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Emergence of *Acinetobacter pittii* Harboring New Delhi Metallo-β-Lactamase Genes in Daejeon, Korea

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Carbapenemase production has been reported worldwide in gram-negative bacteria, including *Acinetobacter* species. We detected carbapenemase-producing *Acinetobacter pittii* in clinical isolates in Daejeon, Korea. Twenty-one ertapenem-resistant *A. pittii* isolates screened with a disk diffusion method were characterized by using the Epsilon test, four multiplex PCR assays, and a multilocus sequence typing (MLST) scheme. A total of 21 *A. pittii* isolates harbored the metallo-β-lactamase (MBL) gene bla_{IMP-1} or bla_{NDM-1}. Nineteen isolates containing bla_{IMP-1} were resistant to imipenem and meropenem, but two isolates harboring bla_{NDM-1} were susceptible to them. The sequence types (STs) of the two New Delhi MBL (NDM-1)-producing *A. pittii* isolates were ST70 and ST207, which differed from the STs (ST63, ST119, ST396, and a novel ST) of the IMP-1-producing *A. pittii*. This is the first report on NDM-1-producing *A. pittii* isolates in Korea. Our results emphasize that the study of NDM-1-producing gram-negative bacteria should involve carbapenem-susceptible as well as carbapenem-resistant isolates.

Key Words: *A. pittii*, Carbapenem, Metallo-β-lactamase, NDM-1

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Acinetobacter species are important nosocomial and opportunistic pathogens that cause frequent outbreaks in intensive care units. Most strains involved in nosocomial infections are highly resistant to various antimicrobial agents and cause life-threatening illnesses. Carbapenems have been the drugs of choice to treat *Acinetobacter* infection because they are stable in response to extended-spectrum and AmpC β-lactamases. However, carbapenem-resistant *Acinetobacter* strains have been increasingly reported [1, 2].

Carbapenemase production is the most critical mechanism of carbapenem resistance, and various carbapenemases, such as the Ambler class B metallo-β-lactamases (MBLs) of IMP, GIM, SIM, SPM, and VIM types and the Ambler class D carbapenemases of OXA-23, OXA-40, OXA-58, and OXA-143 types, have been reported in *Acinetobacter* species worldwide [3]. In addition, New Delhi MBL (NDM-1) has recently emerged as a key carbapenemase that compromises the efficiency of almost all β-lactams.

Although carbapenemase genes are widely disseminated among *Acinetobacter* species isolates, few data are available for *Acinetobacter* pittii isolates harboring carbapenemase. Clinically, *Acinetobacter baumannii* is the most commonly isolated species, but *A. pittii* isolates are also detected frequently and show resistance to various antimicrobial agents. In this study, we investigated carbapenemase genes in *A. pittii* isolates from a university hospital in Daejeon, Korea. In addition, multilocus sequence typing (MLST) was performed to analyze the relationship between carbapenemase types and *A. pittii* isolates harboring carbapenemases.

A. pittii isolates were collected at a university hospital laboratory in Daejeon, Korea, between January 2006 and December 2013. *A. pittii* was identified by using the Vitek 2 Automated Instrument ID System (bioMérieux; Marcy l'Etoile, France) and by sequencing the partial *rpoB* housekeeping gene as described previously [4].

To investigate the presence of carbapenemases, we per-

formed disk diffusion tests using 10-μg ertapenem disks (BD, Franklin Lakes, NJ, USA). All isolates with inhibition zone diameters of ≤21 mm on the ertapenem disks were subjected to carbapenemase detection. The minimum inhibitory concentrations (MICs) of *A. pittii* isolates for imipenem, meropenem, ceftazidime, and cefepime were determined with the Epsilon test (Etest; bioMérieux). The data were interpreted according to the criteria approved by CLSI [5]. *Escherichia coli* ATCC 25922 was used as a reference strain.

A total of 21 ertapenem-resistant *A. pittii* isolates were recovered from urine, sputum, or wound specimens. Most of these isolates (85.7%) were obtained from urine specimens (Table 1). The isolates were subjected to four multiplex PCR assays for the detection of carbapenemase genes. These assays were defined as multi-1, multi-2, multi-3, and multi-4 and were composed of specific primer sets [6-8]. Total DNA was obtained from each target strain by using a DNA purification kit (SolGent, Daejeon, Korea) in accordance with standard protocols. Each multiplex PCR was performed in a total volume of 25 μL with 50 ng total DNA, 2.5 μL 10× Taq buffer, 0.5 μL 10 mM dNTP mix, 20 pmol each primer, and 0.7 U Taq DNA polymerase (SolGent). PCR was performed in a GeneAmp PCR System 9600 thermal cycler (Perkin-Elmer Cetus Corp., Norwalk, CT, USA) with predenaturation of the reaction mixture at 95°C for 5 min, followed by 35 cycles at 95°C for 30 sec, 52°C for 40 sec, and 72°C for 30 sec, with a final extension at 72°C for 5 min. The amplified products were separated via electrophoresis on 1.5% (w/v) agarose gels containing ethidium bromide and visualized with a BioDoc-14TM Imaging system (UVP, Cambridge, UK).

All positive isolates from the multiplex PCR were subjected to PCR assays with specific primers to identify carbapenemase genes [9]. NDM-1-positive *A. pittii* isolates were also subjected to PCR with previously described primers to analyze the surrounding structure of bla_{NDM-1} [10]. The amplicons were purified

Table 1. Characteristics of *Acinetobacter pittii* isolates harboring bla_{MBL} genes

Isolate No.	Source	Year	ST	Allele profile*	MBL gene	MIC (μg/mL)			
						IP	MP	TZ	PM
601	Urine	2006	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	>256
603	Urine	2006	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	>256
605	Sputum	2006	119	36-20-38-16-38-18-20	bla_{IMP-1}	>32	>32	>256	128
606	Urine	2006	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	24
608	Urine	2006	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	48
610	Urine	2006	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	48
611	Wound	2006	396	60-21-46-10-20-18-20	bla_{IMP-1}	>32	>32	>256	48
909	Urine	2009	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	32
923	Sputum	2009	396	60-21-46-10-20-18-20	bla_{IMP-1}	>32	>32	>256	48
931	Urine	2009	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>64	8
952	Urine	2009	63	17-20-23-10-20-13-20	bla_{IMP-1}	>32	>32	>256	>256
1114	Urine	2011	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
1115	Urine	2011	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
1147	Urine	2011	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
1148	Urine	2011	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
12200	Urine	2012	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
12247	Urine	2012	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
12273	Urine	2012	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	>256
12280	Urine	2012	N	45-58-46-10-20-18-56	bla_{IMP-1}	>32	>32	>256	48
13049	Urine	2013	207	45-20-44-16-25-29-20	bla_{NDM-1}	0.5	0.25	32	8
13055	Urine	2013	70	23-20-23-16-25-18-20	bla_{NDM-1}	1	0.25	>256	16

*Indicates profiles of allele numbers attributed to each distinct allele sequence.
Abbreviations: IP, imipenem; MBL, metallo-β-lactamase; MIC, minimum inhibitory concentration; MP, meropenem; N, novel sequence type; PM, cefepime; ST, sequence type; TZ, ceftazidime.

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with a PCR purification kit (SolGent) and sequenced using a BigDye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster City, CA, USA) and an ABI PRISM 3730XL DNA analyzer (PE Applied Biosystems). The various DNA sequences were confirmed with the Basic Local Alignment Search Tool (BLAST) paired alignment facility (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

An MLST scheme with seven housekeeping genes (*cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, and *rpoB*) was used to determine the sequence types (STs) of *A. pittii* isolates [11]. Each ST number was assigned by comparing the allele sequences to those in the MLST databases (http://www.pasteur.fr/mlst and http://pubmlst.org/abaumannii).

All 21 consecutive *A. pittii* isolates resistant to ertapenem contained bla_{IMP-1} or bla_{NDM-1} (Table 1). However, other MBL genes and OXA-type carbapenemases were not found within the cohort.

Most of the ertapenem-resistant *A. pittii* isolates (90.5%) harbored bla_{IMP-1}, and all of these isolates showed high resistance to imipenem (MICs ≥32 μg/mL) and meropenem (MICs ≥32 μg/mL). The frequency of these isolates was in accordance with that of previously reported bla_{IMP-1}-containing *Acinetobacter* species isolated in Korea [12]. Our results indicate that bla_{IMP-1} is widely disseminated among ertapenem-resistant *A. pittii* isolates at the university hospital in Daejeon, Korea.

Interestingly, two ertapenem-resistant *A. pittii* isolates in this study contained bla_{NDM-1}, which has been detected in *Enterobacteriaceae* clinical isolates in South Korea [13]. This study is the first to report an NDM-1-type MBL in *A. pittii* isolated in Korea. Because NDM-1 was first described in carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* isolates, this MBL has now been found in various *Enterobacteriaceae*, *Pseudomonas*, and *Acinetobacter* species [14]. Although NDM-1-producing isolates are usually resistant to carbapenems, the isolates harboring bla_{NDM-1} in our study were susceptible to imipenem and meropenem [10]. This result is similar to that of previous reports that some NDM-1 producers among *Enterobacteriaceae* and *Acinetobacter* showed lower MIC values to imipenem and meropenem [15, 16]. Therefore, ertapenem rather than other carbapenems has been proposed for the detection of NDM producers [17].

Consequently, we analyzed the genetic environment of bla_{NDM-1} in the two *A. pittii* isolates and confirmed an approximately 3,500-bp nucleotide segment containing aminoglycoside resistance gene *aphA6* and transposase IS*Aba125* upstream and a truncated bleomycin resistance gene and *trpF* genes downstream of bla_{NDM-1}. The segment detected in our study was identical to that reported in the previous study of *Acinetobacter* species [10], which reported that IS*Aba125* upstream of bla_{NDM-1} may be the main explanation for bla_{NDM-1} gene dissemination.

In this study, we performed MLST for epidemiological typing of ertapenem-resistant *A. pittii* isolates. The two bacterial isolates containing bla_{NDM-1} belonged to ST70 and ST207, which were different from the STs of the isolates harboring bla_{IMP-1} (ST63, ST119, ST396, and a novel ST). In previous studies, bla_{NDM-1} was identified in *A. pittii* isolates of ST63 in China [18], and NDM-1-producing strains isolated in Paraguay displayed ST320 and ST321 [19]. MLST profiles for four isolates containing bla_{NDM-1} identified in China differed from those described previously [10]. In addition, *A. pittii* isolates with ST63 and ST119 in Japan harbored bla_{IMP-11} and bla_{IMP-19}, respectively [20]. Although the databases of MLST profiles for *A. pittii* are poor, our results suggest that ST was not directly related to MBL production in *A. pittii* isolates. Furthermore, the two strains harboring bla_{NDM-1} were all isolated in 2013, whereas the other strains were isolated before 2012. Our results suggest that *A. pittii* isolates harboring bla_{NDM-1} emerged only recently in Korea.

The emergence and dissemination of carbapenemase genes, including the NDM gene, in *Acinetobacter* species has become a major concern in the treatment of bacterial infections with antimicrobial agents. Early recognition and detection of carbapenemases are critical in controlling infection by clinical *Acinetobacter* isolates showing high resistance to carbapenems. On the basis of the findings that some carbapenemase producers can show susceptible phenotypes and that the NDM gene has a high propensity to disseminate, we suggest that the detection of carbapenem-producing isolates should extend to clinical isolates of carbapenem-susceptible *Acinetobacter* species in addition to carbapenem-resistant ones. In particular, additional tests are needed to detect carbapenemase producers with carbapenem susceptibility.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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