebrospinal fluid, semen, stool and nasal swab. A total of 970 samples were examined for the presence of *Klebsiella*.

**Isolation and Characterization of the Organisms**
It was carried out as described by Olutiola, *et al*., (2001) and Fawole and Oso (2001). All the swabs samples were cultured directly on MacConkey agar (oxoid) and incubated overnight at 37°C. Bacterial colonies with characteristic mucous and pinkish colour were presumptively identified as *Klebsiella* spp. Further confirmation was done by carrying out certain biochemical tests.
Standardization of Inoculum
The inoculum was standardized according to the method of Bauer et al., (1998) where the turbidity of the broth was made equivalent to a 0.5 McFarland standard.

Detection of Extended-Spectrum-Beta-Lactamase (ESBL) production
For the detection of ESBL production in Klebsiella isolates, One Hundred (100) representative organisms selected based on their antibiotic resistance phenotypes were subjected to double-disk tests with Ceftriaxone (CRO), aztreonam (AZM), Cefpodoxime (CPO) and Amoxicillin-Clavulanic acid discs (Oxoid, Basingstoke, UK). A cotton swab was dipped into a standardized bacterial suspension and the surface of the sensitivity test agar was inoculated evenly. The surface of the plate was allowed to air dry. After 15 minutes, the discs were placed 15mm apart at equal distance centre-to-centre with the amoxicillin-clavulanic acid placed at the centre. The plates were incubated at 37°C for 18hrs. Enhancement of the zone of inhibition around one or more of the β-lactam containing disc towards the clavulanic acid containing disc was recorded.

RESULTS AND DISCUSSION
The results of distribution of Klebsiella spp recovered from clinical samples in selected states are shown in Table 1. It showed that out of 970 clinical samples examined for the presence of the organism, only 544 (56.1%) of Klebsiella isolates were recovered. The distribution of the organisms was as follows: Ekiti state had 260 (47.8%), Lagos state 74 (13.6%), Ondo state had 104 (19.1%) and Osun state had 106 (19.5%). Klebsiella spp were most recovered from Ekiti (47.8%) while Lagos state showed least recovery (13.6%).

Table 1: Distribution of Klebsiella isolates from the Four States.

<table>
<thead>
<tr>
<th>Location</th>
<th>No. of specimens examined (%)</th>
<th>No. of Klebsiella recovered (%)</th>
<th>Relative distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ekiti</td>
<td>401 (41.3)</td>
<td>260 (47.8)</td>
<td>64.8</td>
</tr>
<tr>
<td>Lagos</td>
<td>102 (10.5)</td>
<td>74 (13.6)</td>
<td>72.5</td>
</tr>
<tr>
<td>Ondo</td>
<td>224 (23.1)</td>
<td>104 (19.1)</td>
<td>46.4</td>
</tr>
<tr>
<td>Osun</td>
<td>243 (25.1)</td>
<td>106 (19.5)</td>
<td>43.6</td>
</tr>
<tr>
<td>Total</td>
<td>970 (100.0)</td>
<td>544</td>
<td>56.1</td>
</tr>
</tbody>
</table>

The results of detection of ESBL production among the 100 representative Klebsiella isolates selected using double-disc method are shown in Table 2. The result of this study showed that two types of ESBLs were obtained namely: SHV-type and TEM-type. Among isolates from Ekiti, 17 produced the SHV-type, 1 produced the TEM-type totalling 18 ESBLs. Among Lagos isolates, 17 was detected to be SHV-type while the state had TEM-type from two isolates totaling 19 ESBLs type produced. SHV-type detected among isolates was 18 and TEM-type was not detected in Ondo state. The isolate from Osun produced 1 SHV-type and none produced TEM-type. These results imply that ESBL was detected in 56% of the isolates. This percentage is considered to be high compared to prevalence of ESBL production worldwide among Klebsiella species when compared to 27.3% in K. pneumoniae reported by Akram, et al., (2007) who conducted a survey on the urinary tract isolates in India.
Table 2: Detection of ESBL-producing isolates

<table>
<thead>
<tr>
<th>State</th>
<th>Types of ESBL</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SHV</td>
<td>TEM</td>
<td>Total</td>
</tr>
<tr>
<td>Ekiti</td>
<td>17</td>
<td>1</td>
<td>18</td>
</tr>
<tr>
<td>Lagos</td>
<td>17</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Ondo</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>Osun</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>3</td>
<td>56</td>
</tr>
</tbody>
</table>

Similarly, Khurana, et al., (2002) reported 38.5% in K. pneumoniae in a survey on urinary tract isolates of family enterobacteriaceae. Also, Sader et al., (2001) reported ESBL production among K. pneumoniae to be 48.4%. However, this percentage of ESBL producers in this work is lower when compared to 72.4% in Klebsiella pneumoniae reported by Raymond, et al., (2009).

In this study, the two types of ESBL were detected in the ratio 53 : 3. There was a larger number of isolates harbouring SHV-type as compared to TEM-type. This is in contrast to the data from 1999 survey which showed that there was no significant change in the prevalence of isolates carrying TEM-type alone or both TEM-type and SHV-type determinants(45.5% versus 46.8% and 11.0% versus11.0% respectively)(Spanu, et al., 2002).

However, the most notable variation in 2003 was the marked increase in E.coli isolates harbouring non-TEM and non-SHV determinants (22.3% versus 7.6% in 1999). This was mostly due to the emergence and spread of CTX-M-type enzymes in E. coli isolates. The abnormally high percentage of ESBL production among the Klebsiella isolates may indicate the presence of a previously undetected source of a nosocomial infection (Raymond et al., 2009). In interpreting the phenotype of ESBL-positive strains with regard to β-lactams, it should be born in mind that drug resistance may also result from the combined activity of a specific ESBL together with other β-lactamase (The chromosomal AmpC) or the plasmid –borne one (Walter-Rasmussen and Hoiby, 2002). The increasing frequency of ESBL-producing entrobacteria among hospitalized patients is an important problem for both microbiologists and clinicians. Therefore when detecting ESBL-positive strains, microbiology laboratories should provide the clinician with reliable therapeutic options for successfully treating infected patients.

CONCLUSION

Since ESBL distribution has been shown to differ among countries, monitoring the changing epidemiology of ESBL producers contribute to defining the degree of problem in a specific geographical area and establishing a proper treatment protocol.

REFERENCES