Research letters

Transparency declarations

None to declare.

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Journal of Antimicrobial Chemotherapy
doi: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: carabepens, 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 cefepime (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/iminipen–EDTA; AB Biodisk) for all isolates (data not shown). PCR targeting blaIMP (FW, S‘-GGC GTT TAT GTT CAT ACT TG T; RV, S‘-TCG AGA ATT AAG CCA CTC TAT TCC), blaVIM (FW, S‘-TGG CCG TGA TGG TGA GTG; RV, S‘-ATT CAG CCA GAT CGG CAT C), various ESBL genes (blaTEM, blaVEB, blaPER, blaGES, blaHEL-1, blaOXA of Groups 1, 2 and 3, blaOXA-20, blaOXA-18), and penicillinase genes (blaCARB: FW, S‘-TGG AAA CGG GAA AAC GGT GG; RV, S‘-CAG GGC ACC CAT AAC CAC CA; blaCARB 1 to 4 and 6: FW, S‘-GGA TTA CAA TGG CAA TCA GC; RV, S‘-TGT CTT ATC CTT CAA ATC ACC) was only positive for the blaVIM gene in all 10 isolates and for the blaPER gene in a single isolate (no. 6). Sequencing of the variable region of class I integrons obtained for the different strains revealed two distinct integrons. The first one, isolated from five isolates from hospital 1, harboured an aacA4 allele coding for the AAC(6’)-Ib aminoglycoside-modifying enzyme explaining the resistance to amikacin, followed by the blaVIM gene. This same integron has already been identified in Pseudomonas aeruginosa isolates reported from Poland and Hungary1,2 and presents a specific 170 bp 3′-terminal repeat of the blaVIM-1 gene. The second class I integron, obtained from the five strains isolated in hospital 2, revealed a blaVIM-2 gene cassette, following an unidentified open reading frame of 318 nucleotides named orfavr. This last sequence is referenced in GenBank under number EU284133. PCR sequencing confirmed that the blaPER gene detected in isolate no. 6 was a blaPER-1 allele. The co-presence of blaPER and blaVIM-2 has been reported in P. aeruginosa3,5 and Providencia,5 but to the best of our knowledge, this is the first description in P. putida. PFGE analysis revealed five PFGE types among the 10 P. putida isolates. Types A and B were recovered from hospital 1, whereas types C, D and E were found in hospital 2. A cluster of four patients showing PFGE type B was found in hospital 1 and another cluster of three patients with PFGE type C was present in hospital 2. Further, the content of the gene cassettes of the P. putida strains also clearly differed between the two centres.
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>
<th>resistance mechanisms</th>
<th>PFGE</th>
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<tr>
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<td>TZP</td>
<td>CAZ</td>
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</table>

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

*Hospital 1, Saint-Luc; hospital 2, Erasme.*
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.2,3 Altered PBP1a, PBP2b and PBP2x are most important for β-lactam resistance in clinical isolates.2,3

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.4 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 PBP2b substitutions (T338A and R384G). Five penicillin-intermediate isolates with penicillin MICs of 1 mg/L additionally had PBP2b substitutions (T371T 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 T371S 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 T371S in PBP1a and D432T 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 T371S in the STMK motif of PBPs (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 T624G PBP2b substitution. Among the penicillin-resistant isolates (genotypes 3–5), all of which had elevated MICs, 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 changes were associated with 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.6 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 PBPs, 2x and 2b substitutions. In this study, the PBPs 1a, 2b and 2x substitutions found in, and adjacent to, the penicillin-binding motifs SXXX, SXN and KT/SG were similar to substitutions reported by others.6,7 Carbapenems, like penicillins, are thought to have PBP2b as their primary target.8 In a study examining clinical isolates from Japan, a T624G PBP2b substitution was associated with carbapenem resistance.8 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...