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A Case of IMP-4-, OXA-421-, OXA-96-, and CARB-2-Producing *Acinetobacter pittii* Sequence Type 119 in Australia

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An IMP-4-producing *Acinetobacter pittii* strain coproducing oxacillinases was isolated from a leg wound of a 67-year-old female patient. Identification to the species level by *rpoB* and *gyrB* sequencing and multiplex-PCR-based analysis revealed that the isolate was *A. pittii*. Whole-genome sequencing of this *A. pittii* isolate determined the presence of bla_{OXA-96}, bla_{CARB-2}, and a novel $bla_{OXA-421}$ gene. The position of this novel $bla_{OXA-421}$ gene was similar to that of bla_{OXA-51} in *A. baumannii*, downstream of the phosphinothricin *N*-acetyltransferase gene and upstream of *fxsA* in the chromosome. This *A. pittii* isolate was found to belong to sequence type 119 (ST119). Here, we report the first isolation of IMP-4-producing *A. pittii* ST119 with a novel $bla_{OXA-421}$ gene from a patient in Australia and characterize its draft genome.

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, cephalexin, 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., ceftazidime, 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_{OXA-23} and bla_{OXA-51} in *Acinetobacter baumannii* (7, 8). A PCR assay and sequencing for all of the bla_{OXA} genes frequently present in *Acinetobacter* species, i.e., $bla_{OXA-23-like}$, $bla_{OXA-51-like}$, $bla_{OXA-40-like}$, and $bla_{OXA-58-like}$, were performed (7–9). The isolate was positive for the $bla_{OXA-58-like}$ subclass and negative for other subclasses of bla_{OXA}. A PCR assay for IS*Aba1*, 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_{IMP}, bla_{NDM}, bla_{KPC}, and bla_{VIM}, were positive for bla_{IMP-4}. 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 30× 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 showed that *Acinetobacter calcoaceticus* PHEA-2 (score, 503) and *Acinetobacter* sp. strain SH024 (score, 436) are the two closest neighbors of *A. pittii* CR12-42. Our isolate was related to only one other *A. pittii* strain, TG6411, but with a lower score of 221. A total of 13 *A. pittii* draft genomes have been described in the BioProject (http://www.ncbi.nlm.nih.gov/bioproject/); however, draft ge-

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

Antimicrobial(s)	MIC (mg/liter)	Interpretation[a]
Ertapenem	>32	Resistant
Imipenem	24	Resistant
Meropenem	12	Resistant
Doripenem	>32	Resistant
Cefepime	64	Resistant
Ceftazidime	>256	Resistant
Cefotaxime	>32	Resistant
Ceftriaxone	>32	Resistant
Cefuroxime	>256	Resistant
Cefoxitin	>256	Resistant
Piperacillin-tazobactam	12	Resistant
Ampicillin-sulbactam	2	Susceptible[b]
Ticarcillin-clavulanic acid	256	Resistant
Piperacillin	>256	Resistant
Amikacin	12	Intermediate
Gentamicin	>256	Resistant
Netilmicin	24	Resistant
Ciprofloxacin	3	Resistant
Tetracycline	0.75	Susceptible[b]
Minocycline	0.023	Susceptible[b]
Trimethoprim-sulfamethoxazole	>32	Resistant
Colistin	0.094	Susceptible
Tigecycline	0.094	Susceptible

[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_{IMP} variant, bla_{IMP-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_{IMP-4}, bla_{OXA-96}, and bla_{CARB-2} were identified. bla_{OXA-96} has a single nucleotide difference (a guanine-for-adenine substitution at position 483) from bla_{OXA-58}. bla_{OXA-96} had been reported within an *A. baumannii* isolate from Singapore that also harbored bla_{OXA-23} and bla_{OXA-64} (18). In our isolate, bla_{OXA-96} had a genetic context similar to that of bla_{OXA-58}, which was bracketed by IS*Aba3* (GenBank accession number JX968506) (Fig. 1).

In addition, a novel bla_{OXA} gene, $bla_{OXA-421}$, was identified (Fig. 1). This gene had a genetic environment identical to that of the chromosomal bla_{OXA-51} gene in *A. baumannii* (19), which includes two genes that are usually present upstream and downstream of bla_{OXA-51} in *A. baumannii*, the phosphinothricin *N*-acetyltransferase-encoding gene and *fxsA*, respectively. $bla_{OXA-421}$ has 95% identity with the previously reported bla_{OXA} 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 $bla_{OXA-421}$ was bla_{OXA} of *Acinetobacter oleivorans*, with 89% similarity (GenBank accession number CP002080, locus tag AOLE_1170) (21). The other bla_{OXA} genes similar to $bla_{OXA-421}$ were $bla_{OXA-324}$, $bla_{OXA-325}$, $bla_{OXA-326}$, $bla_{OXA-332}$, and $bla_{OXA-354}$ (88 to 89% similarity), which were recently identified in *A. calcoaceticus* (22). The carbapenemase activity of OXA-421 warrants further investigation.

The bla_{IMP-4} gene in *A. pittii* CR12-42 was located inside a class 1 integron. Downstream from bla_{IMP-4} were *qacG2* and the aminoglycoside and chloramphenicol resistance genes *aacA4* and *catB2* (Fig. 1). This genetic context of bla_{IMP-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).

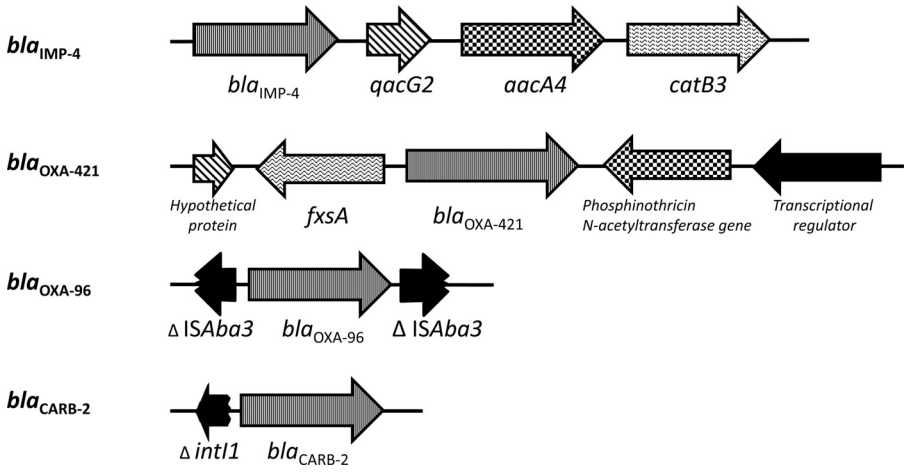

FIG 1 Genetic contexts of the four β-lactamase-encoding genes in *A. pittii* CR12-42.

bla_{IMP-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 bla_{IMP-4} in the IncHI2-type plasmid from *Enterobacter cloacae* and *Escherichia coli* and the IncL/M plasmid carrying bla_{IMP-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 bla_{IMP-4} is located on a plasmid or the chromosome of CR12-42.

A carbenicillinase gene, bla_{CARB-2} was identified with ResFinder. bla_{CARB-2}, which was also designated bla_{PSE-1}, was first reported in *Pseudomonas aeruginosa* (26). The genetic context of bla_{CARB-2} in CR12-42 was also potentially a class 1 integron with a truncated integrase (*intI1*) located upstream of bla_{CARB-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_{50} 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 bla_{IMP-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 bla_{IMP} 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 bla_{IMP}, such as bla_{IMP-1}, bla_{IMP-8}, bla_{IMP-11}, and bla_{IMP-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 Netherland 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 Committee (HREC/13/QRBW/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 $bla_{OXA-421}$ is KM401566. The GenBank accession number of the draft genome of *A. pittii* CR12-42 is JQNT00000000.

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