Prevalence and Antimicrobial Susceptibility of Extended-spectrum β- Lactamase- producing \textit{klebsiella pneumoniae} at a Microbiology Diagnostic Center in Kashmir

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ABSTRACT

Objectives: To determine the prevalence and antimicrobial susceptibility of extended-spectrum β- lactam (ESBL) producing \textit{Klebsiella pneumoniae} isolated from different clinical specimens.

Methods: A total of 144 (10.2%) isolates of \textit{Klebsiella pneumoniae} were recovered from 1409 different clinical specimens like urines, wound swabs, sputum and blood at Al-Haram Diagnostic, Research and Training Center, Kashmir, India over a period of 24 months from December, 2005 to November, 2007. All these isolates were tested for ESBL production.

Results: Of 144 isolates of \textit{K. pneumoniae}, 18 (12.5%) were positive for ESBL production. Most ESBL producers (50%) were from urines, followed by infected wounds (22.2%), sputum (16.7%) and blood (11.1%). ESBL producing strains of \textit{K. pneumoniae} showed the highest susceptibility to imipenem (~89%). The non β- lactam antibiotics with greatest activity against these ESBL strains were ciprofloxacin (72%), Amikacin (66%), tobramycin (61 %) and gentamicin (50%).
Conclusion: Our findings demonstrate a high percentage of ESBL producers among clinical isolates of *K. pneumoniae* and a high rate of multidrug resistance. Two (11.1%) of strains of ESBL producing *K. pneumoniae* were resistant to imipenem. Therefore, regular monitoring of imipenem sensitivity and routine testing of newer carbapenems like meropenem and ertapenem should be carried out further. (Rawal Med J 2009;34:68-72).

Key Words: *K. pneumoniae*, ESBLs, Multidrug resistance.

INTRODUCTION

*K. pneumoniae* is well known as a cause of community-acquired bacterial pneumonia occurring mostly in alcoholics, persons with diabetes mellitus and those with underlying chronic bronchopulmonary disease.\(^1\)\(^-\)\(^2\) The main population at risk is neonates, immunocompromised hosts and patients predisposed by prior surgery, diabetes, malignancy etc.\(^3\) It is also responsible for nosocomial epidemic infections in hospitals, particularly in intensive care and pediatrics units.\(^4\)\(^-\)\(^5\) Extensive and often indiscriminate use of the late-generation (extended-spectrum), cephalosporin in particular, Ceftazidime, Cefotaxime and Ceftriaxone, is associated with the emergence and spread of multi-drug resistant *K. pneumoniae*.\(^6\) There is a growing concern for the increasing antimicrobial resistance among the ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*.\(^7\) Most ESBLs are mutan forms of TEM-1, TEM-2 and SHV-1 enzymes coded by genes located on transferable plasmids that can be easily spread from one organism to another.\(^8\) These enzymes, which are most commonly produced by *Escherichia coli* and *Klebsiella pneumoniae*, are capable of inactivating a variety of β-lactam drugs, including third-generation cephalosporins, extended-spectrum penicillins and monobactams.\(^9\) The therapeutic choices in infections caused by ESBL-producing organisms are limited because of cross-resistance.\(^10\) The carbapenemes are the most active antibiotics against these organisms.\(^11\)\(^-\)\(^12\)
determine the prevalence of ESBLs among the clinical isolates of *K. pneumoniae* from patients at a Microbiology Diagnostic Center in Kashmir, India.

**METHODS**

A total of 1409 different clinical specimens like urines, wound swabs, sputum and blood received at Al-Haram Diagnostic, Research and Training Center, Kashmir, India over a period of 24 months from December, 2005 to November, 2007 were processed. All specimens were processed according to standard procedure.\(^1\) *Klebsiella pneumoniae* isolates were identified using standard techniques.\(^2\) Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion technique on Muller-Hinton agar and commercial antibiotic discs (Oxoid, UK) were used for antimicrobial testing.\(^3\) The antibiotic discs used were Ampicillin (10 µg), Amoxicillin-Clavulanic Acid (20/10 µg), Naldixic Acid (30 µg), Norfloxacin (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Tobramycin (30 µg), Trimethoprim-Sulphamethoxazole (1.25/ 23.75 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg), Cefepime (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Cefuroxime (30 µg), Aztreonam (30 µg) and Imipenem (10 µg). The antibiotic disc impregnated culture plates were incubated at 37°C for overnight. The diameter of the zone of inhibition was measured and recorded as resistant or susceptible according to the National Committee for Clinical Laboratory Standards (NCCLS) interpretative criteria.\(^4\)

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Number (%) of ESBL-producing <em>K. pneumoniae</em></th>
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<tbody>
<tr>
<td>Urine</td>
<td>09 (50.0)</td>
</tr>
<tr>
<td>Wound swabs</td>
<td>04 (22.2)</td>
</tr>
<tr>
<td>Sputum</td>
<td>03 (16.7)</td>
</tr>
<tr>
<td>Blood</td>
<td>02 (11.1)</td>
</tr>
<tr>
<td><strong>Total number</strong></td>
<td>18 (100)</td>
</tr>
</tbody>
</table>
A total of 144 isolates of *K. pneumoniae* were tested for ESBL production. Minimum Inhibitory Concentration (MIC) against Cefotaxime, Ceftazidime, Ceftriaxone and Cefepime was carried out by agar dilution method for all isolates positive for ESBL. Testing for ESBL production was carried out using Muller-Hinton agar plates that were inoculated with standardized inoculum conforming to 0.5 Mc Farland standards of the suspected ESBL strain (as screened by the Kirby –Bauer disk diffusion technique)\textsuperscript{15} to form a lawn culture. Separate commercial discs containing Cefotaxime (30 µg), and Ceftazidime (30 µg) with and without clavulanic acid (10 µg) (Oxoid, UK) were placed over the lawn culture. A distance of 15 mm between the discs was maintained. An increase in zone size of more than or equal to 5 mm for cefotaxime and ceftazidime with and without clavulanic acid was considered to indicate ESBL producing strain.

**RESULTS**

A total of 144 (10.2%) isolates of *K. pneumoniae* were recovered from 1409 different clinical specimens. Of these, 18 (12.5%) isolates were positive for ESBL production. The ESBL- producing strains of *K. pneumoniae* were recovered from all specimen sources, with the majority (9 [50%]) from urine of patients with urinary tract infection. Four (22.2%) were from infected wounds, 3 (16.7%) from patients with lower respiratory tract infections and 2 (11.1%) from the blood of bacteremic patients (Table-1).

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Minimum inhibitory concentration (No. of isolates)</th>
</tr>
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<tbody>
<tr>
<td>Cefotaxime</td>
<td>≥256mcg/ml (18)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≥256mcg/ml (18)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≥256mcg/ml (18)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≥256mcg/ml (18)</td>
</tr>
</tbody>
</table>

Table 2. MIC of extended-spectrum β-lactamase producing strains of *K. pneumoniae*. 
All these ESBL-producing *K. pneumoniae* were found to be resistant to 3rd Generation Cephalosporins (cefotaxime, ceftazidime and ceftriaxone) and these isolates showed multidrug resistance. All showed an MIC of >256 mcg/ml against cefotaxime, ceftriaxone, ceftazidime and cefepime (Table-2). ESBL producing isolates showed the highest susceptibility to imipenem (~89%). Ciprofloxacin (72%), Amikacin (~67%), Tobramycin (61%) and Gentamicin (50%) showed greatest in vitro activity against the ESBL strains (Table-3).

**DISCUSSION**

The ESBL-producing organisms are often multidrug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics. The prevalence of ESBL-producing *K. pneumoniae* in our study was found to be 12.5 % (18 of 144). Our findings are similar to reported in a study from Saudi Arabia, higher than those reported from Trinidad and India and much lower than those reported from a teaching hospital in Saudi Arabia.

Among the different clinical specimens the β-lactamase producing strains of *K. pneumoniae* were found most commonly in isolates from cases of urinary tract infection (50%) followed by wound infections (22%) and respiratory tract infections (16.7%).

**Table 3.** Non-β –lactam antibiotic active against extended-spectrum β-lactamase producing *K. pneumoniae*.

<table>
<thead>
<tr>
<th>Antimicrobial Agent</th>
<th>Number (Percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>16 (88.8)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>13 (72.2)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>12 (66.6)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>11 (61.1)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>9 (50.0)</td>
</tr>
</tbody>
</table>

Of all the antimicrobial agents tested, imipenem had the highest activity against the ESBL – producing *Klebsiella pneumoniae* (~89%), similar to other studies. The carbapenems are known to be stable against ESBL enzymes and effective in the treatment of infections caused
by ESBL-producing bacteria.\textsuperscript{23} The non-\(\beta\)-lactam antibiotics with the greatest activity against the ESBL producing strains of \textit{K. pneumoniae} in our study were ciprofloxacin (72.2%), followed by Amikacin (66.6%), Tobramycin (61.1%) and Gentamicin (50%). These antibiotics could be used as effective alternatives in the treatment of patients infected with ESBL strains of \textit{K. pneumoniae}.

In conclusion, our study showed a high percentage of ESBL producers among clinical isolates of \textit{K. pneumoniae} and a high rate of multidrug resistance. As the available treatment options are limited, prevention of ESBL infections by restricting the use of antimicrobial agents along with implementation of infection control measures remains of primary importance. Because of the new challenges presented by the changing nature and distribution of these enzymes, clinicians should be familiar with the clinical importance of these enzymes and potential strategies for dealing with them. In addition, regular monitoring of imipenem sensitivity and routine testing of newer carbapenemes like meropenem and ertapenem should be carried out further.

REFERENCES


16. Performance standards for antimicrobial susceptibility testing. Tenth information


