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Short Communication

Detection of *Acinetobacter pittii* ST220 co-producing NDM-1 and OXA-820 carbapenemases from a hospital sink in a non-endemic country of NDM

Masaki Iimura[a,1], Wataru Hayashi[b,1], Eriko Arai[c], Tatsuya Natori[c], Kazuki Horiuchi[c], Go Matsumoto[c], Hayato Tanaka[a], Eiji Soga[a], Yukiko Nagano[d], Noriyuki Nagano[a,b,*]

[a] Department of Health and Medical Sciences, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[b] Department of Medical Sciences, Shinshu University Graduate School of Medicine, Science and Technology, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[c] Department of Laboratory Medicine, Shinshu University Hospital, 3-1-1 Asahi, Matsumoto, Nagano 390-8621, Japan
[d] Department of Bacteriology, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya, Aichi 466-8550, Japan

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ABSTRACT

Objectives: NDM-1 is by far one of the most commonly prevalent carbapenemases in Enterobacteriaceae and *Acinetobacter baumannii*. This study presented an *Acinetobacter pittii* (*A. pittii*) isolate co-harboring bla_{NDM-1} and $bla_{OXA-820}$ from a university hospital sink, where New Delhi metallo-β-lactamase (NDM) producers have not been found in either patients or their environments.
Methods: Whole-genome sequencing was performed on the HiSeq 4000 platform, and the reads were de novo assembled using the A5-miSeq Assembly pipeline. Annotation of the resulting scaffolds were performed by using the DDBJ Fast Annotation and Submission Tool (DFAST). The bla_{NDM-1}-carrying plasmid was determined.
Results: The *A. pittii* ST220 strain SU1805 detected from a sink strainer in the treatment room was resistant to imipenem and meropenem. Antimicrobial resistance genes bla_{NDM-1}, $bla_{OXA-820}$, bla_{ADC-43}, and *aphA6* were found in this strain. The bla_{NDM-1} was found to be located downstream of an IS*Aba125* element on a plasmid pSU1805NDM with a size of 41,022 bp, and GC content of 38.3% harbouring 48 protein-coding genes. The *aphA6* gene was also located upstream of the IS*Aba125* on the same plasmid. The *A. pittii* intrinsic $bla_{OXA-213}$-like gene $bla_{OXA-820}$ was located between *fxsA* and *yncA* genes in the chromosome. The strain also harboured biofilm-associated genes such as *ompA*, the *csu* operon and their regulating genes *bfmRS*.
Conclusion: This study described the first isolation of NDM-1-producing *A. pittii* in Japan, and highlighted the importance of proper implementation of measures against AMR for sink drainage systems, since NDM producers may have already been hidden in such environments in a non-endemic country of NDM.

1. Introduction

The global dissemination of carbapenemase-producing organisms is of serious health concern, particularly in clinical settings due to limited therapeutic options. The predominant types of carbapenemases vary by regions and countries: New Delhi metallo-β-lactamase (NDM) in the Indian subcontinent; *Klebsiella pneumoniae* carbapenemase (KPC) in the United States, Italy and Greece; OXA-48-like in Turkey, Indian subcontinent and North African countries; and IMP in the Asia-Pacific region [1].

NDM-1 is by far one of the most commonly prevalent carbapenemases in Enterobacteriaceae and *Acinetobacter baumannii*. It has recently been recognised that NDM-positive *Acinetobacter pittii* (*A. pittii*) was associated with sporadic human infection, intestinal carriage, and hospital transmission in various countries, serving as a potential reservoir of the bla_{NDM-1}-carrying plasmid [2–4]. In Japan, the IMP type is the most common carbapenemase enzyme, whereas NDM-positive organisms have rarely been reported. Moreover, NDM-producing *A. pittii* has so far

* Corresponding author at: Department of Health and Medical Sciences, Shinshu University Graduate School of Medicine, 3-1-1 Asahi, Matsumoto, Nagano 390-8621 Japan.
E-mail address: naganon@shinshu-u.ac.jp (N. Nagano).
[1] These authors contributed equally to this work.

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not been identified. This study report the isolation of NDM-1-producing *A. pittii* from a sink for washing medical instruments in the Shinshu University Hospital, where NDM producers have not been found among patients.

2. Materials and methods

2.1. Identification and characterisation of A. pittii isolate

A carbapenem-resistant *A. pittii* isolate was recovered from a sink strainer in the treatment room of the gastrointestinal surgical ward during surveillance of patient rectal cultures and environmental cultures following the detection of OXA-48-type carbapenemase-producing *Klebsiella pneumoniae* from an inpatient in the same ward. The organism was subjected to matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics Japan, Yokohama, Japan) analysis for bacterial identification. *rpoB* gene-based and 16S rRNA gene-based molecular identification was also performed [5,6]. Antimicrobial susceptibility testing was performed by the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) using Dry plate DP31 (Eiken Chemical Co., Tokyo, Japan) and the results were interpreted according to CLSI M100-28th ed. Guidelines [7]. MIC of colistin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was determined by an in-house prepared panel according to the CLSI broth microdilution method. MIC determination of tigecycline was performed using the Etest (bioMérieux, Marcy l'Étoile, France). Identification of metallo-β-lactamase genes, including IMP genes, NDM genes, and VIM genes, was performed by PCR and sequence analysis [8].

2.2. Transformation experiments

Transformation of *Escherichia coli* (*E. coli*) DH10B with plasmid DNA extracted from *A. pittii* was carried out by electroporation. Transformants were selected on LB agar plates supplemented with 50 mg/L ampicillin, and the presence of the carbapenemase gene was confirmed by PCR and sequencing. The antimicrobial susceptibility of the transformants was determined by the broth microdilution method as previously described.

2.3. Whole-genome sequencing analysis

Whole genome sequencing (WGS) was performed on the HiSeq 4000 platform (Illumina, San Diego, USA), and raw reads were de novo assembled using the A5-miSeq Assembly pipeline into scaffolds [9]. Those assembled contigs were queried with ResFinder 3.2 and PlasmidFinder 2.0 available from the Center for Genomic Epidemiology (http://www.genomicepidemiology.org). Annotation of the resulting scaffolds was performed by using the DDBJ Fast Annotation and Submission Tool (DFAST) (https://dfast.nig.ac.jp/). In-depth exploration of biofilm-associated genes and type IV pilus genes were manually performed on WGS sequence data using the BLAST tool.

3. Results

Acinetobacter pittii SU1805 was identified by MALDI-TOF MS with a 2.036 score, and confirmed the identity by exhibiting 100% nucleotide identity of the *rpoB* (4089 bp) and 16S rRNA (1541 bp) gene sequences to the reference sequence of *A. pittii* strain ST220 (GenBank accession number CP029610, [10]), which was later confirmed by WGS. The *A. pittii* SU1805 was resistant to imipenem (MIC > 8 mg/L), meropenem (MIC > 8 mg/L), ceftazidime (MIC > 16 mg/L), cefepime (MIC > 16 mg/L), and cefotaxime (MIC > 32 mg/L) (Table 1). It remained susceptible to aminoglycosides, levofloxacin, minocycline, and trimethoprim-sulfamethoxazole. The strain had low MICs for colistin (0.5 mg/L) and tigecycline (0.19 mg/L). PCR and sequencing analysis identified the presence of a $bla_{\text{NDM-1}}$ gene.

The *E. coli* DH10B transformants acquiring $bla_{\text{NDM-1}}$-carrying plasmid pSU1805NDM exhibited lower MICs of imipenem and meropenem (MIC 1 mg/L) compared with the donor strain (Table 1). The reduction of aztreonam MIC against transformants (MIC ≤ 0.5 mg/L) relative to the donor strain (MIC 16 mg/L) was also noted.

The genome sequences of *A. pittii* SU1805 were assembled into 515 contigs with a total length of 4,327,310 bp and an L50 contig number of 9. Genome annotation identified 3876 protein-coding genes, including antimicrobial resistance genes $bla_{\text{NDM-1}}$, $bla_{\text{OXA-820}}$, $bla_{\text{ADC-43}}$, and *aphA6* encoding a 3'-aminoglycoside phosphotransferase type VI [11]. No plasmids were detected by PlasmidFinder 2.0, which is intended for the identification of plasmids originated from Enterobacteriaceae species [12]. Multilocus sequence typing by the Pasteur scheme identified the *A. pittii* SU1805 as ST220 (http://pubmlst.org/abaumannii). The $bla_{\text{NDM-1}}$ was found to be located downstream of an IS*Aba125* element on a plasmid pSU1805NDM (GenBank accession number LC483156) with the size of 41,022 bp, GC content of 38.3% harbouring 48 protein-coding genes (Fig. 1). The *aphA6* gene was also located upstream of the IS*Aba125* on the same plasmid. The *virB* gene cluster for a type IV secretion system exhibiting 100% sequence identity among bla_{NDM}-carrying plasmids harboured by *Acinetobacter* spp. was identified. The plasmid pSU1805NDM had high sequence similarity with a previously reported plasmid (GenBank accession number CP027532) harboured by *A. baumannii* strain AR_0088 in the USA (99.96% identity), and pNDM-JN02 (GenBank accession number KM210088) harboured by *Acinetobacter lwoffii* JN247 from a human stool sample in China (99.95% identity). *A. pittii* intrinsic $bla_{\text{OXA-213}}$-like gene, $bla_{\text{OXA-820}}$ (only nucleotide sequence data is available in the GenBank database under the accession number NG_064778) was located between *fxsA* and *yncA* genes in the chromosome. Intrinsic cephalosporinase gene $bla_{\text{ADC-43}}$ was also identified.

In-depth exploration of WGS sequence data allowed identification of virulence factors associated with biofilm formation, including outer membrane protein OmpA gene, *pga* operon (*pgaABCD*) encoding a poly-β-1,6-N-acetyl-D-glucosamine (PGA) that is important for biofilm development, *csu* operon (*csuA/BABCDE*) encoding Csu pili, and the two-component regulatory system BfmRS genes involved in Csu expression. Type IV pili genes encoding pilus adhesion protein, pillins, basal apparatus and motor proteins, signal transduction proteins, and DNA transport proteins were also detected [13].

4. Discussion

NDM-1-producing *A. pittii* isolates have mostly been reported in China and other several countries in Asia, Europe and South America since its first description in 2012 [4]. Most of those cases have been associated with human sporadic infections due to diverse sequence types of *A. pittii* such as STs 70, 119, 207, 220, 320, 321, 457, and 838. Dissemination of NDM-1-producing *A. pittii* ST63 in both patients and the environment (hospital surfaces, water taps and air) in an ICU has also been documented in China [4]. In Japan, NDM producers remain very rare, where most of those have been found among Enterobacteriaceae isolates recovered from patients who had some history of receiving medical treatment while abroad, or had visited Japan for medical purposes. Those NDM producers have not been associated with nosocomial infections. Among *Acinetobacter* spp., NDM has only been reported in *A. baumannii* isolated from a patient transferred from India [14]. To date, there are no reports of NDM in *A. pittii* isolates of humans,

Table 1
MICs of antimicrobials for New Delhi metallo-β-lactamase (NDM)-1-producing *Acinetobacter pittii* SU1805 and *Escherichia coli* DH10B transformants.

Antimicrobials	MICs (mg/L)		
	Acinetobacter pittii SU1805	*Escherichia coli* DH10B (pSU1805NDM)	*Escherichia coli* DH10B
Piperacillin	>64	64	≤0.5
Ampicillin/sulbactam	>16/8	>16/8	≤4/2
Cefazolin	>16	>16	1
Cefotiam	>16	>16	≤0.5
Cefotaxime	>32	>32	≤0.5
Ceftazidime	>16	>16	≤0.5
Cefpodoxime	>4	>4	≤0.5
Cefepime	>16	>16	≤0.5
Flomoxef	>16	>16	≤0.5
Aztreonam	16	≤0.5	≤0.5
Imipenem	>8	1	≤0.5
Meropenem	>8	1	≤0.5
Gentamicin	≤0.25	0.5	0.5
Amikacin	2	≤1	≤1
Minocycline	≤0.25	0.5	0.5
Levofloxacin	≤0.25	≤0.25	≤0.25
Fosfomycin	≤32	≤32	≤32
Trimethoprim-sulfamethoxazole	≤9.5/0.5	≤9.5/0.5	≤9.5/0.5
Colistin	0.5	≤0.25	≤0.25
Tigecycline[a]	0.19	0.094	0.094

[a] determined by Etest.

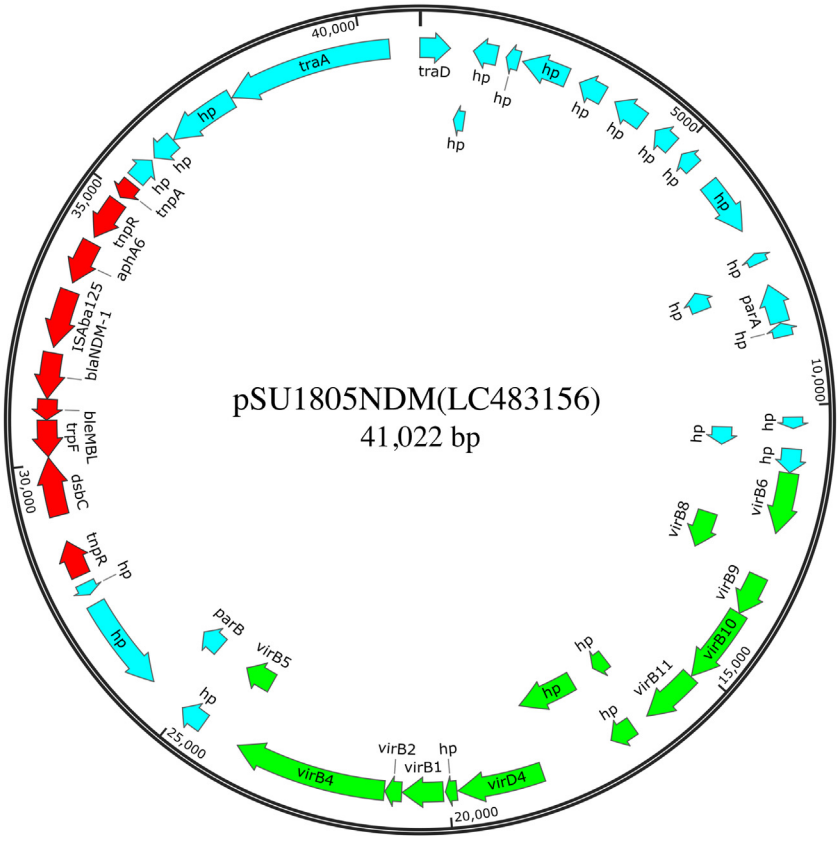

Fig. 1. Circular genetic map of *Acinetobacter pittii* SU1805 plasmid pSU1805NDM.
Arrows show the direction of transcription of open reading frames. The plasmid shows a backbone structure composed of *virB* gene cluster encoding type IV secretion system (green) and putative transfer and replication region (blue) with accessory module region including IS*Aba125-bla*$_{NDM-1}$ (red) integrated into the backbone.

companion animals, food animals, foods, and environmental sources. In the Shinshu University Hospital, the isolation rate of *A. calcoaceticus-baumannii* complex (85.2%) was higher than that of non-*baumannii Acinetobacter* species (14.8%) during the last year (2018), all of which were susceptible to carbapenems (meropenem and doripenem). Moreover, hospital patients and staff were found to have no recognised epidemiological links to China, where NDM-1-producing *A. pittii* belonging to ST220 has been previously reported [10].

Hospital sinks have been reported to be potential environmental reservoirs for the transmission/outbreak of carbapenemase-producing Enterobacteriaceae [15]. An experimental study in the

USA found that the dispersal of *E. coli* originated from the sink strainer and/or the bowl after biofilm growth upward from the microbial reservoir of the P-trap [16]. NDM-1-producing *A. pittii* SU1805 ST220 in this study was characterised to harbour repertoires of biofilm-associated genes. Those genes have been well recognised in *A. baumannii*, enabling survival and persistence of the organism on biotic and abiotic surfaces through biofilm formation, leading to their dissemination in hospital environments [17]. Although there have so far been no studies reporting the biofilm-associated genes in *A. pittii*, a sequence similarity search with the Basic Local Alignment Search Tool revealed that those biofilm-associated genes of NDM-1-producing *A. pittii* SU1805 ST220 shared a high level of sequence similarities (>97.8%) with the corresponding genes in *A. pittii* strains only deposited in the GenBank database. *A. pittii* isolates have been reported to retained, or increased their biofilm-forming ability by feeding with nutrient media after long-term desiccation [18]. A recent study of invasive *Acinetobacter* isolates from paediatric patients has shown that *A. pittii* isolates had significantly higher biofilm production compared with *A. baumannii* isolates by crystal violet retention assay [19]. Although *Acinetobacter* spp. are nonmotile due to the lack of flagella, some strains display twitching motility and surface-associated motility. Those motilities have more frequently been associated with *A. pittii* compared with *A. baumannii*, and the current *A. pittii* strain was found to possess type IV pili genes that are suggested to be necessary for both surface-associated motility and twitching motility. Thus, NDM-1-producing *A. pittii* SU1805 ST220 harboured both biofilm-associated genes and type IV pili genes, which may allow successful colonisation and spread of this strain in hospital environments [19]. Moreover, the plasmid pSU1805NDM harboured by NDM-1-producing *A. pittii* SU1805 ST220 is considered *Acinetobacter* specific; hence, highlighting the pivotal role of clinically important *Acinetobacter* including *A. baumannii* and *A. pittii* in its diffusion to other species. Also, *Acinetobacter* infection due to this strain may pose a threat to patients because invasive *Acinetobacter* isolates have more frequently been associated with *A. pittii* compared with *A. baumannii* [19].

In conclusion, this study identified *A. pittii* co-producing NDM-1 and OXA-820 from a strainer of a hospital waste sink. In this hospital, NDM producers have not been isolated so far. Moreover, IMP-1-producing Enterobacteriaceae isolates were observed during surveillance of hospital environments, while they were not detected from patients. Therefore, it is more likely that the NDM-1- and OXA-820-producing *A. pittii* is directly sourced from the hospital sink, but not from patients. Thus, the study highlights the importance of proper implementation of AMR measures for sink drainage systems since NDM producers may have already hidden in such an environment in Japan, which is a non-endemic country of NDM.

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Competing interests

None declared.

Ethical approval

Not required.

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