transparency declarations

None to declare.

References

1. DANMAP. DANMAP 2005-Use of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Foods, and Humans in Denmark. Danish Institute for Food and Veterinary Research, Copenhagen, Denmark, 2006.

Journal of Antimicrobial Chemotherapy
do:10.1093/jac/dkm529
Advance Access publication 31 January 2008

Nosocomial infections caused by multidrug-resistant Pseudomonas putida isolates producing VIM-2 and VIM-4 metallo-β-lactamases

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Keywords: carbapenems, MBLs, ESBLs, P. putida, β-lactams

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Sir,

Nosocomial infections caused by multidrug-resistant and carbapenem-resistant Pseudomonas putida isolates have been occasionally reported in severely ill or immunocompromised patients hospitalized in the intensive care unit (ICU).1 Here, we report briefly the microbiological characteristics of several carbapenem-resistant P. putida isolates producing VIM metallo-β-lactamases (MBLs) at two Belgian university hospitals located in the Brussels area.

Between January 2004 and May 2007, multidrug-resistant P. putida strains originating from 10 inpatients hospitalized at Saint-Luc (hospital 1) and Erasme (hospital 2) university hospitals were characterized for resistance mechanisms to β-lactams. All the isolates were high-level resistant to imipenem and meropenem by disc diffusion testing (no inhibition zone). The 10 patients presented with severe underlying diseases (Table 1) had been hospitalized more than 9 days in ICUs and had all previously received broad-spectrum antimicrobial therapy. All but one of the isolates had been recovered from urine specimens. Bacterial identification to the species level was achieved with Vitek2-GN (bioMérieux) and control growth at 42°C on trypticase soy agar complemented with sheep blood. MICs determined by Etest (AB Biodisk) showed that all isolates were resistant to piperacillin/tazobactam, ceftazidime, aztreonam, imipenem and meropenem and all but one were resistant to ceftizime (Table 1). Isolates recovered from hospital 1 were resistant to amikacin, whereas isolates from hospital 2 remained susceptible to this aminoglycoside. Resistance to ciprofloxacin was variable but all isolates remained susceptible to colistin. The MBL screening test was positive both by double-disc method (imipenem versus imipenem-EDTA; Rosco Diagnostica A/S) and by MBL double-sided Etest (imipenem/imipenem-EDTA; AB Biodisk) for all isolates (data not shown). PCR targeting blaIMP (FW, 5′-GGC GTT TAT TAT CAT TGT TG; RV, 5′-TCG AGA ATT AAG CCA CTC TAT TCC), blaVIM (FW, 5′-TGT CGT ATC CCT), blaPER, blaGES, blaHEL1, blaOXA of Groups 1, 2 and 3, blaOXA-20, blaOXA-18, and penicillinase genes (blaCARB, FW, 5′-TGG AAA CGG GAA AAC GTT GG; RV, 5′-ATT CAG CCA GAT CGG CAT C), various ESBL genes (blaTEM, blaVEB, blaPER, blaGES, blaHEL1, blaOXA of Groups 1, 2 and 3, blaOXA-20, blaOXA-18), and various β-lactamases among ampicillin-resistant Escherichia coli and Salmonella isolated from food animals in Denmark. Microb Drug Resist 2004; 10: 334–40.

Isolates obtained from hospital 1 were resistant to amikacin, whereas isolates from hospital 2 remained susceptible to this aminoglycoside. Resistance to ciprofloxacin was variable but all isolates remained susceptible to colistin. The MBL screening test was positive both by double-disc method (imipenem versus imipenem-EDTA; Rosco Diagnostica A/S) and by MBL double-sided Etest (imipenem/imipenem-EDTA; AB Biodisk) for all isolates (data not shown). PCR targeting blaIMP (FW, 5′-GGC GTT TAT TAT CAT TGT TG; RV, 5′-TCG AGA ATT AAG CCA CTC TAT TCC), blaVIM (FW, 5′-TGT CGT ATC CCT), blaPER, blaGES, blaHEL1, blaOXA of Groups 1, 2 and 3, blaOXA-20, blaOXA-18, and penicillinase genes (blaCARB, FW, 5′-TGG AAA CGG GAA AAC GTT GG; RV, 5′-ATT CAG CCA GAT CGG CAT C), various ESBL genes (blaTEM, blaVEB, blaPER, blaGES, blaHEL1, blaOXA of Groups 1, 2 and 3, blaOXA-20, blaOXA-18), and various β-lactamases among ampicillin-resistant Escherichia coli and Salmonella isolated from food animals in Denmark. Microb Drug Resist 2004; 10: 334–40.
Table 1. Case history, MIC data for selected antimicrobial agents, and integron structure of *P. putida* clinical isolates expressing VIM MBL

<table>
<thead>
<tr>
<th>Isolate</th>
<th>duration of stay before isolation (days)</th>
<th>MIC (mg/L)</th>
<th>PCR-sequencing-typing</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>hospital a site of isolation underlying disease</td>
<td>TZP</td>
<td>CAZ</td>
</tr>
<tr>
<td>1</td>
<td>1 endotracheal aspirate liver cirrhosis</td>
<td>9</td>
<td>&gt;256</td>
</tr>
<tr>
<td>2</td>
<td>1 urine vascular stroke</td>
<td>10</td>
<td>&gt;256</td>
</tr>
<tr>
<td>3</td>
<td>1 urine meningitis</td>
<td>39</td>
<td>&gt;256</td>
</tr>
<tr>
<td>4</td>
<td>1 urine meningitis</td>
<td>31</td>
<td>&gt;256</td>
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<tr>
<td>5</td>
<td>1 urine polytrauma</td>
<td>32</td>
<td>&gt;256</td>
</tr>
<tr>
<td>6</td>
<td>2 urine cancer</td>
<td>54</td>
<td>128</td>
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<tr>
<td>7</td>
<td>2 urine cancer</td>
<td>22</td>
<td>128</td>
</tr>
<tr>
<td>8</td>
<td>2 urine renal graft</td>
<td>12</td>
<td>128</td>
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<tr>
<td>9</td>
<td>2 urine prostatitis</td>
<td>18</td>
<td>256</td>
</tr>
<tr>
<td>10</td>
<td>2 urine cancer</td>
<td>62</td>
<td>96</td>
</tr>
</tbody>
</table>

TZP, piperacillin/tazobactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam; IPM, imipenem; MER, meropenem; AMK, amikacin; CIP, ciprofloxacin; COL, colistin.

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Activity of doripenem and comparator β-lactams against US clinical isolates of Streptococcus pneumoniae with defined mutations in the penicillin-binding domains of pbp1a, pbp2b and pbp2x

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Sir,

Doripenem, a parenteral carbapenem, was recently approved in the USA for the treatment of complicated intraabdominal infections (cIAIs) and complicated urinary tract infections (cUTIs) including pyelonephritis. In Europe, a marketing authorization application has been filed for the treatment of cIAIs, cUTIs and nosocomial pneumonia. Doripenem has a broad spectrum of activity against clinically important pathogens including Enterobacteriaceae, Gram-negative non-fermenters, anaerobes and many Gram-positive cocci such as methicillin-susceptible Staphylococcus spp., group A streptococci and pneumococci.1

β-Lactam resistance in Streptococcus pneumoniae is caused by mutations in the penicillin-binding domains of one or more of its six penicillin-binding proteins (PBPs) resulting from mosaic genes or point mutations.2 Altered PBP1a, PBP2b and PBP2x are most important for β-lactam resistance in clinical isolates.3,4

This study reports the activity of doripenem against 30 S. pneumoniae US clinical isolates. β-Lactam MICs were determined using panels from Trek Diagnostic Systems (Cleveland, OH, USA) using CLSI recommendations.5 Breakpoints (non-meningitis) for all drugs except doripenem were those approved by CLSI.5 Currently, there are no approved doripenem breakpoints for S. pneumoniae. PBP gene sequencing and competition assays were performed as described previously.5

Seven penicillin-susceptible isolates had β-lactam MICs ≤0.03 mg/L (genotype wild-type) (Table 1). These isolates had no mutations in the penicillin-binding motifs of pbp1a, pbp2b and pbp2x. Of the eight penicillin-intermediate isolates (genotypes 1 and 2), three had penicillin MICs of 0.12 mg/L and MICs for the other β-lactams were ≤0.12 mg/L (genotypes 1A and 1B) (Table 1). They had a T446A substitution in the PBP2b SSNT motif; one isolate (genotype 1B) also had PBP2x substitutions (T338A and R384G). Five penicillin-intermediate isolates with penicillin MICs of 1 mg/L additionally had PBP2x substitutions (T337I and L546V) (genotype 2, Table 1). The carbapenem MICs were the lowest of the agents tested, with imipenem having 2- and 4-fold lower MICs than doripenem and meropenem, respectively (genotype 2A). One isolate (genotype 2B) had substitutions of T371I in PBP1a and D623G in PBP2b. These substitutions were associated with 2–4-fold increases in carbapenem MICs (Table 1).

Among the penicillin-resistant isolates (genotypes 3–5), seven had penicillin MICs of 2–4 mg/L and were non-susceptible to imipenem and meropenem, three were non-susceptible to ceftriaxone and one was non-susceptible to amoxicillin/clavulanic acid; doripenem MICs were 0.5–1 mg/L (Table 1). These seven isolates (genotype 3) had PBP1a substitutions of T371A and P432T in addition to the PBP2x and PBP2b changes mentioned previously (Table 1).

Two isolates with genotype 4 had additional substitutions of A619G and D623G in or near the KTGTA motif of PBP2b and a substitution of T371I in the STMK motif of PBP2x (Table 1). These changes corresponded with increased penicillin MICs (8 mg/L) and resistance to amoxicillin/clavulanic acid, imipenem and meropenem (Table 1). Doripenem MICs were 1–2 mg/L.

Six isolates with genotype 5 had the same PBP substitutions as genotype 4 isolates, with the addition of the M339F change in PBP2x; in four isolates (genotypes 5A, 5B and 5C), there was also a 2–16-fold increase in ceftriaxone MICs leading to resistance, whereas the MICs of the other drugs were within one doubling dilution when compared with genotype 4 isolates (Table 1).

PPB binding studies with a wild-type isolate indicated that the carbapenems had good affinity for all six PBPs with IC50 ≤0.06 mg/L (Table 1). Ceftriaxone bound tightly to all PBPs (IC50 ≤ 0.1 mg/L) except PBP2b (Table 1) as expected, as cephalosporins do not use PBP2b as a primary target.2 In a genotype 5C isolate, the carbapenem binding affinities for all PBPs were reduced: PBP2b and PBP2x had the highest increase in IC50 (2–4 and 3.7–8 mg/L, respectively) (Table 1). The ceftriaxone IC50 for PBP2x was increased at least 200-fold when compared with strain 8865 (Table 1).

β-Lactam MIC increases correlated with increases in the number of PBP1a, 2x and 2b substitutions. In this study, the PBP1a, 2b and 2x substitutions found in, and adjacent to, the penicillin-binding motifs SXKK, SXN and KTSG were similar to substitutions reported by others.2,3 Carbapenems, like penicillins, are thought to have PBP2b as their primary target.1 In a study examining clinical isolates from Japan, a T624G PBP2b substitution was associated with carbapenem resistance.6 No isolates in our study had this mutation; however, a substitution at the adjacent amino acid (D623G) was found in seven of the eight genotype 4 and 5 isolates, all of which had elevated β-lactam MICs. This substitution was reported in a β-lactam-resistant S. pneumoniae recombinant that resulted from transformation with DNA from a β-lactam-resistant Streptococcus mitis strain.2

In summary, doripenem and imipenem (MIC90 1 mg/L) were 2-fold more active than meropenem (MIC90 2 mg/L) against the pneumococcal isolates in this study, including ceftriaxone-resistant...