Genetic and Biochemical Characterization of an Acquired Subgroup B3 Metallo-β-Lactamase Gene, blaAIM-1, and Its Unique Genetic Context in Pseudomonas aeruginosa from Australia

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Three clinical Pseudomonas aeruginosa isolates (WCH2677, WCH2813, and WCH2837) isolated from the Women’s and Children’s Hospital, Adelaide, Australia, produced a metallo-β-lactamase (MBL)-positive Etest result. All isolates were PCR negative for known MBL genes. A gene bank was created, and an MBL gene, designated blaAIM-1, was cloned and fully characterized. The encoded enzyme, AIM-1, is a group B3 MBL that has the highest level of identity to THIN-B and L1. It is chromosomal and flanked by two copies (one intact and one truncated) of an ISCR element, ISCR15. Southern hybridization studies indicated the movement of both ISCR15 and blaAIM-1 within the three different clinical isolates. AIM-1 hydrolyzes most β-lactams, with the exception of aztreonam and, to a lesser extent, ceftazidime; however, it possesses significantly higher kcat values for cephalosporins and carbapenems than most other MBLs. AIM-1 was the first mobile group B3 enzyme detected and signals further problems for already beleaguered antimicrobial regimes to treat serious P. aeruginosa and other Gram-negative infections.

The continuing increase in antibiotic resistance in Gram-negative bacteria is of concern, not least because of the increasing lack of therapeutic options available to treat infections caused principally by Pseudomonas aeruginosa and Acinetobacter baumannii (3, 17, 21). This phenomenon has been exacerbated by the dissemination of metallo-β-lactamases (MBLs) that can confer resistance to nearly all β-lactams, with the exception of aztreonam (4, 5, 43).

Like many resistance mechanisms, MBLs can be encoded by either genes ubiquitously carried on the chromosome or mobile genes (39). The latter genes now include the following subgroups: IMP (15), VIM (26), SPM-1 (36), GIM-1, SIM-1, KMH-1 (25), DIM-1 (20), and the recently described NDM-1 (44). So far, they all belong to MBL subgroup B1. MBL genes are often embedded in class 1 integrons and are carried as gene cassettes. It has also been shown that while many MBL genes are plasmid mediated, some are carried on the chromosome and can be associated with Tn21-like transposons or Tn402 transposons (30, 34). However, SPM-1 is not associated with a standard integron but is flanked by two genetic elements, designated ISCRM 18. ISCRM elements are IS911-like mobile elements and can potentially mobilize and duplicate blaSPM-1 via rolling-circle replication (33, 37).

The B3 subgroup MBLs have hitherto been reported for environmental bacteria, only some of which can cause opportunistic infections. These include Stenotrophomonas maltophilia (L1) (42), Janthinobacterium lividum (THIN-B) (23), Chryseobacterium meningosepticum (GOB-1) (1), Legionella gormanii (FEZ-1) (2), Caulobacter crescentus (CAU-1) (10), CAR-1 from Erwinia carotovora (29), POM-1 (32), and the recently reported ISCR1-associated SMB-1 (38). The MBL genes carried by these environmental bacteria encode subgroup B3 MBLs that are not closely related to the mobile B1 subgroup members (43). They are often GC rich and 2 to 3 kDa larger than the B1 subgroup members, although, with the exception of L1, which is a tetramer, they are all monomeric in structure (29).

Here we describe the full characterization of a new subclass of MBL, AIM-1 (Adelaide imipenemase), which was discovered in three P. aeruginosa isolates from Australia. Furthermore, we also demonstrate that a novel ISCR element, ISCR15, is implicated in the movement of blaAIM-1.

(Preliminary data were presented at the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy [ICAAC], Chicago, Ill., 2007 [45].)

MATERIALS AND METHODS

Clinical cases. (i) Clinical case 1. Patient 1 was a healthy 18-year-old man from the Northern Territory, Australia, with newly diagnosed acute myeloid leukemia and admitted to the Royal Adelaide Hospital in March 2002. One week after presentation, he received broad-spectrum empirical antimicrobial therapy with intravenous ticarcillin-clavulanate and gentamicin, which was later changed to meropenem and vancomycin. On day 26, a multiresistant P. aeruginosa strain (WCH2677, designated the index isolate) was isolated from endotracheal aspirates, and the antimicrobial therapy was changed to intravenous amikacin and ticarcillin-clavulanate. The patient died on day 30 from acute respiratory distress syndrome and multiorgan failure.

(ii) Clinical case 2. Patient 2 was a 59-year-old man with insulin-dependent type 2 diabetes and end-stage renal failure and was admitted at the same time as patient 1. Upon admission, he presented with necrotizing fasciitis of the anterior abdominal wall and was admitted to the intensive care unit (ICU), and empirical treatment with intravenous meropenem and vancomycin was commenced. Antibiotic therapy was modified to include intravenous amoxicillin, ciprofloxacin, and metronidazole. Su-
were separated as previously described (14). and S1 (Invitrogen, Abingdon, United Kingdom), I-CeuI (New England Biolabs, Beverly, MA), prepared and digested with the restriction enzymes SpeI (Roche Diagnos-

P. aeruginosa clinical strains. Clinical isolates were identified by using the BD (Baltimore, MD) Phoenix automated microbiology system.

Susceptibility testing. Susceptibility testing was performed by using the Phoenix 100 system (Becton Dickinson, Oxford, United Kingdom) and by using Etest strips (bioMérieux, La Plane, France), and results were interpreted according to European Committee on Antimicrobial Susceptibility Testing breakpoints (http://www.eucast.org/clinical_breakpoints/).

Phenotypic and molecular detections of MBL. The Hodge test using MacConkey agar, an inipemem-EDTA double-disc synergy test, and MBL Etest strips (bioMérieux, La Plane, France) were used to screen for class B β-lactamase production (40). In addition, the carbapenemase activities of cell sonicates from broth cultures grown overnight were determined by spectrophotometric assays, which were carried out as previously described (6). The presence of known MBL genes (including blaTEM, blaIMP, blaSPM-1, blaGIM-1, and blaNDM-1) was screened for by PCR using primers designed for all known MBL subgroups and class 1 integron structures (20,44). Characterized strains carrying known MBL genes were used as positive controls.

DNA cloning and sequence analysis. Cloning experiments were performed by using cloning vector pK8135 (35). Restriction endonucleases BamHI and Sau3AI and T4 ligase (Promega, Madison, WI) were used for cloning. Transformation was carried out by using electroporation and Escherichia coli TOP10 cells (Invitrogen Corp., Carlsbad, CA). The selection for transformants was performed on LB agar plates containing X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) (30 µg/ml), cefazidime (8 µg/ml), and kanamycin (50 µg/ml). Recombinant plasmids were recovered by using a QiAprep Spin miniprep kit (Qiagen, West Sussex, United Kingdom). The plasmid containing the MBL gene was designated pAIM-1 and contained an insert of 3.5 kb, and it was sequenced on both strands. The nucleotide sequences, deduced amino acid sequences were compared to sequences available on the Internet (http://www.ebi.ac.uk/fasta33/).

Genomic DNA digestion and pulsed-field gel electrophoresis (PFGE). The genomic DNA digestion of the clinical isolates was performed at 37°C overnight with PstI alone, PstI plus HinCI, HindIII, BamHI, EcoRI, EcoRV, KpnI, or NdeI (Promega). The buffers were used according to the manufacturer’s recommendations. Genomic DNA was prepared and digested with the restriction enzymes Spel (Roche Diagnostics, Mannheim, Germany), I-CeuI (New England Biolabs, Beverly, MA), and S1 (Invitrogen, Abingdon, United Kingdom), and DNA fragments were separated as previously described (14).

Hybridization. Hybridization was performed in gel. Briefly, the gel was dried for 5 h at 50°C and then rehydrated in double-distilled water for 5 min before 30-min incubations in denaturing solution (0.5 M NaOH, 1.5 M NaCl) and neutralizing solution (0.5 M Tris-HCl [pH 7.5], 1.5 M NaCl) at room temperature were performed. The gel was then prehybridized at 65°C using prehybridization solution (20 ml) (6× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 0.1% [wt/vol] polyvinylpyrrolidone 400, 0.1% [wt/vol] Ficoll, 0.1% [wt/vol] bovine serum albumin [BSA] [Cohn fraction V], 0.5% [wt/vol] SDS). The hybridization solution was the same as the prehybridization solution apart from 150 µg/ml de-natured calf thymus DNA, which was added at least 4 h before the addition of the probe. The 32P-labeled probe was prepared by using the random primer technique (Stratagene, La Jolla, CA), as previously described (13). Gels were washed in 2% SSC followed by 0.1% SSC.

β-Lactamase purification and characterization. Cultures of E. coli carrying PAM-1 were grown overnight at 37°C in 4 liters of LB broth. A periplasmic protein preparation (30 mM Tris [pH 8.0], as previously described [41]) was obtained, thereby discarding cytoplasmic proteins and, thus, 70% of E. coli proteins. This protein solution was treated with 30% and 60% ammonium sulfate solutions to precipitate the proteins, which were removed by centrifugation. The clarified supernatant was loaded onto a Q-Sepharose column (1.5 by 12 cm with a 25-ml bed volume; Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) equilibrated by running 50 volumes of 30 mM Tris (pH 8.0) at 2 ml/min at pH 7.5 through the column. The protein was eluted with a 0 to 500 mM NaCl gradient in 30 mM Tris (pH 8.0) at 2 ml/min. The AIM-1 enzyme eluted at <100 mM NaCl. Fractions possessing MBL activity were pooled and loaded onto a Sephacryl S 300 gel filtration column (Amersham Pharmacia Biotech, Buckinghamshire, United Kingdom) preequilibrated with 50 mM Tris HCl with 250 mM NaCl buffer at 0.045 ml/min. Fractions showing the highest degree of carbapenemase activity against imipenem were pooled. An Amicon centrifugal filter (Ultracel-50k, -10k; Millipore, Carrigtwohill Co., Cork, Ireland) was used. Protein recovered in the filtrate and protein also retained in the filter were analyzed by sodium doceyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After visualization with silver staining, the protein was deemed 99% pure by eye. The determination of the experimental pl of AIM-1 was undertaken, as previously described (19).

Determination of kinetic values. Purified β-lactamase was used to determine the kinetic parameters kcat and Km. Reactions were performed at 22°C with 1 ml of assay buffer (50 mM cacodylate and 100 µM zinc chloride at pH 7.5) and carried out in duplicate. The standard deviation varied from 3 to 8.5%. The rate of hydrolysis of each β-lactam was calculated for at least 10 different concentrations of substrate based on the extinction coefficients for each substrate. The assays were performed with a Lambda 35 UV-visible (UV-Vis) spectrophotometer (Perkin-Elmer, Cambridge, United Kingdom), by observing the changes in absorption resulting from the opening of the β-lactam ring at the specific wavelengths for each of the 15 antimicrobial agents evaluated, as previously described (24).

Nucleotide sequence accession number. The nucleotide sequence reported in the present study has been assigned EMBL nucleotide accession number AM998375.

RESULTS AND DISCUSSION

Relatedness and susceptibility profiles of the P. aeruginosa clinical isolates. The susceptibility patterns of the three isolates WCH2677, WCH2813, and WCH2837 were found to be nearly identical (Table 1). Genomic DNA from WCH2677 was digested with PstI alone and in combination with a variety of restriction enzymes and revealed fragments of between 1.8 kb and 3.5 kb when hybridized with the blaAIM-1 probe (results not shown). The three P. aeruginosa isolates seem to be closely related clones, with fewer than 3 bands of difference between them in the PFGE analysis (Fig. 1).

Phenotypic and molecular screening for MBLs. The results of MBL screening tests were positive using the imipenem-EDTA double-disc synergy test, MBL Etest strips, and determinations of carbapenemase activities by spectrophotometry (data not shown) (43). However, PCR analysis failed to detect previously known MBL genes (blaVIM, blaIMP, blaSPM-1, blaGIM-1, and blaNDM-1) as well as integrons.

Nucleotide and deduced amino acid sequences of blaAIM-1. DNA from P. aeruginosa WCH2677, the index strain, was used to
TABLE 1 Antimicrobial susceptibility patterns of the three AIM-1-producing isolates and E. coli TOP10 carrying \( \text{bla}_{\text{AIM-1}} \)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC (µg/ml) for isolate</th>
<th>E. coli TOP10</th>
<th>E. coli TOP10 (pAIM-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>32</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>6</td>
<td>4</td>
<td>0.094</td>
</tr>
<tr>
<td>Cefepime</td>
<td>6</td>
<td>8</td>
<td>0.032</td>
</tr>
<tr>
<td>Imipenem</td>
<td>128</td>
<td>128</td>
<td>0.064</td>
</tr>
<tr>
<td>Meropenem</td>
<td>512</td>
<td>512</td>
<td>0.25</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;32</td>
<td>&gt;32</td>
<td>0.004</td>
</tr>
<tr>
<td>Colistin</td>
<td>4</td>
<td>1.5</td>
<td>0.38</td>
</tr>
</tbody>
</table>

**TABLE 1** Antimicrobial susceptibility patterns of the three AIM-1-producing isolates and E. coli TOP10 carrying \( \text{bla}_{\text{AIM-1}} \).

The amino acid sequence displayed the highest level of identity with THIN-B (42.1%) (22), followed by L1 (31.4%) (42), and is placed phylogenetically within the B3 subgroup cluster (29). The predicted amino acid sequence showed that the active site of AIM-1 has amino acid motifs that are broadly conserved throughout the MBL family of enzymes (Fig. 2), i.e., the zinc binding motif HXHXD (residues 116 to 120) and other residues involved in the coordination of the two Zn\(^{2+}\) ions (His196 and His263, according to the BBL numbering system [11]). While AIM-1 shows reasonable similarity to L1, it lacks the leucines at positions 5 and 8 and the methionine at position 140 essential for polymerization into a tetramer (Fig. 2) (12, 27). These data are consistent with the fact that AIM-1 behaved as a monomeric protein throughout the gel filtration process (data not shown).

**Susceptibility profiles of E. coli (pAIM-1).** The MICs of E. coli TOP10 harboring the cloned AIM-1 gene (pAIM-1) are reported in **Table 1**. pAIM-1 mediated resistance to penicillin, ampicillin, piperacillin, cephalothin, cefoxitin, cefotaxime, ceftazidime, and cefepime but did not confer resistance to the \( \beta \)-lactams aztreonam, cefepime, imipenem, and meropenem. Most MBL-positive *P. aeruginosa* strains (and the majority of *Acinetobacter* and *Enterobacteriaceae* strains) usually exhibit ceftazidime MICs of >100 µg/ml, which has become a signature for MBL detection, as the carbapenem MICs vary significantly and are an unreliable indication of the presence of an MBL (43). Thus, it is worrisome that this rule no longer holds true and that clinically significant MBLs could be missed upon screening due to their lack of resistance to *in vitro* ceftazidime, as seen in *Enterobacteriaceae* (8, 31).

**Genetic context of \( \text{bla}_{\text{AIM-1}} \).** Given that \( \text{bla}_{\text{AIM-1}} \) was not part of a standard class 1 integron, the upstream and downstream sequences were determined. Upstream of \( \text{bla}_{\text{AIM-1}} \), there is an ORF, which we have designated ISCR15A, which displays 93.7% nucleotide identity to ISCR5 (Fig. 3). Thus, unlike the majority of MBL genes, \( \text{bla}_{\text{AIM-1}} \) was not found on a class 1 integron, but instead, it is flanked by two ISCR elements (Fig. 1), rather like \( \text{bla}_{\text{SPM-1}} \) with ISCR4 and \( \text{bla}_{\text{OXA-45}} \) with ISCR5. The spacing between the

**FIG 1** Evidence of movement of ISCR15 and \( \text{bla}_{\text{AIM-1}} \), and relatedness of AIM-1-positive strains. All panels a marker (lane M), WCH2677 (lane 1), WCH28173 (lane 2), and WCH2837 (lane 3). (A) SpeI digestion. (B) I-CeuI digestion. (C) S1 digestion and probing with \( \text{bla}_{\text{AIM-1}} \). (D) SpeI digestion and probing with ISCR15.
ISCR15A and bla<sub>AIM-1</sub> ORFs is 270 bp (Fig. 3). Two hundred thirty-six base pairs downstream from the 3' end of the ISCR15 element is the signature origin of the insertion sequence (ori<sub>IS</sub>), 5' = GCG TTG GAA CTTCCTAT ACC 3' (boldface type indicates consensus sequence). This origin is strikingly similar to the ori<sub>IS</sub> sequences of IS<sub>1294</sub>, IS<sub>CR1</sub>, IS<sub>CR3</sub>, and IS<sub>CR5</sub>. Immediately upstream of the bla<sub>AIM-1</sub> structural gene is a putative ribosomal binding site (RBS), which is only a single base pair downstream of the ori<sub>IS</sub> site, indicating that the promoter for bla<sub>AIM-1</sub> lies within the 3' end of the ISCR15 element (Fig. 3), which has been shown for other ISCR1 and downstream resistance genes. A truncated ISCR15-like sequence (designated ISCR15<sub>B</sub>) of 185 bp was found downstream of bla<sub>AIM-1</sub>, which has a nucleotide identity of 96.7% to ISCR5 (16). However, the mobility of bla<sub>AIM-1</sub> is unlikely to be dependent on ISCR15A, as this element is in the wrong orientation and ISCR15B is truncated despite being in the correct orientation. However, the transposase of ISCR15A may still use the intact ori<sub>IS</sub> site of the truncated ISCR15B element and thus may still be capable of mobilizing bla<sub>AIM-1</sub> in trans (37). The ori<sub>IS</sub> of ISCR15A is positioned just 13 bp upstream of the start codon of bla<sub>AIM-1</sub>. Figure 3 shows a credible RBS, but we cannot identify an appropriate −10 or −35 promoter. However, given the close proximity of the start of bla<sub>AIM-1</sub> to the end of ISCR15A (as depicted by the ori<sub>IS</sub>), the promoter for bla<sub>AIM-1</sub> expression must lie within the transposase, as was demonstrated previously for ISCR1 and downstream resistance genes (22, 33). bla<sub>AIM-1</sub> and ISCR15 have GC ratios of 69.6% and 68.8%, respectively, indicating that they are likely derived from similar sources but also confirming their nonpseudomonal origin, which normally has a GC ratio of 66%.

However, the Southern blot patterns using the bla<sub>AIM-1</sub> and ISCR15 probes were not identical. These two genes were used as DNA probes to determine their genetic locations and examine any differences between the strains. While WCH2677 and WCH2837 hybridized as a single band with the bla<sub>AIM-1</sub> probe, WCH2813 gave a double band, indicating two copies of the MBL gene (Fig. 1). Since the ISCR15B element is truncated, this evidence may indicate that ISCR15A has mobilized both itself and bla<sub>AIM-1</sub> in WCH2813 but has replicated only itself in WCH2837. However, the possibility exists that in strain WCH2837, ISCR15 could have replicated both probes but that the second copy of bla<sub>AIM-1</sub> may have been deleted via a recombination event or, indeed, that the genes have been mobilized by another mobile element resident in

**FIG 2** Alignment of the amino acid sequence of AIM-1 with those of L1 and THIN-B. Differences in the amino acid sequences are noted by a single letter representing the amino acid change within a particular sequence. Conserved residues and residues involved in the coordination of the zinc ions are denoted with asterisks. Numbering is according to the updated BBL scheme (11).

**FIG 3** Schematic representation of WCH2677 carrying bla<sub>AIM-1</sub>. (The arrows in the gene boxes indicate the direction of transcription). The sequence upstream of bla<sub>AIM-1</sub> and the ori<sub>IS</sub> for ISCR1, ISCR3, ISCR5, and IS1294 is highlighted. The putative ribosomal binding site is underlined, and the start codon of bla<sub>AIM-1</sub> is indicated in boldface type. GenBank accession numbers are in parentheses.
these isolates (37). Interestingly, when these same digests were probed with ISCR15, only WCH2677 gave the single band, and WCH2837 had an additional band, suggesting the movement of ISCR15 within this isolate. The probing of genomic DNA with SpeI, S1, and I-CeuI indicated that \(\text{bla}_{\text{AIM-1}}\) is carried on the chromosome in all three strains (Fig. 1).

Functional properties of AIM-1. Analysis of purified AIM-1 by SDS-PAGE showed a single band corresponding to a molecular mass of 32 kDa (data not shown). Under our experimental conditions, AIM-1 readily hydrolyzed most compounds, with the exception of aztreonam and clavulanic acid (Table 2). Apart from the values for ceftazidime, these values are broadly similar to those of the \(\text{L1}\) enzyme. The turnover rates for the carbapenems are higher than those for most other MBLs due to the very high mass of 32 kDa (data not shown). Under our experimental conditions, the standard deviation ranged from 3 to 8.5%.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(k_{\text{cat}}) (s(^{-1}))</th>
<th>(K_m) ((\mu\text{M}))</th>
<th>(k_{\text{cat}}/K_m)</th>
<th>(k_{\text{cat}}/K_m)</th>
<th>(k_{\text{cat}}/K_m)</th>
<th>(k_{\text{cat}}/K_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>778 31</td>
<td>2.6 \times 10^3</td>
<td>410 75</td>
<td>5.5 \times 10^3</td>
<td>10^7 10^4</td>
<td>NR</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>594 41</td>
<td>1.4 \times 10^3</td>
<td>580 300</td>
<td>1.9 \times 10^3</td>
<td>480 1300</td>
<td>3.7 \times 10^3</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>337 17</td>
<td>2.2 \times 10^3</td>
<td>420 200</td>
<td>2.1 \times 10^3</td>
<td>100 500</td>
<td>2 \times 10^3</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>529 39</td>
<td>1.4 \times 10^3</td>
<td>65 43</td>
<td>1.5 \times 10^3</td>
<td>NR</td>
<td>2.4 \times 10^6</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>145 26</td>
<td>5.7 \times 10^3</td>
<td>140 120</td>
<td>8.8 \times 10^3</td>
<td>80 40</td>
<td>2 \times 10^6</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>609 49</td>
<td>1.2 \times 10^3</td>
<td>140 160</td>
<td>8.8 \times 10^3</td>
<td>140 50</td>
<td>2 \times 10^6</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>292 29</td>
<td>9.9 \times 10^3</td>
<td>53 130</td>
<td>4.1 \times 10^3</td>
<td>80 40</td>
<td>2 \times 10^6</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>7 148</td>
<td>4.9 \times 10^3</td>
<td>27 145</td>
<td>0.2 \times 10^3</td>
<td>20 140</td>
<td>1.4 \times 10^3</td>
</tr>
<tr>
<td>Cefepine</td>
<td>93 594</td>
<td>1.6 \times 10^3</td>
<td>0.33 130^a</td>
<td>2.5 \times 10^4</td>
<td>&gt;2.3</td>
<td>&gt;300</td>
</tr>
<tr>
<td>Aminopenem</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Meropenem</td>
<td>1,000 163</td>
<td>6.8 \times 10^4</td>
<td>77 13^f</td>
<td>5.9 \times 10^6</td>
<td>200 40</td>
<td>5 \times 10^6</td>
</tr>
</tbody>
</table>

\(a\) The standard deviation ranged from 3 to 8.5%.

\(b\) L1 described previously by Crowder et al. (3).

\(c\) Reported previously by Simm et al. (27).

\(d\) Reported previously by Miller et al. (37).

\(e\) THIN-B data were reported previously by Docquier et al. (9). IMP-1 data were reported previously by Laraki et al. (14). VIM-2 data were reported previously by Poirel et al. (19).

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References


