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First Report of the Carbapenemase Gene $bla_{OXA-499}$ in *Acinetobacter pittii*

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ABSTRACT We identified the carbapenemase gene $bla_{OXA-499}$, a variant of $bla_{OXA-143}$, from a clinical isolate of *Acinetobacter pittii* for the first time. OXA-499 shared 93.1% amino acid identity with OXA-143, and the gene was located on the chromosome. By cloning the OXA-499-encoding gene into the pWH1266 vector and transforming it into susceptible *Acinetobacter* spp., we were able to show that OXA-499 confers resistance to carbapenems.

KEYWORDS *Acinetobacter pittii*, carbapenemase resistant, OXA-143-like, OXA-499

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Acinetobacter spp. are opportunistic pathogens that cause various nosocomial infections, such as pneumonia, bacteremia, wound infections, and meningitis (1). Over the course of time, these pathogens have acquired resistance genes to nearly all antibiotic classes, including fluoroquinolones, aminoglycosides, and carbapenems, which make them difficult to treat. There has been a rapid increase in carbapenem-resistant *Acinetobacter* spp. in Korea, which may be due to a significant increase in carbapenem usage (2). Carbapenem resistance in *Acinetobacter* spp. is associated mainly with carbapenem-hydrolyzing class D β-lactamases (CHDLs), such as OXA-23, OXA-24, OXA-58, OXA-51, OXA-143, and their variants (3–8). *Acinetobacter pittii* is frequently associated with hospital-associated infections and was the most commonly isolated *Acinetobacter* sp. in a recent German study (9). The substantial increase in carbapenem resistance in *A. pittii* needs to be further studied for us to understand its clinical significance (10–12). Here, we report the occurrence of carbapenemase OXA-499, a variant of OXA-143, in carbapenem-resistant *Acinetobacter pittii* clinical isolate YMC2010/8/T346 belonging to the novel sequence type 1385 (ST1385), recovered from a patient in a university-affiliated hospital in South Korea.

In 2010, a 69-year-old male patient was admitted to the emergency room in a university-affiliated hospital in South Korea with signs of fever and abdominal pain. A week earlier, the patient had undergone an esophagojejunostomy, and peritonitis due to leakage was suspected. The patient's initial white blood cell count was 34,480/μl, and his C-reactive protein and procalcitonin levels were elevated. Empirical therapy with piperacillin-tazobactam (4.5 g intravenously [i.v.] thrice daily [TID]) was administered for 2 days. Due to the unstable vital signs of the patient, the regimen was changed to metronidazole (500 mg i.v. twice daily for 4 days), teicoplanin (400 mg i.v. once daily for 10 days), and meropenem (1 g i.v. TID for 13 days). In the culture of isolates obtained from the peritoneal catheter tip, *Enterococcus faecium*, coagulase-negative staphylococci, and *A. pittii* were identified by using the Bruker Biotyper matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (MS) system.

MICs were determined by Etest (bioMérieux, Marcy-l'Étoile, France). *A. pittii* isolate YMC2010/8/T346 was susceptible to piperacillin (MIC, 32 μg/ml), ceftazidime (MIC, 4 μg/ml), cefepime (MIC, 4 μg/ml), ciprofloxacin (MIC, 0.25 μg/ml), and imipenem (MIC, 2 μg/ml) but resistant to meropenem (MIC, 16 μg/ml) according to CLSI guidelines (13). Genomic DNA was isolated using the Wizard genomic DNA purification kit (Promega, Madison, WI), and a whole-genome library was constructed and sequenced on a 318 chip using the Ion Torrent PGM system and Ion Sequencing 400 kit (Life technologies, CA, USA). Additionally, PacBio single-molecule real-time (SMRT) sequencing and genome assembly were performed to identify the location of the $bla_{OXA-499}$ allele. Annotation was performed with RAST (http://rast.nmpdr.org) and the NCBI Prokaryotic Genome Annotation Pipeline. Genomic analysis was performed using Geneious pro 8.0 (https://www.geneious.com), and resistance genes were screened using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) with manual scrutiny.

Whole-genome analysis indicated the presence of $bla_{OXA-506}$, a variant of the *A. pittii* intrinsic $bla_{OXA-213-like}$ and bla_{ADC-41} alleles, which were not associated with insertion sequence (IS) elements, as well as the acquired $bla_{OXA-499}$ gene. Since previous studies indicated that the intrinsic bla_{OXA} from *A. pittii* has thus far not been shown to confer resistance, we considered $bla_{OXA-499}$ as the probable candidate conferring carbapenem resistance (14). Further analysis of the $bla_{OXA-499}$ gene revealed that this enzyme is a variant of OXA-143, first described in *A. pittii* isolated in Germany, which has been deposited under GenBank accession number NG_049775. However, the related data on its carbapenemase characteristics have not been reported to date. OXA-143-like variants have been identified worldwide. OXA-143 and OXA-231 were identified in Brazil, OXA-253 in Honduras and Brazil, OXA-182 in South Korea, and OXA-255 in the United States (8). OXA-499 shared amino acid identities of 93.1%, 92.7%, 96.0%, 92.7%, and 98.9% with OXA-143, OXA-231, OXA-253, OXA-182, and OXA-255, respectively (see Table S1 in the supplemental material). As a result, all of these were grouped together as an OXA-143-like clade in the amino acid phylogeny of OXA-carbapenemase genes (see Fig. S1 in the supplemental material). The deduced protein sequence showed 3 amino acid differences between OXA-499 and OXA-255, namely, Thr22-Ser, Lys29-Asn, and Ser158-Asn. The amino acids conserved in residues STFK (position 81–84), FGN (position 154–156), and KSG (position 218–220) were the same as those in other class D β-lactamases and CHDLs (see Fig. S2 in the supplemental material).

The open reading frame of the $bla_{OXA-499}$ gene was amplified using primers OXA-499A (5′-ATGAAAAAATTTATACTTCCTATCTTCAGC-3′) and OXA-499B (5′-TTATATAATCCCTAAATTTTCTAATG-3′) and ligated into the PstI-digested shuttle vector pWH1266 before transforming it into electrocompetent carbapenem-susceptible *Acinetobacter baumannii* ATCC 19606 and *A. pittii* YMC2013/1/R3000 as described previously (15). This led to increases in the MICs of imipenem, meropenem, and ertapenem from 0.38, 0.75, and 4 μg/ml to 4, 8, and ≥32 μg/ml, respectively; i.e., an 8- to 10-fold increase in carbapenem resistance was observed. Additionally, cloning encompassing the endogenous promoter of $bla_{OXA-499}$, as described by Zander et al. (16), was performed, which increased the MICs of all three carbapenems used in this study to ≥32 μg/ml (Table 1). In addition, positive results were seen for the three-dimensional extract and modified-Hodge tests (17) with carbapenem discs when the $bla_{OXA-499}$ gene was cloned into pET28a(+) and transformed into *Escherichia coli* BL21(DE3) (see Fig. S3 in the supplemental material). Despite repeated transformation of the $bla_{OXA-499}$ gene using a plasmid preparation from *A. pittii* YMC2010/8/T346 to either *A. baumannii* or *A. pittii*, the gene was not transferable. PacBio SMRT sequencing indicated the presence of the $bla_{OXA-499}$ gene in the chromosome. Genomic sequence comparisons of various *Acinetobacter* spp. (see Fig. S4 in the supplemental material) indicated the insertion of a 4.5-kb fragment consisting of a plasmid-associated putative peptidase gene present upstream and a TonB-dependent receptor plug domain present downstream of the $bla_{OXA-499}$ gene (Fig. 1). The Xer C/D-like sites were located 40 bp upstream (5′-ATTT

TABLE 1 Carbapenem susceptibility of *A. pittii* YMC2010/8/T346, *A. pittii* YMC2013/1/R3000, *A. baumannii* ATCC19606, and transformants harboring $bla_{OXA-499}$- encoding recombinant pWH1266

Strain and vector	MIC (μg/ml) and interpretation of[a]:		
	Meropenem	Imipenem	Ertapenem[b]
A. pittii YMC2010/8/T346	16, R	1.5, S	≥32
A. baumannii ATCC19606	0.75, S	0.38, S	4
A. baumannii ATCC19606 + pWH1266	1, S	0.38, S	6
A. baumannii ATCC19606 + pWH1266::Oxa499__P[c]	≥32, R	≥32, R	≥32
A. baumannii ATCC19606 + pWH1266::Oxa499	8, I	4, S	≥32
A. pittii YMC2013/1/R3000	0.75, S	0.38, S	4
A. pittii YMC2013/1/R3000 + pWH1266	0.75, S	0.38, S	8
A. pittii YMC2013/1/R3000 + pWH1266::Oxa499__P[a]	≥32, R	≥32, R	≥32
A. pittii YMC2013/1/R3000 + pWH1266::Oxa499	8, I	4, S	≥32

[a]R, resistant strains; I, intermediate strains; S, susceptible strains.
[b]CLSI did not provide MIC interpretation guidelines for ertapenem in *Acinetobacter* spp.
[c]Oxa499_P indicates that the cloning was performed including the natural promoter of the $bla_{OXA-499}$ gene.

AATATAATACGCCTTATACGAAAT-3′) of the putative peptidase, 79 bp upstream (5′-ATT TAACATAATGGGCGTTATGTTAAGT-3′) of $bla_{OXA-499}$, and 44 bp downstream (5′-TTACG CATAAGCCGTATTATGTTAATT-3′) of the TonB-dependent receptor plug domain, which indicates the mobility and probable recombination of this fragment into the chromosome. The putative peptidase gene showed 77% similarity to a peptidase encoded on *A. baumannii* plasmid p3ABSDF (GenBank accession number CU468233), and the TonB-dependent receptor plug domain showed 55% amino acid identity with a hypothetical protein in *Acinetobacter* spp. NIPH1867 (locus tag WP_005210788), which was similar to findings reported previously (16). In addition, the regions surrounding this 4.5-kb fragment insertion showed similarity with the plasmids identified in *Acinetobacter* spp. in a BLAST search. The above findings implied that the initial location of $bla_{OXA-499}$ was in a plasmid, which subsequently integrated into the chromosome.

Initially described in *A. baumannii* isolated from Brazil (18), OXA-143 variants are now being detected all around the globe in *Acinetobacter* spp. The detection of OXA-499 in a novel sequence type of *A. pittii*, including the previously detected OXA-182, in South Korea is a cause of great concern and indicates the possibility that more variants of the carbapenemase gene $bla_{OXA-143}$ exist.

Nucleotide sequence accession number(s). The nucleotide sequence of the $bla_{OXA-499}$ gene and the whole-genome sequences of *A. pittii* isolate YMC2010/8/T346 generated from Ion Torrent PGM and PacBio SMRT sequencing are available under the GenBank accession numbers KX828713, MKHF00000000, and CP017938, respectively.

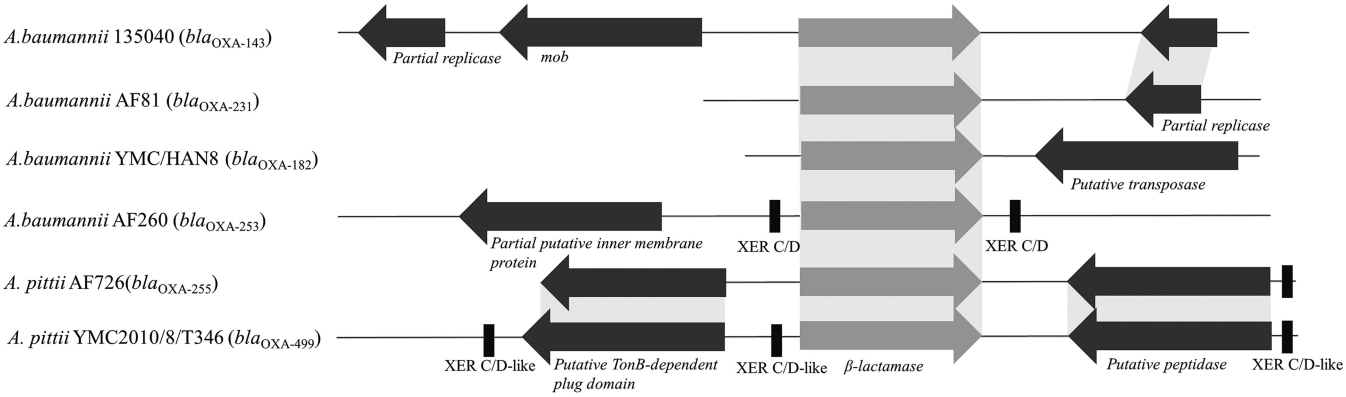

FIG 1 Schematic drawing of the $bla_{OXA-143-like}$ flanking regions in *A. baumannii* 135040 (GenBank accession no. NG_049441), *A. baumannii* AF81 (NG_049527), *A. baumannii* HAN8 (NG_049483), *A. baumannii* AF260 (NG_049548), *A. pittii* AF726 (NG_049550), and *A. pittii* YMC2010/8/T346 (KX828713). Shaded regions indicate the similarities between the isolates.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02676-16.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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