DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASE IN
KLEBSIELLA PNEUMONIAE ISOLATED FROM SPUTUM IN
KHARTOUM, SUDAN

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ABSTRACT
The aim of this study was to detect extended spectrum β-lactamase (ESBL) in Klebsiella pneumoniae isolated from sputum. A total of 100 sputum specimens were collected from Alshaab Teaching Hospital during April to June 2012. Bacteriological tests of sputum specimens were performed for Klebsiella pneumoniae including inoculation on MacConkey’s agar and blood agar. The identity of the isolates was confirmed by biochemical tests. Out of 100 sputum specimens; 21 (21%) Klebsiella pneumoniae was recovered from patients with different age groups. Susceptibility test and extended spectrum β-lactamase production were carried out for each isolate. The isolates were resistance to nitrofurantoin, nalidixic acid and co-trimoxazole and sensitive to imipenem. Screening tests for ESBL were performed against four types of third generation cephalosporin (3GC) and amoxycylave. Out of 21 isolates, 19 were sensitive and two were resistance. Double disc synergy test was done for the two isolates that were found resistance to the 3GC to detect ESBL and the result was positive. This study showed that Klebsiella pneumoniae considered to be one of ESBL producer.

KEYWORDS: Klebsiella pneumonia, EBSL, 3GC, Beta-lactamase, resistance.

INTRODUCTION
Klebsiella pneumoniae (K. pneumoniae) is a member of family Enterobacteriaceae. The organism can produce infection at a variety of sites with the risk of being increased in
patients with impaired host defenses (e.g. Diabetes mellitus, alcoholism, malignancy, chronic obstruction pulmonary disease and glucorticoid therapy). The introduction of the third generation cephalosporins (3 GC) was very much helpful in fighting against the beta-lactamases in clinical practice (Paterson and Bonomo, 2005). However resistance to these antibiotics started to emerge rapidly. Because of their increased spectrum of activity, especially against 3GC, these enzymes were called extended spectrum beta-lactamases (ESBLs) (Bradford, 2001).

These enzymes are produced by Enbterobacteriaceae mainly by Escherichia coli, Klebsiella pneumoniae and K. oxytoca. They have been detected in other Gram-negative bacilli such as Proteus species, Salmonella species, Pseudomonas aeruginosa and other Enteobacteriaceae. The first ESBL-producing organism was isolated in Germany in 1983. Thereafter, such organisms were reported in the USA following outbreaks of infections caused by these pathogens. The ESBL enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. In addition, ESBL producing organisms exhibit co-resistance to many other classes of antibiotics resulting in the limitation of therapeutic option (Astal, et al., 2004).

For this reason, the significance of such ESBL–mediated infections has been increasingly reported worldwide. The ESBL have serine at their active site and attack the amide bond in the lactam ring of antibiotics causing their hydrolysis. Because of inoculum effect and substrate specificity, their detection is a major challenge. Two indicators of ESBL are eight-fold reductions in MIC and potentiation of the inhibitor zone of third generation cephalosporin in the presence of clavulanic acid. For this reason, detection of ESBL, using conventional antimicrobial susceptibility methods and delay in the recognition and reporting of ESBL production by Gram negative bacilli is associated with prolonged hospital stay, increase morbidity, mortality and health care expenses. So, it becomes necessary to know the prevalence of these organisms and to formulate the treatment policy (Mehrgan and Rahbar, 2008).

The National Committee for Clinical Laboratory Standards (NCCLS) recommended that microbiology laboratories reported ESBL-producing isolates of E. coli and Klebsiella species are resistant to all penicillins, cephalosporins (including cefepime), and aztreonam, irrespective of their individual in vitro test results. The presence of ESBL in some K. pneumoniae and E. coli strains poses an important challenge in clinical practice, since these
organisms are common causes of serious infections (Mehrgan and Rahbar, 2008).

*Klebsiella* is ubiquitous in nature, in human they colonize the skin, pharynx or gastrointestinal tract (GIT), they may also colonize sterile wounds and urine, carriage rate varies with different studies. *Klebsiella* may be regarded as normal flora in many parts of colon, intestinal tract and in the biliary tract, oropharyngeal carriage has been associated with endothelial incubation, impaired host defenses and antimicrobial use. *K. pneumoniae* and *K. oxytoca* are the 2 members of the genus responsible for the most human infections. They are opportunistic pathogens found in the environment and in the mammalian mucosal surfaces. The principle pathogenic reservoirs of infection are the GIT of patient and the hands of personnel. Organisms can spread rapidly even leading to nosocomial outbreaks (Baron, *et al*., 1994).

Infection with *Klebsiella* organisms occur in the lung, where they cause destruction changes, necrosis, inflammation and hemorrhage occur within lung tissue, sometimes producing thick, bloody, mucoid sputum described as currant jelly sputum. Pneumonia caused by *Klebsiella* is a necrotizing process with predilection for debilitated people; it has a high mortality rate with approximately 50% even with antimicrobial therapy. The mortality rate approaches 100% for person with alcoholism and bactremia. *Klebsiella pneumoniae* infection it causing inflammation of lung characterized by fever, chills, muscle stiffness, chest pain, cough and short of breath. Pneumonia may be caused by bacteria, virus or fungus and sometimes by physical and chemical irritants. It occurs in patients with allergic but young children and the elderly as well as immunocompromised and immunodeficit patients are especially at risk. Septic patients may be at increase risk for acquiring antimicrobial resistant pneumonia because of the prior exposure to various types of antibiotics, factors that is known to play an important role in the generating of antimicrobial resistance. The bacterial spectrum and antimicrobial resistance may vary temporarily and geographically, each institution must undertake its own local evaluation. Such an evaluation may also be useful to detect emerging trends of antimicrobial resistance (Wagenlehner, *et al*., 2008).

*K. pneumoniae* are resistant to multiple antibiotics which is plasmid mediated propriety of *K. pneumoniae* due to production of beta lactamase enzyme. Carbapenemase producing *K. pneumoniae* is the recent addition to the pool of multi drug resistant nosocomial pathogen (Desphande, *et al*., 2006). Extensive use of broad spectrum antibiotic in hospitalized patients has lead to both increased carriage of *Klebsiella* and subsequently the development of
multidrug resistant strains that produce extended spectrum beta lactamase (ESBL). These strains are highly virulent show capsular type K55, and have an extraordinary ability to spread. Most outbreaks are due to single clone or single gene. The bowel is the major site of colonization with infection of the urinary tract, respiratory tract and wounds. Bactremia and significant increased mortality have resulted from infection with these species. In addition to prior antibiotic use, risk factors for infection include the presence of an indwelling catheter, feeding tube or central venous catheter, poor health status and treatment in intensive care units or nursing home. Acquisition of these species has major problem in most hospital because of resistance of multiple antibiotic and potential transfer of plasmid to other organisms (Tortora, et al., 2004). Morbidity and mortality rate are comparable to those for other Gram-ve organisms that cause sepsis and septic shock. In neonatal units, outbreaks caused by ESPL producing strains present a more serious problem and may be associated with increased mortality (Tortora, et al., 2004).

ESBLs are beta-lactamas that hydrolyse expanded spectrum cephalosporin with an oxyimino side chain these include cefotaxime, ceftriaxone and ceftazidime as well as the oxyimino-monobactam (aztreonam). The ESBLs confer resistant to these antibiotics and related oxyimino-beta lactams. Typically they derived from genes for TEM-1, TEM-2 or SHV-1 by mutations that alter the amino acid configuration around the active site of these beta lactamases. This extends the spectrum of β.lactam antibiotics susceptible to hydrolysis by these enzymes. An increasing number of ESBLs not of TEM or SHV lineage has recently been described. The ESBLs are frequently plasmid encoded (Emery and Weymouth, 1997). Carbapenems are the treatment of choice for serious infections due to ESBL producing organisms, yet carbapenem resistant isolates have recently been reported. ESBL producing organisms may appear susceptible to some extended spectrum cephalosporins. However, treatment with such antibiotic has been associated with high failure rates (Paterson, et al., 2003).

MATERIALS AND METHODS

In this study a one hundred sputum specimens were collected in sterile sputum container from patients suffering from lower respiratory tract infection (pneumonia) who attended Alshaab teaching hospital, Khartoum, Sudan. All sputum specimens were cultured on Blood agar and MacConkey agar medium under aseptic condition, both medium incubate aerobically at 37°C overnight, and then observed for the presence of bacterial growth. The identification of bacteria was carried out using different tests as described by Cowan.
**In vitro antimicrobial susceptibility test**
All isolated *K. pneumoniae* were subjected to antimicrobial susceptibility test using modified Kirby-Bauer disc diffusion method. The antimicrobials used were nitrofurantoin (300 μg/disc), co-trimoxazole (25 μg/disc), nalidixic acid (30 μg/disc) and imipenem (10 μg/disc).

**ESBL detection**
**Double disk synergy test**
Standard and modified double disk synergy test (DDST) was performed using disks of 30 mg each of cefepime (CPM), ceftriaxone (CI), ceftazidime (CAZ) and cefotaxime (CTX) along with amoxyclav (AMCA) (amoxycillin 20 mg+ clavulanic acid 10 mg). The disks were placed at a distance of 30 and 16 mm from each other (centre to centre) and incubated at 37°C overnight. The organism was considered harboring ESBLs, if the zone of inhibition around one or more of the four antibiotic discs (CAZ, CPM, CI and/or CTX disk) showed a clear cut increase towards the AMCA disk (Ananthakrishanan, *et al.*, 2000).

**RESULTS**
One hundred sputum specimens were collected from patients suffering from lower respiratory tract infection (pneumonia) who attended Alshaab teaching hospital. Among these patients, 69 (69%) were males and 31 (31%) were females, twenty one isolates were revealed, 15 isolates from male and only 6 isolates from female (Table. 1). The age of the patients ranges from (5 to 70) years. Out of the total 100 patients, 22 were children, 30 were adult, and 48 were elder. The results showed that the age group ranging between (41-70) years was more infected *K. pneumoniae* isolates from 16 patients.

Eighty five (85%) of the specimens showed significant growth, while the remaining 15 (15%) showed no growth. Out of the 85 positive cultures, 47 were lactose fermented (LF) while the remaining 38 were non lactose fermented (NLF). Out of the 47 lactose fermented isolates, 21 were *K.pneumoniae* according to morphological, microscopic and biochemical tests, which produce large (2-4mm) grey-white mucoid colonies on blood agar and large pink mucoid colonies on MacConkey agar and was Gram negative capsulated rods.

**Antibiotic susceptibility results**
The result of susceptibility testing against some different antibiotics nitrofurantoin, nalidixic acid, imipenem and co-trimoxazole) showed that the sensitivety rate of *K. pneumoniae* was
(100%) to imipenem and co-trimoxazole followed by (65%) to nitrofurantoin, while the resistance rate was (100%) to nalidixic Acid (Table. 2).

ESBL results
Screening test results
The result of standard disk diffusion (SDD) as screening method for identifying potential ESBL producers was done against group of third generation cephalosporine which include ceftazidime (CAZ), cefotaxime (CTX), ceftriaxone (CI) and cefepime (CPM) (Table. 3). The test showed that only 2 organisms from the 21isolated K. pneumoniae were resistant to all the investigated, while the remaining 19 were sensitive.

Double disk synergy test (DDST) results
The double disk synergy test (DDST) was done to these 2 organisms which showed resistant to the group of cephalosporine (3GC) antibiotics, which indicated that, these 2 organisms could be considered as ESBL producers.
None of the ESBL harbouring isolates was sensitive to combination of amoxacilin and clavulinic acid.

Table 1. Distribution of study samples and isolation rates according to gender:

<table>
<thead>
<tr>
<th>Gender</th>
<th>Frequency</th>
<th>Percent</th>
<th>Positive isolates</th>
<th>Negative isolates</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>69</td>
<td>69%</td>
<td>15</td>
<td>54</td>
<td>69</td>
</tr>
<tr>
<td>Female</td>
<td>31</td>
<td>31%</td>
<td>6</td>
<td>25</td>
<td>31</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>100%</td>
<td>21</td>
<td>79</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial susceptibility test results for K. pneumoniae isolates.

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>No. of isolates</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>21</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>17</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Nalidixic Acid</td>
<td>0</td>
<td>0</td>
<td>21</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3. Antimicrobial susceptibility of *K. pneumoniae* against third generation Cephalosporine.

<table>
<thead>
<tr>
<th>Type of antibiotic</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>19</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>19</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>19</td>
</tr>
<tr>
<td>Cefepime</td>
<td>19</td>
</tr>
</tbody>
</table>

DISCUSSION

This study was conducted to evaluate ESBL profile of *K. pneumoniae*. In this study one hundred sputum specimens were investigated, 85(85%) showed bacterial growth. When standard DDST (30mm distance center to center) was used as confirmatory test for detection of ESBL producing isolates, maximum of 2 isolates were detected as ESBL producers using ceftriaxone (CRO). However reducing the distance to 16mm center-to-center markedly increased the sensitivity of the test.

The distance between the disks is critical for each enzyme, as the test depends on the concentration of both beta-lactam antibiotic and inhibitor. Several attempts may be required to detect an ESBL producer. Several modifications, including the choice of drugs tested and varying the distance between the disks, have been recommended. Different distances, 30, 20 and 16 mm for detection of ESBL harbouringn isolates were tried. Best results were obtained by using 16mm distance center-to-center between the two disks.

Increasing the distance further between the two disks resulted in missing a large number of ESBL harbouring isolates. On the other hand, decreasing the distance further interfered with the interpretation of the results. A study from India done by Ananthakrishanan, *et al.* (2000) also used 16 mm distance and reported good results.

In the present study, the use of cefotaxime and ceftriaxone in the DDST resulted in the detection of a larger number of ESBLs harbouring isolates than ceftazidime. The results were similar to the combined disk method currently recommended by the NCCLS. Thus, for laboratories that perform susceptibility testing by disk diffusion, modified DDST could be easily incorporated into an already existing system. It has added benefit that there is no need to measure zone sizes hence removing the subjectivity and is easy to read by recording presence or absence of synergy. Further, it requires no extra time in setting up the test and reading it.
This study showed that all isolates were sensitive to the imipenem which are the most common alternative drugs used for treatment of ESBL producing bacteria. Similar results were observed by Kadar and Angamathu (2005) who revealed that, more than 89% of the ESBL producers were susceptible to imipenem and meropenem. However, the use of an alternative drug which is very broad spectrum and expensive drug as first line for treatment of ESBL-positive bacteria will significantly increases cost of treatment and will contribute to carbapenem resistance in other organism.

The much higher frequency of ESBLs of this study was found in inpatients’ samples this disagree with Sorlózano, et al (2004) who found the higher frequency in outpatient sample. This occurs because the 3rd generation cephalosporins is not indicated as a first-line drugs for infections in the outpatient setting and they are not, therefore, subjected to regular laboratory testing.

In this study several risk factors for infections due to ESBL-producing strains in hospitalized patients were identified: previous admission to a hospital, diabetes mellitus, recurrent UTI, fluoroquinolone used during the previous 2 months, and older age in male patients, so this result was similar to that it obtain by Rodríguez-Baño, et al., (2004). In the course of risk factors for infections with ESBL-producing organisms, it should be noted that the medium age of our patients was 45 years, and male patients were more frequently infected with ESBL-producing strains.

CONCLUSION

In conclusion, *Klebsiella pneumoniae* was found to constitute about 21% of the respiratory tract infected patients among the study population. Two isolates were found to be extended spectrum β-lactamase producers, which explained their resistance to 3GC.

**Recommendations**

- The generates results suggested that additional testing to detect ESBL production in the clinical isolates on a routine basis would be necessary to institute appropriate antibiotic therapy.
- Formulation of proper antibiotic policy and providing appropriate guidelines to prescribe antibiotics can prevent the spread of multidrug resistant organisms in the hospital as well as in the community.
- Proper infection control practices and barriers are essential to prevent spreading and
outbreaks of ESBL producing bacteria. In laboratories where special tests for ESBL detection are not available and interpretative comment should accompany the susceptibility results to indicate that the antibiogram is suggestive of ESBL production, and the physician should consider avoiding therapy with expanded-spectrum cephalosporins or aztreonam.

- Further studies are needed to detect ESBL types in terms of highly different geographical dissemination of these isolates.

REFERENCES


