CASE REPORT

A 67-year-old diabetic woman suffered a fall leading to a displaced distal spiral tibial plateau fracture. In the weeks prior to the fall, she had received multiple antimicrobials (clindamycin, lincomycin, cephalaxin, ciprofloxacin, and ceftazidime) for an infected hematoma of the breast and a series of lower respiratory tract infections. The patient underwent definitive repair of the fracture but postoperatively developed osteomyelitis. Debridement of the leg wound was performed. *Acinetobacter* species and vancomycin-resistant *Enterococcus* strains were isolated from the tissue removed. This *Acinetobacter* species (CR12-42) was carbapenem resistant. Despite ongoing antibiotic treatment, the patient’s leg required amputation in March 2013, after continuous inflammation, infections for more than 5 months, and an episode of severe *Clostridium difficile* infection resulting in colectomy. The leg infection was resolved by the amputation.

The initial identification of this *Acinetobacter* species was done by Vitek 2. Antimicrobial susceptibility testing by Vitek 2 (bioMérieux) showed resistance to carbapenems, ceftazidime, ceftriaxone, cefepime, gentamicin, tobramycin, trimethoprim-sulfamethoxazole, ticarcillin-clavulanic acid, and ciprofloxacin according to the EUCAST standard (1). The isolate was referred to our laboratory at the University of Queensland Centre for Clinical Research. The *Acinetobacter* isolate was identified to the species level by a gyrB multiplex PCR, which revealed that CR12-42 was *Acinetobacter pittii* (2). Partial rpoB sequencing (3) confirmed that CR12-42 was *A. pittii*.

Phenotypic characterization to determine the class of carbapenemase was performed as previously described (4–6). The *A. pittii* isolate showed a metallo-β-lactamase phenotype by producing a larger inhibition zone around carbapenem disks with EDTA than around carbapenem disks alone (>5-mm breakpoint increase in the size of the inhibition zone). The isolate also produced a positive result in the modified Hodge and Carba NP tests for carbapenemase production. MICS were determined with Etest (bioMérieux). The isolate was resistant to all of the carbapenems tested, i.e., cefazidime, cefotaxime, cefepime, cefoxitin, ticarcillin-clavulanic acid, trimethoprim-sulfamethoxazole, and ciprofloxacin (Table 1). Interestingly, this *A. pittii* isolate was susceptible to tetracycline, minocycline, colistin, and tigecycline (Table 1).

Carbapenem resistance in *Acinetobacter* species is commonly associated with the presence of carbapenem-hydrolyzing class D β-lactamase- or oxacillinase-encoding genes such as *bla*<sub>OXA-23</sub> and *bla*<sub>OXA-51</sub> in *Acinetobacter baumannii* (7, 8). A PCR assay and sequencing for all of the *bla*<sub>OXA</sub> genes frequently present in *Acinetobacter* species, i.e., *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-51-like</sub>, *bla*<sub>OXA-40-like</sub>, and *bla*<sub>OXA-58-like</sub>, were performed (7–9). The isolate was positive for the *bla*<sub>OXA-58-like</sub> subclass and negative for other subclasses of *bla*<sub>OXA</sub>. A PCR assay for ISA<sub>ba1</sub>, the common insertion element in *A. baumannii*, was also negative. A PCR assay and sequencing for other carbapenemase-encoding genes (10, 11), i.e., *bla*<sub>IMP</sub>, *bla*<sub>NDM</sub>, *bla*<sub>KPC</sub>, and *bla*<sub>IMP-4</sub>, were positive for *bla*<sub>IMP-4</sub>. A prepared pair-ended library of the whole genomic DNA was sequenced via Illumina MiSeq to further characterize the resistance mechanisms of *A. pittii* CR12-42 and to analyze its genome.

Whole-genome DNA sequencing produced a total of 138,932,382 paired-end reads with 30X average coverage. We used the CLC genomic workbench version 7.5 (CLC Bio, Aarhus, Denmark) for de novo assembly with a 500-bp minimum threshold resulting in 127 contigs. The draft genome consisted of 4,372,178 nucleotides and was annotated by rapid annotations using subsystems technology (RAST) (12). RAST annotation for all of the *bla*<sub>OXA</sub> genes frequently present in *Acinetobacter* species, i.e., *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-51-like</sub>, *bla*<sub>OXA-40-like</sub>, and *bla*<sub>OXA-58-like</sub>, were performed (7–9). The isolate was positive for the *bla*<sub>OXA-58-like</sub> subclass and negative for other subclasses of *bla*<sub>OXA</sub>. A PCR assay for ISA<sub>ba1</sub>, the common insertion element in *A. baumannii*, was also negative. A PCR assay and sequencing for other carbapenemase-encoding genes (10, 11), i.e., *bla*<sub>IMP</sub>, *bla*<sub>NDM</sub>, *bla*<sub>KPC</sub>, and *bla*<sub>IMP-4</sub>, were positive for *bla*<sub>IMP-4</sub>. A prepared pair-ended library of the whole genomic DNA was sequenced via Illumina MiSeq to further characterize the resistance mechanisms of *A. pittii* CR12-42 and to analyze its genome.

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TABLE 1 MICs of antimicrobials for A. pittii CR12-42 as determined by Etest

<table>
<thead>
<tr>
<th>Antimicrobial(s)</th>
<th>MIC (mg/liter)</th>
<th>Interpretation$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ertaepenem</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Imipenem</td>
<td>24</td>
<td>Resistant</td>
</tr>
<tr>
<td>Meropenem</td>
<td>12</td>
<td>Resistant</td>
</tr>
<tr>
<td>Doripenem</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefepime</td>
<td>64</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>&gt;256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>&gt;256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>12</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>2</td>
<td>Susceptible$^b$</td>
</tr>
<tr>
<td>Ticaricillin-clavulanic acid</td>
<td>256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Piperacillin</td>
<td>&gt;256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Amikacin</td>
<td>12</td>
<td>Intermediate</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>&gt;256</td>
<td>Resistant</td>
</tr>
<tr>
<td>Netilmicin</td>
<td>24</td>
<td>Resistant</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>3</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.75</td>
<td>Susceptible$^b$</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.023</td>
<td>Susceptible$^b$</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>&gt;32</td>
<td>Resistant</td>
</tr>
<tr>
<td>Colistin</td>
<td>0.094</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>0.094</td>
<td>Susceptible</td>
</tr>
</tbody>
</table>

$^a$ Unless noted otherwise, MIC interpretations are based on EUCAST criteria (1).
$^b$ Ampicillin-sulbactam, tetracycline, and minocycline MIC interpretations are based on CLSI criteria (33).

nomes of only three isolates were published, including one draft genome of an NDM-1-producing A. pittii strain from China (13).

In silico identification of CR12-42 to the species level by using rpoB and gyrB showed it to be 100% identical to A. pittii. A. pittii belongs, together with Acinetobacter nosocomialis, within the A. calcoaceticus-baumannii complex and was formerly named Acinetobacter genomic species 3 (14). In silico analysis of A. baumannii multilocus sequence typing (MLST) by the Pasteur scheme (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Abaumannii.html) identified A. pittii CR12-42 as being of sequence type 119 (ST119). The alleles found were cpn-60 (n = 36), fusA (n = 20), gltA (n = 38), pyrG (n = 16), recA (n = 38), rplB (n = 18), and rpoB (n = 20). It has been reported that MLST by the Pasteur scheme is capable of providing the ST of A. pittii (15). The clinical significance of A. pittii ST119 is indicated by the fact that it has been reported to be the predominant clone among the A. pittii strains (18 out of 25) isolated in four hospitals in Japan (16). Interestingly, these Japanese A. pittii isolates possessed a different bla<sub>IMP</sub> variant, bla<sub>IMP</sub>-19 (16). Of note, A. pittii ST119 has not been reported previously in Australia.

The resistance genes were screened with ResFinder (17). The β-lactamase-encoding genes bla<sub>IMP</sub>-4, bla<sub>OKA</sub>-96, and bla<sub>CARB</sub>-2 were identified. bla<sub>OKA</sub>-96 has a single nucleotide difference (a guanine-for-adenine substitution at position 483) from bla<sub>OKA</sub>-58, bla<sub>OKA</sub>-96 had been reported within an A. baumannii isolate from Singapore that also harbored bla<sub>OKA</sub>-23 and bla<sub>OKA</sub>-64 (18). In our isolate, bla<sub>OKA</sub>-96, had a genetic context similar to that of bla<sub>OKA</sub>-58, which was bracketed by ISA<sub>Baa3</sub> (GenBank accession number JX968506) (Fig. 1).

In addition, a novel bla<sub>OKA</sub> gene, bla<sub>OKA</sub>-421, was identified (Fig. 1). This gene had a genetic environment identical to that of the chromosomal bla<sub>OKA</sub>-51 gene in A. baumannii (19), which includes two genes that are usually present upstream and downstream of bla<sub>OKA</sub>-51 in A. baumannii, the phosphinothricin N-acetyltransferase-encoding gene and fxsA, respectively. bla<sub>OKA</sub>-421 has 95% identity with the previously reported bla<sub>OKA</sub> gene (GenBank accession number CP002177, locus tag BDGL_000903) from the genome of A. calcoaceticus PHEA-2 (20), which is the closest neighbor of our CR12-42 isolate, as previously mentioned. The second closest relative of bla<sub>OKA</sub>-421 was bla<sub>OKA</sub> of Acinetobacter oleivorans, with 89% similarity (GenBank accession number CP002080, locus tag AOLE_1170) (21). The other bla<sub>OKA</sub> genes similar to bla<sub>OKA</sub>-421 were bla<sub>OKA</sub>-324, bla<sub>OKA</sub>-325, bla<sub>OKA</sub>-326, bla<sub>OKA</sub>-332, and bla<sub>OKA</sub>-334 (88 to 89% similarity), which were recently identified in A. calcoaceticus (22). The carbapenemase activity of OXA-421 warrants further investigation.

The bla<sub>IMP</sub>-4 gene in A. pittii CR12-42 was located inside a class 1 integron. Downstream from bla<sub>IMP</sub>-4 were qacG2 and the aminoglycoside and chloramphenicol resistance genes aacA4 and catB2 (Fig. 1). This genetic context of bla<sub>IMP</sub>-4 in CR12-42 was found to be identical to that in an IMP-4-producing A. baumannii strain from Singapore (GenBank accession number DQ532122) (18).

FIG 1 Genetic contexts of the four β-lactamase-encoding genes in A. pittii CR12-42.
**blaIMP-4** has also been reported in *Acinetobacter junii* from Australia; however, the genetic context was not characterized (23). Our genetic context was also similar to that of *blaIMP-4* in the IncH1 type plasmid carrying *blaIMP-4* in an *E. cloacae* strain from Australia (24, 25). However, the plasmid backbone of these sequences could not be identified within our draft genome. Further investigation is needed to determine if *blaIMP-4* is located on a plasmid or the chromosome of CR12-42.

A carbenicillinase gene, *blaCARB-2*, was identified with ResFinder. *blaCARB-2* which was also designated *blaPSE-1* was first reported in *Pseudomonas aeruginosa* (26). The genetic context of *blaCARB-2* in CR12-42 was also potentially a class 1 integron with a truncated integrase (intI1) located upstream of *blaCARB-2* (Fig. 1). Other resistance genes found in this strain included *sul1* (sulfonamide resistance), *msr(E)* and *mph(E)* (macrolide resistance), and *aac-3-IId* (aminoglycoside resistance). Consistent with this, the *A. pittii* strain was resistant to gentamicin and tobramycin but susceptible to amikacin. Of note, no 16S rRNA methylase was found in this isolate.

Regardless of its resistance to multiple antimicrobials, *A. pittii* CR12-42 remained susceptible to tetracycline and minocycline, which was consistent with the absence of a tetracycline resistance gene within the draft genome. In addition, the MIC of ampicillin-sulbactam remained low (2 mg/liter), despite the presence of multiple carbapenemase-encoding genes. Further, sulbactam is known to have activity against *A. baumannii* (27). In a study by Higgins et al., the ampicillin-sulbactam MIC of 115 A. baumannii strains was 2 mg/liter (27). Ampicillin-sulbactam susceptibility was also shown in the majority of the previously reported *A. pittii* ST119 strains harboring *blaIMP-19* (94%) in Japan (16). In addition, 94% of these were susceptible to minocycline, similar to the antimicrobial phenotype of CR12-42 (16). Apart from the difference in *blaIMP* variants, CR12-42 has an antimicrobial phenotype and genotype identical to those of *A. pittii* ST119 from Japan.

**IMP-producing Enterobacteriaceae** strains have been frequently reported in Australia. Although OXA-23-like is the main subclass of carbapenemases identified in *A. baumannii*, IMP-4 is occasionally identified in *A. pittii* in locations such as Hong Kong and Singapore (18, 28). Other variants of *blaIMP*, such as *blaIMP-1*, *blaIMP-8*, *blaIMP-11*, and *blaIMP-19* have been described in *A. pittii* in Southeast Asia (16, 29, 30). *A. pittii* has also recently been reported to produce NDM (31, 32).

Generally, *A. baumannii* is considered the most important and the most prevalent *Acinetobacter* species causing infections. However, *A. pittii* has caused hospital outbreaks in The Netherlands and China (32, 33) and was reported as the most common *Acinetobacter* species causing nosocomial infections in Germany (34). Our study illustrates the emergence of a multidrug-resistant *A. pittii* strain in Australia. Therefore, accurate identification to the species level and characterization of the prevalence of *A. pittii* among the *Acinetobacter* species isolated in our region and its antibiotic resistance warrant further investigation.

This work was approved by the Royal Brisbane and Women’s Hospital Human Research Ethics Committe (HREC/13/QRWB/391: epidemiology, clinical significance, treatment, and outcome of infections by carbapenem-resistant *Enterobacteriaceae* and *Acinetobacter* species in Queensland). This project is registered as BioProject PRJNA255268 and BioSample SAMN03003652.

**Nucleotide sequence accession numbers.** The GenBank accession number of *blaOXA-21* is KM401566. The GenBank accession number of the draft genome of *A. pittii* CR12-42 is JQNT00000000.

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**REFERENCES**


