Comparative in vitro activity of beta-lactam/beta-lactamase inhibitor combinations against Gram negative bacteria

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Background & objectives: Currently, the use of beta-lactamase inhibitors in combination with beta-lactam antibiotics represents an effective measure to combat a specific resistance mechanism of beta-lactamase producing organisms. Knowledge about the susceptibility profile of bacteria to different combination agents available is essential to guide appropriate treatment of severe infections in hospitalized patients. The present study compares the in vitro activity of three commercially available beta-lactam/beta-lactamase inhibitor combinations (piperacillin/tazobactam, cefoperazone/sulbactam, ticarcillin/clavulanic acid) against β-lactamase producing Gram negative bacteria in a tertiary care hospital in north India.

Methods: A total of 9004 consecutively isolated extended spectrum beta-lactamase (ESBL) producing Gram negative bacteria isolated from various clinical samples from patients admitted to the All India Institute of Medical Sciences, New Delhi, from September 2003 to August 2004 were included in the study. These isolates were screened for ESBL production by the inhibitor based test recommended by the National Committee for Clinical Laboratory Standards (NCCLS). Antibiotic susceptibility testing was carried out by disc diffusion method as per NCCLS guidelines.

Results: Of the 9004 isolates tested, 3232 (35.89%) were sensitive and 568 (6.31%) were resistant to all three combination agents, and rest 5204 (57.80%) were resistant to at least one of the combinations. Susceptibility to piperacillin/tazobactam, cefoperazone/sulbactam, and ticarcillin/clavulanic acid was 81.37, 76.06 and 45.48 per cent respectively. Piperacillin/tazobactam exhibited significantly (P<0.05) greater antimicrobial activity against Pseudomonas spp., Escherichia coli and Klebsiella spp. compared to cefoperazone/sulbactam.

Interpretation & conclusion: Overall piperacillin/tazobactam was observed to be the best combination agent followed by cefoperazone/sulbactam in our setting. This difference in activities of these combination agents needs to be evaluated further by ascertaining their efficacy in clinical studies.

Key words Beta-lactam/beta-lactamase inhibitors - cefoperazone/sulbactam - extended spectrum beta-lactamase - piperacillin/tazobactam - ticarcillin/clavulanic acid
With the extensive use of third and fourth generation cephalosporins as an important component of empirical therapy in intensive care units and high risk wards, resistance to these drugs has become a major problem all over the world\textsuperscript{1,2}. Resistance has developed in bacteria by possessing extended spectrum beta-lactamases (ESBLs) capable of hydrolyzing these newer cephalosporins. Beta-lactamase mediated resistance may be overcome by combining beta-lactam antibiotics with beta-lactamase inhibitors which bind irreversibly to the beta-lactamases and render them inactive thus sparing the beta-lactam antibiotic. Currently, three beta-lactamase inhibitors (tazobactam, sulbactam and clavulanic acid) are in clinical use, and in combination with beta-lactam antibiotics, represent a successful strategy to combat a specific resistance mechanism\textsuperscript{2}. With a high prevalence of infections due to ESBL positive bacteria in our hospital\textsuperscript{3-5}, a parallel increase in the use of these combinations is being observed. Hence there is a need to determine the susceptibility pattern of different microorganisms against the commercially available combination agents, a knowledge of which is essential to guide empiric as well as appropriate therapy of severe infections in hospitalized patients. The present study was carried out to evaluate the comparative in vitro activities of three commercially available beta-lactam/beta-lactamase inhibitor combinations: piperacillin/tazobactam (P/T), cefoperazone/sulbactam (C/S), ticarcillin/clavulanic acid (T/C) against ESBL producing bacteria causing infections in a tertiary care hospital in north India.

Material & Methods

The retrospective study was conducted in the Clinical Bacteriology Laboratory of the All India Institute of Medical Sciences, New Delhi, India, over a one year period from September 2003 to August 2004. Samples (blood, urine, tracheal aspirates/bronchoalveolar lavage, soft tissue samples and sterile body fluids) received from in-patients of the hospital were processed for isolation and identification of bacterial pathogens according to standard microbiological techniques\textsuperscript{6}.

All Gram negative bacteria isolated from these samples were tested for ESBL production by using four disks (concentration in µg) ceftazidime [HiMedia, Mumbai, India (30)], ceftazidime/clavulanic acid [Pfizer, India (30/10)], cefotaxime [HiMedia, Mumbai, India (30)], and cefotaxime/clavulanic acid [Pfizer, India (30/10)]. \textit{Escherichia coli} ATCC 25922 (beta-lactamase negative), \textit{Pseudomonas aeruginosa} ATCC 27853 (beta-lactamase negative), and \textit{Klebsiella pneumoniae} ATCC 700603 (ESBL positive) strains were used as control organisms. The performance and interpretation of the test was done as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines\textsuperscript{7}.

Antimicrobial sensitivity testing was performed on Mueller Hinton agar (MHA) (HiMedia, Mumbai, India) plates by the disk diffusion method according to NCCLS guidelines\textsuperscript{7}. The following disks (drug concentrations in µg) were tested - piperacillin/tazobactam, [Oxoid, United Kingdom (100/10)]; cefoperazone/sulbactam, [BBL, Becton Dickinson, India (75/10)]; ticarcillin/clavulanic acid, [Oxoid, United Kingdom (75/10)]; amikacin, (30); ciprofloxacin, (5) and meropenem (10) all from HiMedia, Mumbai, India. The diameter of the zones of inhibition of growth was recorded and interpreted as sensitive, intermediate resistant or resistant based on the NCCLS guidelines\textsuperscript{7}. Organisms with “intermediate” levels of resistance to the antibiotics were included in the percentage of resistant organisms for final analysis.

Statistical analysis: Statistical analysis was carried out using Chi-square test.

Results & Discussion

A total of 80,116 samples were received in the Bacteriology Laboratory from intensive care units (ICUs), medical oncology, surgery and medicine wards during the study period. Of these, there were 27,462 blood samples, 23,083 urine samples, 11,013 soft tissue samples, 10,411 tracheal aspirates/bronchoalveolar lavage samples, and 8147 sterile body fluids.
A total of 13,091 Gram-negative bacteria were isolated, of which 9004 (68.78%) were found to be ESBL producers. Overall, P/T exhibited the best activity (81.37% organisms susceptible) followed by C/S (76.06% organisms susceptible). Ticarcillin/clavulanic acid (45.48% organisms susceptible) was found to have a poor activity against all the organisms (Table). When P/T and C/S were compared, P/T exhibited significantly greater antimicrobial activity against *Pseudomonas* spp, *Esch. coli* and *Klebsiella* spp, whereas C/S exhibited the same against *Acinetobacter* isolates. P/T exhibited a marginally better activity against *Enterobacter* and *Citrobacter* spp, whereas both P/T and C/S demonstrated equal activity against *Proteus* spp. All the three agents demonstrated best activity against *Proteus* spp. In total, 3232 (35.89%) ESBL positive bacteria were sensitive and 568 (6.31%) were resistant to all three combinations, and rest 5204 (57.80%) were resistant to at least one of the combinations. The susceptibility rates of the isolates to other antibiotics tested were as follows: amikacin (5041/9004, 56%), ciprofloxacin (1536/9004, 17.06%) and meropenem (8286/9004, 92.02%).

The present study demonstrated that in vitro P/T was the best combination followed by C/S, a finding similar to others. In another study, however, P/T exhibited greater in vitro activity only against *Esch. coli* and *P. vulgaris* as compared to piperacillin/sulbactam. Both combinations, though were equally effective against the other *Enterobacteriaceae* and *P. aeruginosa* isolates. The relatively superior activity of sulbactam against *Acinetobacter* isolates observed in our study was at par with others including one previous study from our institute. Sulbactam may have higher intrinsic activity against *Acinetobacter* isolates and thus may be considered a drug of choice where *Acinetobacter* is a suspected pathogen.

In a controlled clinical trial, P/T and T/C were found to be almost equally effective for the treatment of infections caused by beta-lactamase producing strains. The overall efficacy rates were 90.5 per cent for the P/T group and 88.5 per cent for the T/C group, respectively. In contrast, another clinical trial found T/C to be associated with a poor outcome as compared to P/T.

Studies comparing the relative efficacy of different combination agents against different organisms from India are limited. An in vitro study from south India on a limited number of *P. aeruginosa* isolates revealed a resistance rate of 12, 20 and 36 per cent respectively to P/T, C/S and T/C indicating P/T to be the best combination. Similarly, another study from central India on *E. coli, Klebsiella* and nonlactose fermenters reported a lower resistance to P/T (14.7-20.5%) as compared to C/S (27-34.2%).

In conclusion, the results of our in vitro study indicated that of the currently available β-lactam/β-lactamase inhibitor combinations, piperacillin/tazobactam has the best activity against nosocomial Gram-negative pathogens followed by cefoperazone/

### Table. In vitro activity of piperacillin/tazobactam (P/T), cefoperazone/sulbactam (C/S) and ticarcillin/clavulanic acid (T/C) against ESBL positive organisms

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ESBL positive</th>
<th>P/T</th>
<th>C/S</th>
<th>T/C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas</em> spp.</td>
<td>2479</td>
<td>1994 (80.43)</td>
<td>1398 (56.39)*</td>
<td>924 (37.27)</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>2251</td>
<td>1705 (75.74)</td>
<td>1953 (86.76)*</td>
<td>1084 (48.15)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>2081</td>
<td>1725 (82.89)</td>
<td>1669 (80.20)*</td>
<td>969 (46.56)</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>1272</td>
<td>1113 (87.50)</td>
<td>1050 (82.55)*</td>
<td>647 (50.86)</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp.</td>
<td>460</td>
<td>397 (86.30)</td>
<td>388 (84.35)</td>
<td>188 (40.87)</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp.</td>
<td>264</td>
<td>213 (80.68)</td>
<td>210 (79.54)</td>
<td>131 (49.62)</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>197</td>
<td>180 (91.37)</td>
<td>181 (91.87)</td>
<td>152 (77.16)</td>
</tr>
<tr>
<td>Total</td>
<td>9004</td>
<td>7327 (81.37)</td>
<td>6849 (76.06)</td>
<td>4095 (45.48)</td>
</tr>
</tbody>
</table>

*P<0.05 compared to P/T
sulbactam. The difference in activities in these combination agents needs to be evaluated further by ascertaining their efficacy in clinical study.

References


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