Dissemination of the Metallo-β-Lactamase Gene \( \text{bla}_{\text{IMP}-4} \) among Gram-Negative Pathogens in a Clinical Setting in Australia

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Background. The clinical utility of carbapenems is under threat because of the emergence of acquired metallo-β-lactamase (MBL) genes. We describe the first outbreak in Australia of infection and/or colonization with gram-negative pathogens carrying the MBL gene \( \text{bla}_{\text{IMP}-4} \).

Methods. MBL-producing organisms were identified using susceptibility data in conjunction with MBL screening methods. PCR and sequence analysis were performed to characterize the resistance gene and identify the presence of integrons. DNA profiles were determined by ribotyping. Clinical and epidemiological data were prospectively collected from January–July 2004.

Results. A total of 19 isolates were recovered from 16 patients: Serratia marcescens (10 isolates), Klebsiella pneumoniae (4 isolates), Pseudomonas aeruginosa (3 isolates), Escherichia coli (1 isolate), and Enterobacter cloacae (1 isolate). Isolates were resistant to most β-lactams except aztreonam, and variable resistance to carbapenems was observed (MIC range, 2 to \( 18 \) mg/L). PCR and sequence analysis identified the \( \text{bla}_{\text{IMP}-4} \) gene and a class 1 integrase (\( \text{IntI1} \)) in all isolates. Of the 16 patients, 12 (75%) had infection; 5 had septicemia, 5 had ventilator-associated pneumonia, 1 had a urinary tract infection, and 1 had a superficial central venous line infection. Six (38%) of the 16 patients died, and 5 of those 6 (31% of the group of 16) had clinical infection with an MBL-producing organism. All except 2 patients had spatio-temporal epidemiological links in the intensive care unit. All \( K. \) pneumoniae isolates were of different ribogroups, whereas the \( S. \) marcescens and \( P. \) aeruginosa isolates were predominately of the same ribogroup.

Conclusions. MBL-producing gram-negative organisms have now emerged in Australia. The resistance gene, \( \text{bla}_{\text{IMP}-4} \), appears highly mobile; this outbreak involved 5 different gram-negative genera from patients with close epidemiological links.

Carbapenems are important therapeutic agents for the treatment of infection with multidrug-resistant gram-negative bacteria, particularly those carrying genes for extended-spectrum and derepressed AmpC β-lactamases. Of the β-lactams, carbapenems have the broadest spectrum and greatest stability against hydrolysis by β-lactamases. However, the clinical utility of these antimicrobials is under threat with the emergence of acquired genes for carbapenemases, particularly those coding for Ambler class B metallo-β-lactamases (MBLs), now reported in Asia [1], Europe [2, 3], North America [4], and South America [5]. These enzymes (IMP-, VIM-, SPM- and GIM-types) have a broad substrate profile and often confer high-level resistance to all β-lactams except aztreonam. Unlike class A β-lactamases, MBLs are not inhibited by clavulanic acid, tazobactam, or sulbactam; but, because of their zinc dependence, they are susceptible to the ion chelator EDTA. Acquired MBLs have now been detected in a range of organisms, including \( Pseudomonas aeruginosa \) [2], \( Acinetobacter \) species [6], and Enterobacteriaceae [7], but rarely has a hospital outbreak been described in which multiple gram-negative genera are involved [8].

The first IMP-type MBL enzyme was described in Japan in 1991 [9]. Since then, 18 IMP-variant enzymes have been reported [10]. The \( \text{bla}_{\text{IMP}-4} \) gene, coding the
IMP-4 enzyme, has recently been described in Acinetobacter species isolates from Hong Kong [1] and a Citrobacter youngae isolate from the People’s Republic of China [11]. Of particular concern, the blaIMP-4 gene was identified on a class 1 integron residing on a large conjugative plasmid [11, 12]. Such genetic elements are critical for the acquisition, maintenance, and dissemination of resistance in gram-negative organisms [13].

We recently reported a carbapenem-resistant P. aeruginosa isolate carrying the blaIMP-4 gene that was recovered from blood culture [14]. Subsequently, an outbreak of infection and/or colonization with MBL-producing gram-negative organisms occurred at our institution. The objective of this study was to describe the microbiological, clinical, and epidemiological features of this outbreak.

MATERIALS AND METHODS

The study was conducted between January and July, 2004, at The Alfred Hospital, a 320-bed tertiary care institution in Melbourne, Australia. The hospital has a 36-bed intensive care unit (ICU) which is divided into medical and surgical ICUs and specializes in trauma, burns, and heart-lung transplantation. Gram-negative organisms cultured from clinical isolates underwent identification and initial susceptibility testing using an automated system (Vitek; bioMérieux). Isolates with resistance to carbapenems and/or resistance to other broad-spectrum β-lactams, such as ceftazidime or ticarcillin-clavulanate, underwent phenotypic MBL screening. Organisms known to inherently carry an MBL gene, such as Aeromonas hydrophilia, Flavobacterium species, and Stenotrophomonas maltophilia, were excluded.

A phenotypic MBL screening plate was designed, which included 5 components: (1) a double-disc synergy test that used an imipenem disc containing 10 μg of imipenem placed 20 mm (center to center) from a filter disc containing 10 μL of EDTA at a concentration of 100 mmol/L; (2) 2 imipenem discs placed 25 mm apart (center to center), 1 containing 10 μL of EDTA at a concentration of 100 mmol/L, to detect an inhibition zone size difference of ≥4 mm; and (3) an aztreonam disc containing 30 μg of aztreonam to detect aztreonam susceptibility. IsoSensitest agar (Oxoid) with a 1-in-10 dilution of a 0.5 McFarland standard inoculum was used.

If the presence of EDTA inhibited imipenemase activity, the organism was categorized as MBL-positive. These isolates underwent genotypic testing to confirm the presence of an MBL gene. The antibiotic MICs of all confirmed isolates were determined using broth microdilution according to the Clinical and Laboratory Standards Institute (i.e., NCCLS) guidelines [15, 16]. Only the first resistant isolate of a given genus from each patient was included in the study. Of the included isolates, those of the same genus underwent ribotyping with the automated RiboPrinter Microbial Characterisation System (Qualicon) using the restriction enzymes EcoR1 (for Enterobacteriaceae) or PVUII (for P. aeruginosa), as recommended by the manufacturer. We also examined, in a single patient, the riboprint pattern of an Enterobacter cloacae isolate before and after the detection of an MBL gene.

PCR analysis was performed with primers specific for blaVIM [17, 18] and blaIMP [17, 18]. DNA sequencing was performed with ABI Prism Big Dye Terminator Cycle sequencing (Applied Bioscience) and the ABI Prism 3700 (Applied Biosystems) automated sequencer. Primers to amplify the entire sequence of the blaIMP-4 gene were used [18]. DNA sequence analysis software (Lynnon Biosoft) was used for alignment of DNA sequences and deduced amino acid sequences. The presence of class 1, 2, or 3 integrons was detected by PCR amplification of an integrase-specific fragment of the IntI1, IntI2, and IntI3 genes, respectively [19].

Medical records of patients with confirmed isolation of MBL-producing organisms were reviewed for clinical and epidemiological data. The clinical significance of each isolate (i.e., whether it represented infection or colonization) was determined. Infection was defined as either (1) the presence of an acute systemic inflammatory response together with local symptoms or signs of infection, including purulent sputum and pyuria [8]; or (2) isolation of an organism from a sterile site.

RESULTS

From January to July 2004, a total of 2100 nonduplicate gram-negative isolates from clinical specimens were processed at The Alfred Hospital. A total of 204 (10%) of the 2100 isolates met the criteria for MBL screening. Of these, 20 isolates from 16 patients were found to be MBL-positive according to our phenotypic definition. These included 5 different genera of gram-negative pathogens: 10 isolates of Serratia marcescens, 4 isolates of Klebsiella pneumoniae, 4 isolates of P. aeruginosa, 1 isolate of E. cloacae, and 1 isolate of Escherichia coli. PCR analysis for the presence of blaVIM or blaIMP showed that 19 isolates (90.9% of the 204 screened) from 16 patients carried blaIMP. For 1 P. aeruginosa isolate, no gene product could be amplified. DNA sequencing confirmed the 19 isolates were all carrying the same MBL gene, blaIMP. Further PCR analysis showed that all isolates were also carrying IntI1, signifying the presence of a class 1 integron.

The results of susceptibility testing for the 19 blaIMP-positive isolates are shown in table 1. All isolates demonstrated high-level resistance to most broad-spectrum cephalosporins and inhibitor-protected penicillins. Resistance to carbapenems was variable (MIC range, 2 to >8 mg/L), except among P. aeruginosa isolates, which all demonstrated high-level resistance. Aztreonam susceptibility was not seen in the P. aeruginosa or E.
cloacae isolates. Resistance to non-β-lactam antibiotics, including aminoglycosides, quinolones, and sulfonamide derivatives, was variable. All isolates were susceptible to polymyxin B except for the S. marcescens isolates, which have intrinsic resistance to this antimicrobial agent.

The clinical characteristics of the 16 patients from whom β-lactamase-positive gram-negative organisms were isolated are summarized in Table 2. The median age was 45 years (range, 17–85 years) and 9 patients (56%) were male. Eight patients (50%) had no underlying disease prior to admission. Two patients (patients 1 and 12) had non-Hodgkin lymphoma, 3 patients (6, 11, and 16) had type 2 diabetes mellitus, 2 patients (10 and 15) were receiving prednisolone therapy, and 1 patient (14) had congenital heart disease. Twelve patients (75%) received a carbapenem prior to the isolation of an MBL-producing organism, with a median duration of treatment of 13 days (range, 3–23 days). The remaining 4 patients received broad-spectrum β-lactam antibiotics, including cephalosporins and β-lactam-β-lactamase inhibitor combinations.

All isolates were considered hospital-acquired. Of the 19 isolates carrying the \( \text{bla}\_\text{IMP-4} \) gene, 8 (42%) were isolated from the respiratory tract, which was the predominant source: 7 isolates were recovered from sputum samples and 1 from a bronchial alveolar lavage fluid specimen. Four isolates (21%) were recovered from urine cultures, and the remaining 7 isolates were recovered from blood cultures (\( n = 3 \)), wound cultures (\( n = 3 \)), and a central venous catheter-tip culture (\( n = 1 \)). In total, \( \text{bla}\_\text{IMP-4} \)-positive gram-negative organisms caused infection in 75% of cases. Five isolates were associated with ventilator-associated pneumonia, 3 were associated with septicemia, 1 was associated with urinary tract infection, and 1 was associated with superficial central venous line infection. Of the 3 urinary isolates representing colonization, 2 were associated with subsequent septicemia.

Six (38%) of the 16 patients from whom an MBL-producing organism was isolated died; 5 of those 6 (31% of the group of 16) had clinical infection with an MBL-producing organism. Three patients died with septicemia; in 2 of these 3 patients (patients 1 and 16), the primary source of bacteremia was central venous catheter–related infection (with \( P. \) aeruginosa and \( S. \) marcescens, respectively), and in 1 of the 3 (patient 10), the source was urinary colonization with \( P. \) aeruginosa. Patient 1 received empirical therapy with ciprofloxacin and teicoplanin, and patient 10 received empirical therapy with cefazolin, but both died before organism identification. Patient 16 received empirical therapy with ciprofloxacin, to which the organism was resistant, and died before susceptibility results were available. The remaining 2 patients (6 and 9) died with ventilator-associated pneumonia (in both cases due to \( S. \) marcescens), and patient 6 also had intra-abdominal sepsis. Patient 6 was initially treated with ciprofloxacin, tobramycin, and ceftazidime, and then treatment was changed to colistin and vancomycin. The treatment history for patient 9 was incomplete.

Seven patients with clinical infection survived. Of the 3 pa-

<table>
<thead>
<tr>
<th>Isolate (code)</th>
<th>Mer</th>
<th>Imi</th>
<th>Caz</th>
<th>Cpm</th>
<th>Tic-Clv</th>
<th>Cip</th>
<th>TMP-SMX</th>
<th>Gen</th>
<th>Tob</th>
<th>Amk</th>
<th>Atz</th>
<th>Pol</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{S. marcescens (WCH 1819)} )</td>
<td>4 (S)</td>
<td>8 (I)</td>
<td>&gt;16 (R)</td>
<td>16 (I)</td>
<td>&gt;128 (R)</td>
<td>2 (I)</td>
<td>1 (S)</td>
<td>8 (I)</td>
<td>8 (I)</td>
<td>4 (S)</td>
<td>0.25 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 1818)} )</td>
<td>8 (I)</td>
<td>8 (I)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>&gt;128 (R)</td>
<td>4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 1820)} )</td>
<td>8 (I)</td>
<td>8 (I)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>&gt;128 (R)</td>
<td>2 (I)</td>
<td>&lt;0.5 (S)</td>
<td>4 (S)</td>
<td>16 (R)</td>
<td>4 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 1822)} )</td>
<td>4 (S)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td>16 (I)</td>
<td>&gt;128 (R)</td>
<td>2 (I)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 1862)} )</td>
<td>8 (I)</td>
<td>8 (I)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>&gt;128 (R)</td>
<td>4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>2 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 1998)} )</td>
<td>&gt;8 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;128 (R)</td>
<td>4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 2026)} )</td>
<td>8 (I)</td>
<td>8 (I)</td>
<td>&gt;16 (R)</td>
<td>16 (I)</td>
<td>&gt;128 (R)</td>
<td>4 (I)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{S. marcescens (WCH 2030)} )</td>
<td>8 (I)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;128 (R)</td>
<td>4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>0.25 (S)</td>
<td>&gt;8 (I)</td>
</tr>
<tr>
<td>( \text{Pseudomonas aeruginosa (WCH 1823)} )</td>
<td>&gt;8 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;128 (R)</td>
<td>&gt;4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>16 (I)</td>
<td>&lt;1 (S)</td>
</tr>
<tr>
<td>( \text{P. aeruginosa (WCH 1874)} )</td>
<td>&gt;8 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;128 (R)</td>
<td>&gt;4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>16 (I)</td>
<td>&lt;1 (S)</td>
</tr>
<tr>
<td>( \text{P. aeruginosa (WCH 1891)} )</td>
<td>&gt;8 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;128 (R)</td>
<td>&gt;4 (R)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>16 (I)</td>
<td>&lt;1 (S)</td>
</tr>
<tr>
<td>( \text{Klebsiella pneumoniae (WCH 1821)} )</td>
<td>8 (I)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>&gt;128 (R)</td>
<td>&gt;0.03 (S)</td>
<td>&lt;0.5 (S)</td>
<td>2 (S)</td>
<td>4 (S)</td>
<td>1 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&lt;1 (—)</td>
</tr>
<tr>
<td>( \text{K. pneumoniae (WCH 1825)} )</td>
<td>4 (S)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td>4 (S)</td>
<td>&gt;128 (R)</td>
<td>&gt;0.03 (S)</td>
<td>&lt;2 (S)</td>
<td>4 (S)</td>
<td>1 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&lt;1 (—)</td>
<td></td>
</tr>
<tr>
<td>( \text{K. pneumoniae (WCH 1933)} )</td>
<td>8 (I)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>&gt;128 (R)</td>
<td>0.12 (S)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>0.5 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&lt;1 (—)</td>
</tr>
<tr>
<td>( \text{K. pneumoniae (WCH 2017)} )</td>
<td>4 (S)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td>8 (S)</td>
<td>&gt;128 (R)</td>
<td>&gt;0.03 (S)</td>
<td>&lt;2 (S)</td>
<td>8 (I)</td>
<td>1 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&lt;1 (—)</td>
<td></td>
</tr>
<tr>
<td>( \text{Escherichia coli (WCH 2025)} )</td>
<td>4 (S)</td>
<td>2 (S)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td>&gt;128 (R)</td>
<td>0.12 (S)</td>
<td>&gt;2 (R)</td>
<td>&gt;8 (R)</td>
<td>&gt;16 (R)</td>
<td>2 (S)</td>
<td>&lt;0.12 (S)</td>
<td>&lt;1 (—)</td>
</tr>
</tbody>
</table>

**Table 1.** Susceptibility profiles of the 19 gram-negative isolates carrying the \( \text{bla}\_\text{IMP-4} \) metallo-β-lactamase gene.
Table 2. Clinical characteristics of the 16 patients with bla<sub>IMP-4</sub>-positive gram-negative organisms isolated, in order of acquisition.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis at admission</th>
<th>Initial isolation of resistant organism</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Burkitt lymphoma</td>
<td>Pseudomonas aeruginosa (WCH 1823)</td>
<td>Death</td>
</tr>
<tr>
<td>2</td>
<td>Multiple trauma</td>
<td>Serratia marcescens (WCH 1819)</td>
<td>Discharge</td>
</tr>
<tr>
<td>3</td>
<td>Complications of endoscopy</td>
<td>S. marcescens (WCH 1818)</td>
<td>Death</td>
</tr>
<tr>
<td>4</td>
<td>Multiple trauma</td>
<td>S. marcescens (WCH 1820)</td>
<td>Discharge</td>
</tr>
<tr>
<td>5</td>
<td>Multiple trauma</td>
<td>Klebsiella pneumoniae (WCH 1821)</td>
<td>Death</td>
</tr>
<tr>
<td>6</td>
<td>Cardiac failure</td>
<td>S. marcescens (WCH 1822)</td>
<td>Death</td>
</tr>
<tr>
<td>7</td>
<td>Multiple trauma</td>
<td>K. pneumoniae (WCH 1825)</td>
<td>Discharge</td>
</tr>
<tr>
<td>8</td>
<td>Severe burns</td>
<td>P. aeruginosa (WCH 1874)</td>
<td>Discharge</td>
</tr>
<tr>
<td>9</td>
<td>Multiple trauma</td>
<td>S. marcescens (WCH 1862)</td>
<td>Death</td>
</tr>
<tr>
<td>10</td>
<td>Guillain-Barré syndrome</td>
<td>P. aeruginosa (WCH 1891)</td>
<td>Death</td>
</tr>
<tr>
<td>11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Myocardial infarct</td>
<td>K. pneumoniae (WCH 1933)</td>
<td>Discharge</td>
</tr>
<tr>
<td>13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Abdominal sepsis</td>
<td>Enterobacter cloacae (WCH 2007)</td>
<td>Death</td>
</tr>
<tr>
<td>14</td>
<td>Cardiac failure</td>
<td>S. marcescens (WCH 2016)</td>
<td>Discharge</td>
</tr>
<tr>
<td>15</td>
<td>Diverticulitis</td>
<td>K. pneumoniae (WCH 2017)</td>
<td>Discharge</td>
</tr>
<tr>
<td>16</td>
<td>Pneumonia and renal failure</td>
<td>S. marcescens (WCH 2026)</td>
<td>Death</td>
</tr>
</tbody>
</table>

**NOTE.** BAL, bronchoalveolar lavage; CVC, central venous catheter.

<sup>a</sup> In addition, a strain of S. marcescens was isolated from a CVC tip culture (on 26 June 2004) and a strain of Escherichia coli was isolated from a urine specimen (on 14 July 2004), both of which represented colonization.

<sup>b</sup> A strain of S. marcescens was isolated from a jejunostomy wound site culture (on 20 June 2004), and it represented colonization.
Metallo-β-Lactamases in Australia

Figure 1. Clinical epidemiology findings for the 16 patients who acquired a metallo-β-lactamase–producing gram-negative organism between January and July 2004. Each gram-negative genera is depicted by a different symbol and is plotted at the time of isolation.

DISCUSSION

Carbapenems play a critical role in the management of severe hospital-acquired infections and are often used as an antibiotic of last resort. The emergence of gram-negative pathogens with acquired MBLs that mediate carbapenem resistance is a worrisome development. This concern is compounded by the paucity of antibacterial drugs being developed to target gram-negative organisms [20]. To our knowledge, this is the first study to illustrate the ease of dissemination of an MBL gene among 5 different gram-negative genera in a clinical setting. It is also the first clinical outbreak of infection with MBL-producing organisms in Australia, a continent that only recently described the presence of this resistance mechanism [14]. Other unique features of this outbreak include the high rate of clinical infection and the detection of the *bla*<sub>IMP-4</sub> gene in isolates of *S. marcescens* and *E. cloacae*. A total of 19 isolates from 16 patients were identified over a 7-month period, representing 0.9% of gram-negative isolates. All isolates were found to carry the

13) had multiple genera of *bla*<sub>IMP-4</sub>-positive gram-negative pathogens isolated during a single hospital stay.

The ribotyping results are shown in figure 2. All *K. pneumoniae* isolates were of different ribogroups. In contrast, all except 1 *S. marcescens* isolate and all of the *P. aeruginosa* isolates (ribogroup 127-415-S-1, data not shown) were of the same ribogroup. The *E. cloacae* strains isolated from the same patient before and after *bla*<sub>IMP-4</sub> detection were of the same ribogroup.

Figure 1.
**Figure 2.** Dendrogram of ribotype patterns determined with the restriction enzyme EcoR1 for the Enterobacteriaceae isolates, indicating patient, species, ribogroup, and metallo-β-lactamase (MBL) coded for. All isolates of *K. pneumoniae* were of different ribogroups. All *S. marcescens* isolates, except one, were of the same ribogroup. Analysis of 2 *E. cloacae* isolates from the same patient (13), taken before and after MBL gene detection, were of the same ribogroup.

*b*\textsubscript{bla}\textsubscript{IMP-4} gene, a resistance gene previously reported in Hong Kong and China [1, 11], and were found to have 95.6% amino acid homology with the first described MBL gene, *bla*\textsubscript{IMP-1} [1].

The emergence of the *bla*\textsubscript{IMP-4} gene at our institution was rapid, and the majority of patients had strong epidemiological links. The most commonly isolated organism was *S. marcescens* (10 isolates), followed by *K. pneumonia* (4 isolates), *P. aeruginosa* (3 isolates), *E. cloacae* (1 isolate) and *E. coli* (1 isolate). Of particular interest, patients with temporal links in the ICU most often had isolates of different genera. Also, *bla*\textsubscript{IMP-4}-carrying isolates of multiple gram-negative genera were recovered from 2 patients during a single hospitalization. With regard to the ribotyping data, all *K. pneumoniae* isolates were unique strains, and the *E. cloacae* isolates recovered from a single patient before and after *bla*\textsubscript{IMP-4} detection, were the same strain; both of these findings suggest horizontal gene acquisition. In contrast, all *S. marcescens* isolates except 1 were the same strain and all *P. aeruginosa* isolates were the same strain, suggesting nosocomial transmission. Given the observed clinical and molecular epidemiology and the presence of a class 1 integron in all isolates, it would appear that horizontal gene transfer between genera was occurring. This important hypothesis needs further confirmation with integron characterization, mobile genetic element assessment, and gene transfer experiments.

Of the 19 *bla*\textsubscript{IMP-4}-positive isolates, only 5 (26%) were found to be carbapenem-resistant (MIC >8 mg/L). Seven isolates (37%) tested sensitive to imipenem (MIC ≤4 mg/L), including 2 isolates of *S. marcescens*, 4 isolates of *K. pneumoniae*, and 1 isolate of *E. coli* (table 1). This variable carbapenem susceptibility has been described previously [21, 22] and can be explained by a number of factors. Firstly, phenotypic expression of resistance is more likely when other resistance mechanisms are present. For example, the uniform appearance of carbapenem resistance in our *P. aeruginosa* isolates most likely represents the copresence of up-regulated efflux pumps and/or membrane impermeability [23]. Other explanations include suppressed MBL gene expression by secondary regulatory systems, leading to a silent or cryptic *bla*\textsubscript{IMP} gene [7], and varied carbapenem hydrolysis depending on the MBL gene dosage effect, which relates to the plasmid copy number [8]. Given the importance of early recognition, diagnostic laboratories must use more sensitive means of screening, such as those described in this and other studies [24]. Also, if available, molecular screening should be used to enable rapid detection of this emerging β-lactamase.

Aztreonam susceptibility is a common feature of MBL-producing organisms and was found in 15 (79%) of our isolates. It was not evident in our *P. aeruginosa* and *E. cloacae* isolates,
most likely due to the presence of derepressed AmpC β-lactamases. The clinical utility of aztreonam in treating human infections caused by MBL-producing organisms has not been studied. Data from animal studies suggest that aztreonam at high doses may be effective [25]. Given the limited therapeutic options and the potential severity of infection, aztreonam may become a more common part of our armamentarium.

Few studies describe the clinical characteristics of infections caused by MBL-producing organisms [8, 26–28]; the majority of those that do report high rates of colonization [8, 26, 28]. In the current study, 12 patients (75%) experienced clinical infection, with ventilator-associated pneumonia and septicemia being the most common. Fifty percent of patients had no pre-existing illness. This finding is in contrast to the findings of previous studies, which have reported higher rates of underlying malignancy [8, 28, 29] or comorbid illness [27]. Overall, 38% of patients died in the hospital. This may relate to the patient population, but of the 6 patients who died, 5 were infected with an MBL-producing organism; 3 had septicemia and 2 had ventilator-associated pneumonia.

The clinical implications of this study are of concern. The blaIMP-4 gene, which mediates resistance to broad-spectrum β-lactams, has now emerged in a new continent. Therapeutic options are limited and the roles of aztreonam and colistin therapy are uncertain. The apparent ease of MBL gene dissemination between gram-negative genera warrants further research to help devise means of prevention. Diagnostic laboratories in Australia and other countries must now be on high alert, because early detection may limit the wide dispersal of MBL genes. Implementation of strict infection control practices and the prudent and cautious use of carbapenems are also critical if we are to preserve the longevity of these invaluable antimicrobial agents.

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