EXTENDED SPECTRUM β-LACTAMASES IN URINARY ISOLATES OF ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE - PREVALENCE AND SUSCEPTIBILITY PATTERN IN A TERTIARY CARE HOSPITAL

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Abstract

A total of 411 urinary isolates (353 Escherichia coli and 58 Klebsiella pneumoniae) were studied for extended spectrum β-lactamase (ESBL) production by double disk approximation test and NCCLS confirmatory test. ESBL production was found to be 41% in E.coli and 40% in K.pneumoniae. Fourteen percent and 12% of ESBL producers showed false susceptibility to ceftazidime and cefotaxime in routine susceptibility testing. The susceptibility of ESBL producers to imipenem, nitrofurantoin and amikacin was found to be 100%, 89% and 86% respectively. A high degree of associated resistance to gentamicin, co-trimoxazole and quinolones was found in ESBL producers. Majority of ESBL producers was detected among patients admitted in medical ICU and surgery ward.

Key words: ESBL, Escherichia coli, Klebsiella pneumoniae

Extended spectrum β-lactamases (ESBLs) are defined as β-lactamases capable of hydrolyzing oxyiminocephalosporins and are inhibited by β-lactamase inhibitors. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years resulting in limitation of therapeutic options. Microorganisms responsible for urinary tract infection (UTI) such as E.coli and Klebsiella spp. have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat. There are not enough data on the prevalence of ESBL producers in urinary tract infection in South India. Hence, the present study was undertaken to find out prevalence of ESBL producers in urinary isolates of E.coli and K. pneumoniae and also their susceptibility to non-β-lactam antibiotics.

Materials and Methods

Between January and December 2002 a total of 2261 urine samples were processed for significant bacteruria in the department of microbiology from patients clinically suspected to have UTI. All E.coli (353) and K.pneumoniae (58) isolated in significant numbers were included in the study. Clinico- demographic data of study patients was noted. Chi-square test was used to analyze the susceptibility pattern of non β-lactam antibiotics in ESBL producers and non-producers.

Antibiotic susceptibility testing

The above isolates were tested for antimicrobial susceptibility by disc diffusion technique according to NCCLS guidelines. The following antibiotic discs (drug concentration in µg) were used: amikacin (30), ceftazidime (30), cefotaxime (30), co-trimoxazole (25), gentamicin (15), imipenem (10), ciprofloxacin (5), nalidixic acid (30), norfloxacin (10) and nitrofurantoin (300).

Test for ESBL production

a) Double disc approximation test

The organism was swabbed on to a Mueller-Hinton agar plate. Antibiotic discs of amoxicillin/clavulanic acid (20/10 µg) and cefotaxime (30 µg) were placed at a distance of 15 mm apart and incubated. Organism that showed a clear extension of cefotaxime inhibition zone towards the disc containing clavulanate was considered as ESBL producer.

b) NCCLS confirmatory test

While performing antibiotic testing, ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30/10 µg) were placed on Mueller-Hinton agar and incubated. Organism was considered as ESBL producer if there was a ≥ 5 mm increase in zone diameter of ceftazidime/clavulanate disc and that of ceftazidime disc alone. Escherichia coli ATCC 25922 and Klebsiella pneumoniae strain 48188 (Jacoby GA USA) were used as negative and positive controls respectively.

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Results

Of the 2261 urine samples processed, 715 samples yielded various bacterial isolates. There were 353 *E. coli* and 58 *K. pneumoniae* among them. ESBL production was observed in 41% of *E. coli* (143/353) and 40% of *K. pneumoniae* (23/58) by NCCLS confirmatory test. The double disk approximation test failed to detect ESBLs in four isolates of *E. coli* and one *K. pneumoniae*. These ESBL positive isolates were obtained from 72 male and 94 female patients with a male female ratio of 1:1.3. They were distributed in the age group of 1 month to 77 years. Ninety two percent of ESBL producers were from in-patients.

The antimicrobial susceptibility results of ESBL producers are shown in figure. Susceptibility of ESBL producers to imipenem, nitrofurantoin and amikacin were found to be 100%, 89% and 86% respectively. False susceptibility to ceftazidime and cefotaxime were observed in 14% and 12%.

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Sensitivity to non-β lactam antibiotics varied from 4 to 26%. Co-resistance to non-β lactam antibiotic was observed more (p<0.01) with ESBL producers (Table). Forty six percent and 30% of ESBL producers were obtained from patients admitted in ICU and surgery units.

Discussion

ESBLs are now a problem in hospitalized patients throughout the world. The prevalence of ESBLs among clinical isolates vary greatly world wide and are rapidly changing over time. The occurrence of ESBL producers in urinary isolates of *E. coli* and *K. pneumoniae* in our study was found to be 41 and 40% respectively. This is higher than the reported figures of *E. coli* and *K. pneumoniae* in USA (2.2/6.6%), Canada (2.7/6.2%) and India (24.7/38.5). Much higher (58%) prevalence of ESBL producers in

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>ESBL producers (n=166)</th>
<th>Non-producers (n=245)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>147</td>
<td>89</td>
</tr>
<tr>
<td>Amikacin</td>
<td>143</td>
<td>86</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>42</td>
<td>25*</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>43</td>
<td>26*</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>15</td>
<td>9*</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10</td>
<td>6*</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>6</td>
<td>4*</td>
</tr>
</tbody>
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* p<0.01
ESBL production coexisted with resistance to several other antibiotics. ESBLs are encoded by plasmids, which also carry resistant genes for other antibiotics. We found such associated resistance with co-trimoxazole - 74%, gentamicin - 75% and fluoroquinolones - 91-96% (p<0.01). Other workers in India have reported such association only with gentamicin. Admission in ICU and surgery were found to be the risk factors for ESBL production in our study.

ESBL production has been observed in large percentage of urinary isolates. Patients infected with these strains cannot be treated with β-lactam antibiotics and monobactams. Since co-resistance to non-β-lactam antibiotics like norfloxacin, co-trimoxazole and gentamicin was observed, amikacin and nitrofurantoin are found to be alternatives for treating such patients at low cost.

References