Table 1. β-Lactamase activity of E. coli Wi harbouring recombinant plasmid pACYC184 containing the blaCTX-M-2, blaCTX-M-3, blaCTX-M-9 or blaCTX-M-15 genes, of E. coli Top 10 harbouring pTEM-3 and of the clinical isolate Klebsiella pneumoniae YC harbouring KPC-2

<table>
<thead>
<tr>
<th></th>
<th>E. coli Wi pCTX-M-2</th>
<th>E. coli Wi pCTX-M-3</th>
<th>E. coli Wi pCTX-M-9</th>
<th>E. coli Wi pCTX-M-15</th>
<th>E. coli Top 10 pTEM-3a</th>
<th>K. pneumoniae YC pKPC-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lactamase activity (mU/mg of protein)</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.3</td>
<td>0.4 ± 0.1</td>
<td>1.6 ± 0.5</td>
<td>0.6 ± 0.2</td>
<td>98 ± 6</td>
</tr>
</tbody>
</table>

3pTEM-3 is a natural plasmid containing the blaTEM-3 gene. 4E. coli Top 10 pTEM-3 and K. pneumoniae YC expressing KPC-2 were used as negative and positive controls, respectively.

approximately 100-fold lower than that of the KPC-2 β-lactamase, and similar to that of TEM-3 (Table 1). The slightly higher activity of CTX-M-15 was not relevant considering the standard deviation values (Table 1). The synergistic effect of ertapenem and clavulanic acid observed on Mueller–Hinton plates with CTX-M-producing Enterobacteriaceae is not explained by a hydrolytic activity of the CTX-Ms towards ertapenem. Inhibition studies were thus conducted for β-lactamases CTX-M-15 and TEM-3 to assess the presence and magnitude of drug–drug interactions. The inhibition constants (Ki) were determined as described by Dixon, i.e. plots were prepared of the reciprocal of the rate of metabolite formation (1/v) as a function of inhibitor concentration at each substrate concentration. The Ki value was recovered at the intersection of the obtained lines. Cefalotin (10, 20, 50 and 100 μM) was used as the substrate and ertapenem as the inhibitor (0.001–0.1 μM). This inhibition study showed that the ertapenem Ki value was 10-fold lower for CTX-M-15 (7 nM) than for TEM-3 (65 nM). The better efficiency of CTX-M-15 towards ertapenem over TEM-3, resulting from a 10-fold lower Ki and a slightly higher Vmax (approximately 2-fold higher), may explain the synergy image observed with CTX-M producers and not with TEM-3. Similarly, it is known that tazobactam has a much higher inhibitory activity against CTX-Ms than against TEM-type ESBLs.

Thus, ertapenem is effective against CTX-M-producing Enterobacteriaceae, taking into account the very low level of its hydrolysis by CTX-Ms. The synergy image that may be observed between ertapenem and clavulanic acid for CTX-M producers may mostly result from the stronger inhibitory effect of clavulanic acid on CTX-Ms associated with a weak hydrolysis of ertapenem. Indeed, the IC50 value of clavulanate is 9 nM for CTX-M-15, whereas it is 26 nM for TEM-3. Finally, the synergy image observed should not lead to a false conclusion of ertapenem inefficacy, whereas a similar synergy image between cephalosporins and clavulanic acid for those CTX-M producers is related to a high hydrolysis of cephalosporins.

Funding

This work was funded by a grant from the Ministère de l’Éducation Nationale et de la Recherche (UPRES-EA 3539), Université Paris XI, and mostly by a grant of the European Community (6th PCRD, LSHM-CT-2005-018705).

Transparency declarations

None to declare.

References


Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkn314
Advance Access publication 30 July 2008

In vitro activity of ME1036 versus other β-lactams against penicillin-resistant Streptococcus pneumoniae serotypes exhibiting higher amoxicillin than penicillin MIC

Asunción Fenoll1, Lorenzo Aguilar28, Olga Robledo1, María-José Giménez2, Juan-José Granizo3, Donald Biek4 and David Tarragon1

1Spanish National Reference Pneumococcal Laboratory, Instituto de Salud Carlos III, ctra. Majadahonda-Pozuelo
Research letters

Km. 2, 28220 Majadahonda, Madrid, Spain; Microbiology Department, School of Medicine, University Complutense, Avda. Complutense s/n, 28040 Madrid, Spain; Grana Datos SL, c/Demetrio de la Guerra 4, 28223 Pozuelo de Alarcón, Madrid, Spain; Cerexa, Inc., 1751 Harbor Bay Parkway, Alameda, CA 94502, USA

Keywords: carbapenems, amoxicillin resistance, serotype 14, serotype 6B, serotype 9V, serotype 19A

*Corresponding author. Tel: +34-91-3941505; Fax: +34-91-3941511; E-mail: laguilar@med.ucm.es

Sir,

It has been postulated that pneumococcal infections should be considered among the severe nosocomial diseases, and that *Streptococcus pneumoniae* should be covered by empirical therapy of hospital-acquired pneumonia together with methicillin-resistant *Staphylococcus aureus* (MRSA), the other principal Gram-positive, nosocomial respiratory pathogen. In a retrospective study carried out in Spain over the period 1995–2002, serotypes 14, 23F and 19 were those most frequently isolated in nosocomial *S. pneumoniae* bloodstream infections.

Several serotypes, together with 6B and 9V, have been reported as some of the most troublesome with regard to antimicrobial resistance. An additional problem is the reported spread of clones with higher amoxicillin than penicillin MIC. The emergence of resistance to amoxicillin within existing penicillin-resistant clones is also related to macrolide and ciprofloxacin resistance, with levofloxacin non-susceptibility rates of 34% among the serotype 14 Spanish multidrug-resistant clone. The genetic relatedness of these multidrug-resistant isolates, which suggests possible clonal expansion, raises the concern that it may be increasingly difficult to find adequate therapies for *S. pneumoniae* infections.

ME1036 is a novel parenteral carbapenem active against MRSA, β-lactamase-producing *Haemophilus influenzae* and ESBL-producing Enterobacteriaceae. The aim of this study was to explore the *in vitro* activity of ME1036 against recent penicillin-resistant isolates of *S. pneumoniae* showing higher amoxicillin versus penicillin MIC.

From the *S. pneumoniae* isolates received in the Spanish Pneumococcal Reference Laboratory (Instituto de Salud Carlos III) in the period January 2005 to September 2007, 220 penicillin-resistant isolates showing higher amoxicillin versus penicillin MIC were tested. Antimicrobial susceptibility was determined by the agar dilution method using Mueller–Hinton agar (Difco Laboratories, Detroit, MI, USA) as culture media supplemented with 5% sheep blood (Biomedics, Madrid, Spain), with final inocula of 10⁵ cfu/mL, and incubating under 5% CO₂ atmosphere. *S. pneumoniae* ATCC 6303, *S. pneumoniae* ATCC 49619 and five clinical isolates were used as quality control strains as in all determinations carried out in the Spanish Reference Laboratory for Pneumococci. Minimum concentrations (mg/L) tested in the plates were 0.001 for ME1036, 0.007 for imipenem and meropenem, 0.015 for penicillin, ampicillin, cefotaxime, ceftriaxone and cefepime, 0.03 for cefuroxime, 0.06 for amoxicillin, 0.12 for erythromycin and 1 for levofloxacin. For all compounds, the maximum concentration tested in the plates was 32 mg/L, except for ampicillin (16 mg/L). Susceptibility breakpoints (mg/L) defined by CLSI were penicillin ≤0.06, amoxicillin ≤2, cefuroxime sodium ≤0.5, cefotaxime, ceftriaxone and cefepime ≤1, imipenem ≤0.12, meropenem ≤0.25, erythromycin ≤0.25 and levofloxacin ≤2. CLSI breakpoints are not defined for ampicillin and ME1036. Serotyping was performed by the Quellung reaction and/or dot blot assay. Of the 220 strains, 69 belonged to serotype 9V, 65 to serotype 14, 33 to serotype 6B, 27 to serotype 19A, 7 to serotype 19F, 5 were non-typeable and the other 14 strains belonged to other serotypes with less than five isolates. Table 1 shows susceptibility to study drugs for serotypes with more than 25 isolates. Susceptibility rates for penicillin, amoxicillin and cefuroxime were 0% and that for cefepime was <28%. Susceptibility rates to cefotaxime ranged from 26.2% for serotype 14 to 82.6% for serotype 9, whereas susceptibility rates to ceftriaxone were >88% for all serotypes. Susceptibility rates for imipenem and meropenem were ≤6.2% in all cases. The MIC<sub>90</sub> value for ME1036 was 1/16/16 — 8/8/8 — 1/1/1 — 1/1/1 — 1/1/1 — 1/1/1 — 1/1/1 — 1/1/1 — 1/1/1.

Table 1. MIC<sub>50</sub>, MIC<sub>90</sub> (mg/L) and percentage of susceptibility to study drugs for penicillin-resistant strains exhibiting higher amoxicillin than penicillin MIC for serotypes with more than 25 isolates with this resistance phenotype

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Serotype 9V (n = 69)</th>
<th>Serotype 14 (n = 65)</th>
<th>Serotype 6B (n = 33)</th>
<th>Serotype 19A (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEN</td>
<td>2/4</td>
<td>2/4</td>
<td>2/2</td>
<td>2/4</td>
</tr>
<tr>
<td>AMP</td>
<td>8/16</td>
<td>8/16</td>
<td>8/16</td>
<td>8/16</td>
</tr>
<tr>
<td>AMX</td>
<td>8/16</td>
<td>8/16</td>
<td>8/16</td>
<td>8/16</td>
</tr>
<tr>
<td>CXM</td>
<td>8/8</td>
<td>8/16</td>
<td>8/16</td>
<td>8/16</td>
</tr>
<tr>
<td>CFX</td>
<td>1/2</td>
<td>2/2</td>
<td>1/2</td>
<td>2/2</td>
</tr>
<tr>
<td>CRO</td>
<td>0.5/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>FEP</td>
<td>2/4</td>
<td>2/4</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>IPM</td>
<td>0.5/0.5</td>
<td>0.5/1</td>
<td>0.5/1</td>
<td>0.5/1</td>
</tr>
<tr>
<td>MEM</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>ME1036</td>
<td>0.1/0.12</td>
<td>0.06/0.12</td>
<td>0.06/0.12</td>
<td>0.06/0.12</td>
</tr>
<tr>
<td>ERY</td>
<td>≤0.12/32</td>
<td>0.12/32</td>
<td>32/32</td>
<td>32/32</td>
</tr>
<tr>
<td>LVX</td>
<td>≤1/16</td>
<td>1/1/1</td>
<td>1/1/1</td>
<td>1/1/1</td>
</tr>
</tbody>
</table>

PEN, penicillin; AMP, ampicillin; AMX, amoxicillin; CXM, cefuroxime; CFX, cefotaxime; CRO, ceftriaxone; FEP, cefepime; IPM, imipenem; MEM, meropenem; ERY, erythromycin; LVX, levofloxacin.
0.12 mg/L for all serotypes, which was at least 4-fold lower than imipenem, meropenem and ceftriaxone. Our findings suggest that against these multidrug-resistant pneumococcal isolates, the ranking of in vitro activity based on MIC90 for the β-lactams would be ME1036 (0.12 mg/L) (most active) > imipenem (0.5 mg/L) > meropenem (1 mg/L) > ceftriaxone (1 mg/L) > cefotaxime (2 mg/L) > penicillin = cefepime (4 mg/L) > amoxicillin (8–16 mg/L) > cefuroxime (16 mg/L) > ampicillin (≥16 mg/L) (least active).

A previous study that employed agar dilution testing against a smaller number of non-selected penicillin-resistant S. pneumoniae isolates reported an MIC90 value of 0.03 mg/L for ME1036,4 a value two dilutions lower than the MIC90 value determined in this study against multidrug-resistant strains belonging to troublesome serotypes exhibiting higher amoxicillin than penicillin MIC. MIC90 values determined for ME1036 by broth microdilution against a small number (11 strains) of penicillin-resistant S. pneumoniae isolates in a previous study showed values similar to those in the present study.5

In conclusion, ME1036 exhibited excellent intrinsic activity against penicillin-resistant S. pneumoniae belonging to serotypes 9V, 14, 6B and 19A, exhibiting higher amoxicillin than penicillin MIC. The spread of multidrug resistance that includes β-lactams (including penicillins, second- and third-generation cephalosporins and previous carbapenems) may challenge empirical hospital treatment of lower respiratory tract infections. The high intrinsic activity of ME1036 against resistant strains of S. pneumoniae may represent an advantage when broad-spectrum activity is required.

Funding
This study was supported by an unrestricted grant from Forest Laboratories, Inc.

Transparency declarations
D. B. is an employee of Cerexa Inc. Others authors: none to declare.

References

Journal of Antimicrobial Chemotherapy
doi:10.1093/jac/dkn344
Advance Access publication 27 August 2008

In vitro activity of tigecycline against Gram-positive cocci: a multicentre study in Greece


1Department of Microbiology, University Hospital of Larissa, Mezourlo, 41110 Larissa, Greece; 2Department of Microbiology, School of Medicine, Rio, 26500 Patras, Greece; 3Department of Molecular Microbiology, Institute of Biomedical Research and Science, 41222 Larissa, Greece; 4Department of Microbiology, University Hospital of Alexandroupolis, Dragana, 68100 Alexandroupolis, Greece; 5Department of Microbiology, University Hospital of Heraklion, Stavrakia, 71110 Heraklion, Crete, Greece; 6Department of Microbiology, University Hospital ‘AHEPA’, 54636 Thessaloniki, Greece; 7Department of Microbiology, General Hospital of Lamia, 35100 Lamia, Greece; 8Department of Microbiology, General Hospital ‘Asclepeion’, Voula, 16673 Athens, Greece; 9Department of Microbiology, General Hospital ‘Georgios Genimatas’, 11527 Athens, Greece; 10Department of Microbiology, ‘Attikon’ University Hospital, Athens, 12462 Athens, Greece; 11Department of Microbiology, General Hospital of Volos, 38222 Volos, Greece

Keywords: resistance, staphylococci, enterococci, streptococci, pneumococci

*Corresponding author. Tel: +30-2410-682517; Fax: +30-2410-682535; E-mail: petinaki@med.uth.gr or petinaki@hotmail.com

Sir,
Tigecycline, a new glycyclcline antibiotic with broad-spectrum activity against aerobic and anaerobic Gram-positive and Gram-negative bacteria, appears to be a therapeutic option for serious infections caused by multidrug-resistant organisms. The purpose of this study was firstly to evaluate the in vitro activity of this drug against Gram-positive cocci in Greek hospitals and secondly to define a baseline for monitoring possible future emergence of resistance to tigecycline in our clinical settings.

From January 2006 to December 2007, a total of 10 420 Gram-positive cocci were tested for their susceptibility to tigecycline. The numbers of isolates of the various genera and species