Research Paper

Activity in vitro of twelve antibiotics against clinical isolates of extended-spectrum beta-lactamase producing Escherichia coli

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Twelve beta-lactam and non-beta-lactam antibiotics were evaluated against 115 clinical isolates of extended-spectrum beta-lactamase-producing (ESBLs) Escherichia coli using a broth microdilution test in accordance with the CLSI guidelines. Susceptibility was 100% with imipenem, ertapenem and amikacin, 95.7% with piperacillin-tazobactam, 91.3% with cefoxitin, 87% with tobramycin, 81.7% with amoxicillin-clavulanate, 80% with cefepime, 67.8% with ceftazidime, 27.8% with ciprofloxacin, 27% with levofloxacin and 13% with ceftriaxone. Ertapenem was the antibiotic with the lowest minimum inhibitory concentrations (MICs) for all isolates. There were no clinically relevant differences in the activity of the antibiotics in the presence of CTX-M-9 or SHV enzymes.

Keywords: Escherichia coli / ESBLs / Microdilution / Susceptibility

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Introduction

Extended-spectrum beta-lactamases (ESBLs) are enzymes produced by Gram-negative bacteria that confer resistance to all penicillins, cephalosporins (with the exception of cephapicyns), and monobactams. Apart from cephapicyns, the only beta-lactams to maintain activity are the beta-lactam combinations with beta-lactamase inhibitors and carbapenem antibiotics (Bradford 2001). The in vivo use of some of these antibiotics has led to therapeutic failure. For example, when using cephapicyns, mutants have been generated which are resistant by virtue of a reduction in permeability (Martinez-Martinez et al. 1996). These antibiotics are therefore not useful in practice. Furthermore, the use of combinations of beta-lactams with beta-lactamase inhibitors is controversial (Johnson et al. 2002, Spanu et al. 2002). In any case, it is not advisable to use these antibiotics until the results of the antibiogram are known, and their use should be reserved for non-serious infections with demonstrated in vitro susceptibility.

Carbapenems have been reported to be the most active beta-lactam antibiotics against ESBL-producing Gram-negative bacilli (Johnson et al. 2002). These are not, however, an alternative in the management of community-acquired infections.

When ESBL-producing organisms are sensitive to other antibiotic groups these are useful as treatment. Nevertheless, it is important to investigate the activity of these other antibiotics, against which co-resistances can be found through plasmids, transposons or integrons (Lautenbach et al. 2001).

Since these enzymes frequently show a multiresistance pattern, treatment options are limited for infections caused by ESBL-producing organisms. Therefore they constitute a health-care challenge of great clinical importance.

With this in mind we designed the present study to determine the activity of different beta-lactam and non-beta-lactam antibiotics in clinical isolates of ESBL-producing strains of E. coli using a broth microdilution test.
Materials and methods

Bacterial isolates
We studied 115 different clinical isolates of ESBL-producing E. coli identified in the Laboratory of Clinical Microbiology at the San Cecilio University Teaching Hospital in Granada (Spain) using the WIDER system (Francisco Soria Melguizo S.A., Spain) (Canton et al. 2000), in which we confirmed the presence or otherwise of ESBLs by the diffusion technique with discs of cefotaxime (30 µg), ceftriaxone/clavulanate (30/10 µg), ceftazidime (30 µg) and ceftazidime/clavulanate (30/10 µg), in adherence to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2006a). Following phenotypic confirmation, determination of beta-lactamase and clonality was carried out by means of biochemical (determination of the isoelectric point) and molecular studies (PCR), following the procedures described elsewhere by our group (Sorlozano et al. 2007).

Sixty-seven isolates produced CTX-M-9 enzymes and 48 produced SHV enzymes. Of the total, 86.1% were from urine samples (58.6% producing CTX-M-9) whilst 77.4% were of community origin (55% producing CTX-M-9). Of the hospital samples, 69.2% produced CTX-M-9.

A total of 41.7% of the isolates were CTX-M-9 producers originating from community and urine samples. In the case of the SHV producers, the figure was 31.3%.

Susceptibility determination
Microdilution was carried out in Mueller-Hinton broth, adjusted for Ca++ and Mg+++, in accordance with CLSI guidelines (CLSI 2006b). Each antibiotic was dissolved according to the manufacturers’ recommendations. The following concentrations (in µg/ml) were tested in the microdilution procedure: amoxicillin-clavulanate 0.125/0.06 to 128/64, piperacillin-tazobactam 0.25 to 256, with a fixed concentration of tazobactam of 4 µg/l, ceftriaxone 0.5 to 512, cefoxitin 0.25 to 256, ceftazidime 0.25 to 256, cefepime 0.25 to 256, imipenem 0.008 to 8, ertapenem 0.008 to 8, amikacin 0.25 to 256, tobramycin 0.125 to 128, ciprofloxacin 0.125 to 128, and levofloxacin 0.125 to 128. The minimum inhibitory concentration (MIC) is defined as the lowest antibiotic concentration to completely inhibit bacterial growth. The isolates were considered to be susceptible, intermediate or resistant according to the recommendations of the CLSI.

Quality controls
Following the CLSI guidelines (CLSI 2006a), we used the following strains as quality control in all procedures: K. pneumoniae ATCC 700603 and E. coli ATCC 25922.

Statistical analysis
The Fisher exact test for $r \times s$ tables was used to compare the clinical categories and MIC distributions between the two groups of ESBL isolates (CTX-M-9 and SHV) for each antibiotic tested. As an alternative hypothesis ($H_1$) we considered the presence of a difference between the groups compared for both variables.

Results
Table 1 shows the values (in µg/ml) of the MIC ranges, the MIC50 and MIC90 values, and percentage susceptibility to the 12 antibiotics tested of the 115 ESBL-producing isolates (67 and 48 producers of CTX-M-9 and SHV respectively). Of note is the fact that imipenem, ertapenem and amikacin were the only three antibiotics to show activity against 100% of the isolates.

The results of the Fisher exact test on comparing the CTX-M-9- and SHV-producing isolates versus the behavior of each antibiotic, from the perspective of MIC distribution and clinical category, were the following ($p$ value for MIC distribution; $p$ value for clinical category): amoxicillin-clavulanate (0.065; 0.342); piperacillin-tazobactam (0.810; 1.000); ceftriaxone (<0.001; <0.001); cefoxitin (0.828; 1.000); ceftazidime (<0.001; <0.001); cefepime (0.003; 1.000); imipenem (0.289; 1.000); ertapenem (0.178; 1.000); amikacin (0.102; 1.000); tobramycin (0.086; 0.049); ciprofloxacin (0.609; 0.674); levofloxacin (0.128; 0.523).

Ceftriaxone showed greater activity against SHV-producing isolates, whilst ceftazidime was more active against CTX-M-9-producing isolates. There were also significant differences for cefepime as regards MIC distribution, these concentrations being lower among the SHV producers, and for tobramycin in the distribution by clinical categories (increased percentage of resistance among SHV producers).

Discussion
In this study the ESBL-producing isolates proved themselves in general to be susceptible to a combination of amoxycillin and clavulanic acid, with no significant difference between the CTX-M-9- and SHV-producing strains. Other authors have obtained similar susceptibility percentages for ESBL-producing E. coli, [cf. for example the study conducted by the Spanish Group for Nosocomial Infections (GEIH) (Hernandez et al. 2005), who arrived at a figure of 69% for isolates susceptible to amoxycillin-clavulanic acid]. Some other authors
from a significantly different distribution of the MICs producing group than in the SHV producers – resulting in the MICs for certain antibiotics being significantly lower in the CTX-M-9 producers compared to the SHV producers. The susceptibility of ESBL isolates to cefoxitin and ceftazidime was also seen to be very active against these organisms, with no significant differences between the two groups of enzymes. To investigate the activity of cephalosporins and carbapenems against ESBL-producing isolates, with no significant differences between the two enzyme groups.

The susceptibility of ESBL isolates to cefoxitin and cephalosporins seems to be variable and depends on the study in question. Sader et al. (2005) found that 91.3% of their isolates were susceptible to cefoxitin. With cephalosporins, the values were 24.3%, 50% and 93.8% in studies by Casellas et al. (2003), Hoban et al. (2005) and Sader et al. (2005) respectively. We found both cefoxitin and cephalosporins to be active against ESBL-producing isolates, with no significant differences between the two groups of enzymes. To investigate the activity of cephalosporins and carbapenems against ESBL-producing isolates, with no significant differences between the two enzyme groups.

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Because of the reduced capacity of CTX-M-9 enzymes to hydrolyze ceftazidime (Bradford 2001) the MICs obtained in this group are significantly lower and fall within the susceptibility range found for the SHV-producing isolates against this antibiotic. Casellas et al. (2003) reported a susceptibility of 7.4%, far lower than our own value, since they investigated CTX-M-2-producing E. coli.

Imipenem and ertapenem were the only beta-lactam antibiotics to show activity against 100% of the isolates, with no significant differences between the two enzymes. In this context, ertapenem was the antibiotic with the lowest MICs for each isolate. Thus this drug constitutes the best in vitro option of all the antibiotics tested. Although ertapenem and imipenem are both active against E. coli, the greater intrinsic activity of ertapenem seems to be due to its greater affinity for PBP-3 compared with imipenem (Kohler et al. 1999).

In the same way as in our study, where ertapenem exhibited the lowest MIC<sub>90</sub> (0.125 µg/ml), other authors have also found the most active carbapenem antibiotic in vitro to be ertapenem, with a MIC<sub>90</sub> of 0.06 µg/ml as opposed to 0.5 µg/ml for imipenem, 16 µg/ml for ceftazidime, or more than 128 µg/ml in the case of piperacillin-tazobactam when tested against ESBL-producing isolates of K. pneumoniae in a study published by Livermore et al. (2001). Alhambra et al. (2004) found an MIC<sub>90</sub> of 0.03 µg/ml for ertapenem when testing against ESBL-producing E. coli isolates.

Amikacin was found to be active against 100% of the ESBL-producing isolates, with no significant differences found between the two groups of enzymes. Tobramycin was also seen to be very active against these isolates.

### Table 1. In vitro activity of the 12 antibiotics tested against clinical isolates of extended-spectrum beta-lactamase producing E. coli.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>ESBL-producers</th>
<th>CTX-M-9-producers</th>
<th>SHV-producers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 115</td>
<td>n = 67</td>
<td>n = 48</td>
</tr>
<tr>
<td>Range (µg/ml)</td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt; (µg/ml)</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt; (µg/ml)</td>
<td>Susceptible (%)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>2/1–32/16</td>
<td>8/4</td>
<td>32/16</td>
</tr>
<tr>
<td>Piperacillin-tazobactam%</td>
<td>≤0.25/4–128/4</td>
<td>2/4</td>
<td>8/4</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>2–&gt;512</td>
<td>16</td>
<td>512</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>0.5–32</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>≤0.25–256</td>
<td>2</td>
<td>64</td>
</tr>
<tr>
<td>Cefepime</td>
<td>≤0.25–128</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.03–2</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>≤0.008–0.5</td>
<td>0.03</td>
<td>0.125</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≥0.25–8</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>≤0.125–128</td>
<td>0.5</td>
<td>8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>≤0.125–128</td>
<td>16</td>
<td>64</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤0.125–128</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

(Hoban et al. 2005), however, have obtained lower values (12.5%).
isolates, particularly against the CTX-M-9 producing organisms.

The quinolones showed lower active, with no significant differences between the CTX-M-9 and SHV-producing isolates. The association between the production of ESBLs and resistance to these antibiotics has been well established (Valverde et al., 2004), though such resistance is not observed in all cases (Sader et al. 2005).

The carbapenems are undoubtedly very active in vitro against ESBL-producing isolates and, in view of the presence of resistances associated to other antibiotic groups, often constitute one of the few treatment options available for such organisms.

References


