In vitro Activity of Ceftriaxone Plus Tazobactam Against Members of Enterobacteriaceae

S Krishna Prakash*, Vijay Arora**, Ram Prashad***, VK Sharma****

Abstract

Aims: To study the in vitro activity of ceftriaxone alone and in combination with the β-lactamase inhibitor tazobactam against bacterial isolates belonging to the Family Enterobacteriaceae.

Methods: One hundred and five consecutive isolates of Escherichia coli and Klebsiella spp. that had been recovered from various high-risk areas of the hospital were included in the study. MIC estimation to ceftriaxone and a combination of ceftriaxone and tazobactam was performed by the agar dilution method.

Results: By the MIC studies, 88.6% of the strains appeared to be resistant to ceftriaxone with the MIC90 value being >256 µg / ml. When the MIC were done to ceftriaxone in combination with tazobactam, the resistance rate dropped to 4.8% with the MIC90 value being 4.0 µg / ml.

Conclusion: The combination of ceftriaxone and tazobactam appears to be an excellent therapeutic alternative with 94.6% of ceftriaxone resistant strains being susceptible in vitro to this combination. ©

INTRODUCTION

The third generation cephalosporins were introduced into clinical practice in the early 1980s, and since then, they have served as efficacious and fairly safe agents for the management of many serious infections.1 The recent appearance of Extended Spectrum Beta Lactamases (ESBLs), which are capable of conferring resistance to these agents in some Enterobacteriaceae, has compromised the effectiveness of the third generation cephalosporins in clinical practice.2,3 However combinations of β-lactam antibiotics and β-lactamase inhibitors seem to have addressed this issue and have proved useful in treating infections caused by ESBL producing bacteria.4 This in turn has generated considerable interest in combinations of some third generation cephalosporins with β-lactamase inhibitors such as Clavulanic acid, Sulbactam or Tazobactam, which are capable of inhibiting many of the novel ESBLs.5 The efficacy of a combination of Ceftriaxone with Tazobactam has been evaluated in certain animal models6,7 and in certain bacterial species.8 However no such work has been carried out in India to the best of our knowledge. The present study was therefore undertaken to compare the in-vitro activity of Ceftriaxone alone and in combination with Tazobactam against members of Enterobacteriaceae.

MATERIALS AND METHODS

One hundred and five consecutive isolates of Escherichia coli and Klebsiella species that had been recovered from various high risk areas of the hospital like Burns ward, post operative ward, intensive care units etc. were included in the study. All the isolates had been identified by conventional methods9 and their antimicrobial susceptibility patterns were determined by the disc diffusion method using the Stokes technique10 to 10 antimicrobial agents. The minimum inhibitory concentrations (MICs) of all the strains to Ceftriaxone alone and in combination with Tazobactam were determined by the agar dilution method as per the standard NCCLS guidelines.11 Ceftriaxone and Tazobactam were tested in combination in a ratio of 8:1, as has been reported.7 Pure powders of ceftriaxone and tazobactam were supplied by Aristo Pharmaceuticals Ltd.

RESULTS

Of the 105 total strains included in the study 47 were Escherichia coli, 48 were Klebsiella pneumoniae and the remaining 10 were Klebsiella oxytoca. The results of their antimicrobial susceptibility is shown in Table 1. The rates of resistance to nearly all the antimicrobials with the exception of the carbapenems: imipenem and meropenem appeared to be high. While 98.0% of the isolates were resistant to amoxycillin, the corresponding figure for cephalexin was 88.6%. For the 3rd generation
Cefalosporins cefotaxime and ceftriaxone the resistance rates were seen to be 79.0% and 77.1% respectively, while for cefpirome, a fourth generation cephalosporin, the figure was slightly lower at 69.5%. Although the resistance rate was comparatively lower at 40.4% for amikacin, the corresponding figure or gentamicin was 73.3%. For the fluoroquinolone, Ciprofloxacin a resistance rate of 62.9% was seen.

The results of the MIC studies are summarized in Table 2. The MIC range for Ceftriaxone was ≤ 0.25 - >256 µg/ml. 93 strains (88.6%) appeared to be resistant (MIC > 64 µg / ml). It was disturbing to note that the MIC50 and MIC90 values were both very high at 256 µg / ml and > 256 µg / ml respectively.

In contrast when ceftriaxone was combined with tazobactam, only 05 strains (4.8%) appeared to be resistant. Although the MIC range was ≤ 0.25 - 128 µg/ml (4 of the 5 resistant strains had an MIC value of 128 µg / ml), the MIC50 and MIC90 values were considerably lower at 2.0 µg / ml and 4.0 µg / ml respectively.

As a result considerable interest is now being generated in combinations of some third generation cephalosporins with β lactamase inhibitors like sulbactam, clavulanic acid or tazobactam, which inhibit many of the ESBLs. 5

Although the efficacy of combination of ceftriaxone and Tazobactam has been evaluated in certain bacterial species like Bacteroides fragilis and Legionella spp there are no reports on the in vitro activity of this combination on isolates belonging to the family Enterobacteriaceae. The only study done using a diffusion chamber in rabbits employed only six strains in all.7 It was with this background that this study was initiated.

All the 105 isolates included in this study had been recovered from various high-risk areas of the hospital. It was therefore not surprising that by the disc diffusion method, the strains showed a high resistance to most of the antimicrobials against which they were tested; nevertheless all the strains were uniformly sensitive to imipenem and meropenem (Table 1). Eighty-one strains (77.1%) appeared to be resistant to ceftriaxone by the disc diffusion method. However, when the MIC of this agent was estimated, a total of 93 strains (88.6%) appeared to be resistant to ceftriaxone (Table 2: - strains exhibiting an MIC value of > 64 µg / ml were considered resistant).

By the MIC studies it was seen that twelve strains in all were sensitive to ceftriaxone (11.4%) i.e. MIC levels < 8 µg / ml. Needless to say all these strains were sensitive to a combination of ceftriaxone and tazobactam too. Also, all the strains were sensitive to ceftriaxone by the disc

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Escherichia coli n=47</th>
<th>Klebsiella pneumoniae n=48</th>
<th>Klebsiella oxytoca n=10</th>
<th>Total Strains n=105</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>97.9</td>
<td>97.7</td>
<td>100.0</td>
<td>98.0</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>89.4</td>
<td>85.4</td>
<td>100.0</td>
<td>88.6</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>74.5</td>
<td>47.9</td>
<td>80.0</td>
<td>62.9</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>74.5</td>
<td>66.7</td>
<td>100.0</td>
<td>73.3</td>
</tr>
<tr>
<td>Amikacin</td>
<td>31.9</td>
<td>39.6</td>
<td>80.0</td>
<td>40.4</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>82.9</td>
<td>72.9</td>
<td>90.0</td>
<td>79.0</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>80.9</td>
<td>70.8</td>
<td>90.0</td>
<td>77.1</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>70.2</td>
<td>64.6</td>
<td>90.0</td>
<td>69.5</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

The increasing use of the new generations of cephalosporins is not without its own share of problems - it may actually create a high risk for the very efficient selection of members of the family Enterobacteriaceae harbouring β-lactamases with an extended spectrum activity.

The first observation of Klebsiella species producing ESBL was made early as in 1983 in Germany.12 Soon ESBL producing Enterobacteriaceae were reported from Europe13 USA14 and South America.15 The first Indian report of ESBL producing gram-negative bacilli was from Delhi in 1997,16 followed by reports from other parts of the country.17,18

ESBL producing bacteria are a matter of concern to the clinician since they are also associated with resistance to other non β lactam antibiotics like aminoglycosides, chloramphenicol and tetracycline.19 This makes the therapeutic options available very limited.

### Table 1: Antimicrobial susceptibility of bacterial isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Escherichia coli</th>
<th>Klebsiella pneumoniae</th>
<th>Klebsiella oxytoca</th>
<th>Total Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxycillin</td>
<td>97.9%</td>
<td>97.7%</td>
<td>100.0%</td>
<td>98.0%</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>89.4%</td>
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<td>100.0%</td>
<td>88.6%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
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<td>73.3%</td>
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<td>82.9%</td>
<td>72.9%</td>
<td>90.0%</td>
<td>79.0%</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>80.9%</td>
<td>70.8%</td>
<td>90.0%</td>
<td>77.1%</td>
</tr>
<tr>
<td>Cefpirome</td>
<td>70.2%</td>
<td>64.6%</td>
<td>90.0%</td>
<td>69.5%</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
</tbody>
</table>

Table 2: MIC values of strains to ceftriaxone and ceftriaxone in combination with tazobactam

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>MIC Range (in µg/ml)</th>
<th>MIC50 (in µg/ml)</th>
<th>MIC90 (in µg/ml)</th>
<th>% of strains resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>≤ 0.25 - &gt; 256</td>
<td>256</td>
<td>&gt;256</td>
<td>88.6</td>
</tr>
<tr>
<td>Ceftriaxone + Tazobactam</td>
<td>≤ 0.25 - 128</td>
<td>2.0</td>
<td>4.0</td>
<td>4.8</td>
</tr>
</tbody>
</table>

Note: Values of ≤ 8 µg / ml have been taken as sensitive and ≥ 64 µg/ml as resistant.
The details of these strains are shown in Table 3.

The MIC estimation of ceftriaxone in combination with tazobactam demonstrated that of the total 105 isolates, only 5 strains (4.8%) appeared to be resistant. This actually meant that of the 93 strains that appeared resistant (MIC > 64 µg / ml) to ceftriaxone, 88 strains (94.6%) became susceptible when ceftriaxone was combined with the β-lactamase inhibitor tazobactam. All these strains exhibited a four fold or greater drop in their MIC values.

Tazobactam has been shown to be a more effective β-lactamase inhibitor than sulbactam and further more, both tazobactam and clavulanic acid are potent inhibitors of not only the conventional spectrum β-lactamases but also of the newer enzymes; also, tazobactam has been shown to be more active than clavulanic acid against OXA - 2 and OXA - 5 enzymes.20

The details of the 5 strains that appeared resistant to ceftriaxone in MIC studies even in combination with the β-lactamase inhibitor tazobactam are shown in Table 4. We suggest that probably, these strains could be producing the inhibitor resistant TEM enzymes (1RTs), which are resistant to the action of β-lactamase inhibitors.

In conclusion, this study shows that a high percentage of bacterial isolates from high-risk areas of a tertiary hospital in Delhi produce ESBLs (88.6%). Of these, 94.6% became susceptible to a combination of ceftriaxone and tazobactam. It is well known that especially when organisms produce multiple β-lactamases, therapeutic options become few.21 However, as seen from this study, a combination of ceftriaxone and tazobactam appears to be an excellent therapeutic alternative.

Acknowledgements

The pure powders of ceftriaxone and tazobactam were supplied by Aristo Pharmaceuticals Ltd. Their gesture is acknowledged with gratitude.

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**Announcement**

**Indian Society of Electrocardiology**

*Indian Society of Electrocardiology* is organizing its first ‘ECG Learning Course’ at RAPICON Hall, Central Railway Headquarters Hospital, Byculla, Mumbai on Saturday 27th August 2005.

An examination will be held on 28th August 2005 at the same venue and the qualified candidates will be given a Certificate of Competence for ECG Reading.

Course is designed for PG students and practicing physicians.

Separate registration for Learning Course and ECG Certification Exam.

Registration for 100 Candidates only for each on First Come First Serve basis.

For further details contact: Dr. SB Gupta, Hon. Secretary, *Indian Society of Electrocardiology; Head, Department of Medicine and Cardiology, Central Railway Headquarters Hospital, Byculla, Mumbai 400 027.*

Tel: 022-22624556; Fax: 022-22651044; Mobile: 09821364565/09821638617

E-mail: sbgupta@vsnl.net  
Website: www.iseindia.com

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**Announcement**

National Update on Allergy, Asthma and COPD on 3rd & 4th September at Hotel Clarks, Varanasi.

For further details contact: Dr. JK Samaria, Organising Secretary, Organised by Department of Chest Diseases, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221005.

Website: nationalallergy2005.com