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Occurrence of $bla_{\text{NDM-1}}$ in a Clinical Isolate of *Acinetobacter lwoffii* in Japan: Comparison of $bla_{\text{NDM-1}}$-Harboring Plasmids between *A. lwoffii* and *A. pittii* Originated from a Hospital Sink

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Acinetobacter spp. are recognized to be significant nosocomial pathogens because of their capability for developing resistance to a wide range of antimicrobials by acquiring resistance mechanisms very quickly (1,2). Thus, they serve as potential hospital reservoirs for resistance genes/elements, which are subsequently spread among other clinically relevant *Acinetobacter* species, such as *A. baumannii*, *A. nosocomialis*, and *A. pittii*. The acquisition of carbapenem resistance by *Acinetobacter* spp. is most commonly due to their production of carbapenemases (e.g., OXA-type carbapenemase) and, less frequently, metallo-β-lactamases. The New Delhi metallo-β-lactamases (NDM), the rapid dissemination of which is causing worldwide concern, are the most prevalent of the metallo-β-lactamases in *Acinetobacter* spp., occurring predominantly in *A. baumannii* (3,4). Herein, we report the discovery of a clinical isolate of *A. lwoffii* in Japan that harbored both the $bla_{\text{NDM-1}}$ gene and a novel $bla_{\text{OXA-915}}$ carbapenemase gene. We also demonstrate that the NDM-1-encoding plasmid harbored by this *A. lwoffii* isolate obtained from an inpatient analyzed in this study exhibited significant sequence identity with that harbored by a previously described *A. pittii* strain that had been isolated one year earlier from a sink in the same hospital ward (5).

The carbapenem-resistant *A. lwoffii* strain was isolated from a bile sample of a male patient who had undergone gastrointestinal surgery in 2019. For bacterial identification, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics Japan, Yokohama, Japan) analysis and *rpoB* and 16S rRNA gene-based molecular identification were performed (6,7). Determination of the minimum inhibitory concentrations (MICs) was carried out with the broth microdilution method using DP31 dry plates (Eiken Chemical Co., Tokyo, Japan), and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100 (28th ed.) guidelines (8). The MIC of colistin (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) was determined using an in-house prepared panel according to the CLSI broth microdilution method. The presence of the bla_{NDM} gene was identified through PCR and sequencing analyses (5). Whole-genome sequencing (WGS) analysis was performed using the Illumina NovaSeq 6000 system, and de novo assembly of the sequence data was executed with the A5-miseq pipeline. The assembled genome was annotated using DFAST and analyzed using ResFinder 4.1, an online tool available from the Center for Genomic Epidemiology for the identification of antimicrobial resistance genes. The average nucleotide identity based on the MUMmer calculation (ANIm) of paired genomes was calculated using JSpeciesWS. An in-depth exploration of virulence-associated genes and heavy metal resistance genes was conducted manually on the WGS data.

A. lwoffii SU1904 was identified by MALDI-TOF MS with a score of 2.139. The WGS-based analysis revealed that its *rpoB* (4,089 bp) and 16S rRNA (1,590 bp) gene sequences had 98.09% and 98.55% nucleotide identity, respectively, to the reference sequence of *A. lwoffii* strain FDAARGOS_552 (GenBank No. CP046296). *A. lwoffii* SU1904 had ANIm values of more than 96.17% with *A. lwoffii* strains ATCC 9957 (GenBank No. GCA_000369125), NIPH 478 (GenBank No. GCA_000369145), and SH145 (GenBank No.

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Table 1. MICs of antimicrobials for NDM-1-producing *Acinetobacter lwoffii* SU1904

Antimicrobials	MICs (mg/L)
Ampicillin	>16
Piperacillin	≤16
Ampicillin/sulbactam	≤4
Cefazolin	>16
Cefotiam	>16
Cefotaxime	>2
Ceftazidime	>16
Cefepime	16
Cefozopran	16
Aztreonam	≤4
Imipenem	>8
Meropenem	8
Gentamicin	≤2
Amikacin	8
Tobramycin	≤4
Minocycline	≤2
Ciprofloxacin	≤0.25
Levofloxacin	≤0.5
Fosfomycin	≤4
Trimethoprim-sulfametoxazole	≤38/2
Colistin	0.5

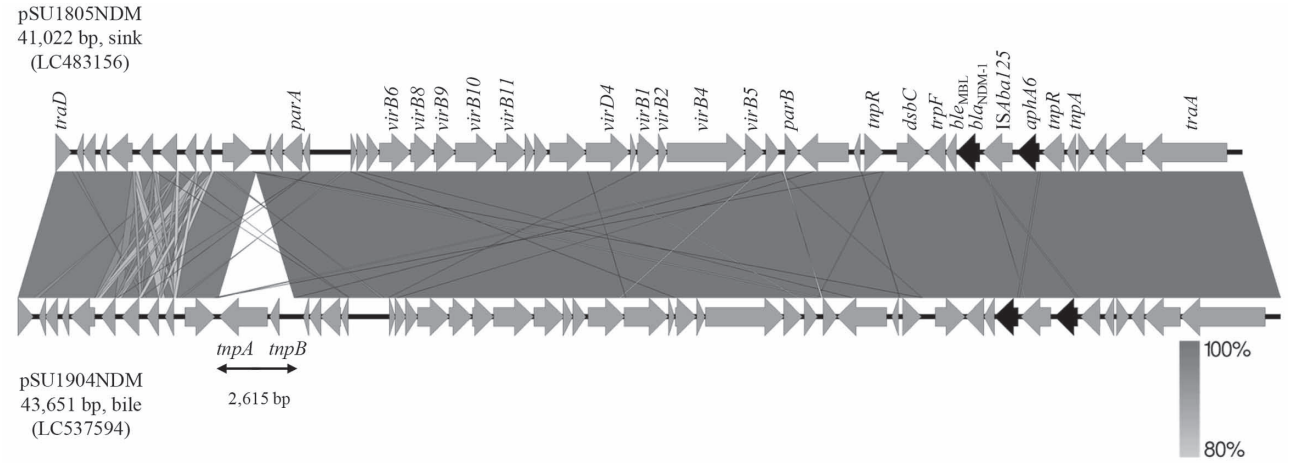

Fig. 1. Linear comparison of the complete sequences of the plasmids pSU1904NDM from *Acinetobacter lwoffii* (this study, LC537594) and pSU1805NDM from *A. pittii* (LC483156). The arrows show the translation orientation of the coding genes. The bla_{NDM-1} and *aphA6* genes are indicated by black arrows. The 2,615-bp region carrying *tnpA* (IS*66* family transposase) and *tnpB* (IS*66* family insertion sequence element accessory protein) is indicated.

GCA_000162095), confirming its designation as *A. lwoffii*. As shown in Table 1, *A. lwoffii* SU1904 was resistant to imipenem, meropenem, and ceftazidime, with MICs of >8, 8, and >16 mg/L, respectively, but was susceptible to piperacillin, gentamicin, amikacin, levofloxacin, ciprofloxacin, and minocycline (8). The de novo assembly of the genome yielded 209 contig sequences with a total length of 3,474,038 bp and a G+C content of 42.7%. Genome annotation identified 3,287 protein-coding genes, including antimicrobial resistance genes (bla_{NDM-1}, $bla_{OXA-134-like}$, and *aphA6*), biofilm-associated genes (*bfmRS* and *ompA*), genes involved in the type II (*gspN*, *gspD*, *gspC*, *gspE*, and *lipA*) and type VI (*vgrG*) secretion systems and type IV pilus system (*comE* and *comF*), genes encoding global regulators of virulence, including pili synthesis, motility, biofilms, and resistance to human serum (*gacSA*), copper resistance genes (*copRS*, *copABCD*, and *actP*), and cobalt-zinc-cadmium resistance genes (*czcCBAD*).

The $bla_{OXA-134-like}$ gene (GenBank No. LC537318), a bla_{OXA} gene intrinsic to *A. lwoffii*, exhibited the highest nucleotide identity (96.17%) to $bla_{OXA-282}$ in *A. lwoffii* NIPH 715 (GenBank No. NG_049580). This $bla_{OXA-134-like}$ gene encoded a novel OXA-134-like variant possessing nine amino acid substitutions and a one-amino-acid stretch from OXA-282, which was

assigned as OXA-915. The bla_{NDM-1} gene, preceded by IS*Aba125* and *aphA6*, was found to be located on a 43,651-bp circular plasmid, pSU1904NDM (GenBank No. LC537594), which had a G+C content of 38.8% and harbored 50 protein-coding genes (Fig. 1). It should be noted that the complete sequence of pSU1904NDM showed a high degree of nucleotide identity (99.8%) with our previously reported 41,022 bp plasmid pSU1805NDM (GenBank No. LC483156), except for a 2,615 bp region harbored by pSU1904NDM. The 2,615 bp region, which carried elements of the IS*66* family (*tnpA* and *tnpB*) and was flanked by 8 bp direct repeats (GTAAGAGT), was located at positions 6936 to 9550 of pSU1904NDM. The pSU1805NDM-harboring *A. pittii* strain had been isolated one year earlier from an instrument washing sink located in the treatment room of the same ward in which the patient of this present study was admitted, and NDM-1-encoding plasmids have so far not been identified from other inpatients of the hospital (5). Plasmids pSU1805NDM and pSU1904NDM are considered to be *Acinetobacter* specific because their complete sequences were highly homologous to plasmids that have only been identified in *Acinetobacter* species listed on the NCBI databases. The replicon types of bla_{NDM-}carrying plasmids in *Acinetobacter* remain largely unknown (9), and these two plasmids were also found to be nontypeable. Conjugal transfer of the NDM-1 plasmid from *A. pittii* to several *Acinetobacter* spp., including *A. lwoffii* and *A. baumannii*, has been reported (10). Thus, the overall similarity between pSU1805NDM and pSU1904NDM suggests that the NDM-1-encoding plasmid may be transferred horizontally from *A. pittii* to *A. lwoffii* within hospital environments, such as sink drains, highlighting the importance of such environments as potential reservoirs of NDM-1-encoding plasmids and NDM-1-producing *Acinetobacter* spp. Biofilm formation, type IV pilus-associated twitching motility, and heavy metal resistance all favor the survival and spread of these *Acinetobacter* spp. in the hospital sink drainage systems, where the horizontal transfer of resistance genes via plasmids can then occur between species in such sink environments (11). Although *A. baumannii*, which has more human virulence potential, is frequently responsible for outbreaks of infections, *A. lwoffii* has also been associated with nosocomial infections in humans (12). There are only a few studies reporting virulence factor genes of *A. lwoffii*. Notably, the *A. lwoffii* SU1904 strain in this study was found to carry many virulence genes that are also carried by *A. baumannii*. Because NDM producers remain very rare among human clinical isolates in Japan, the implementation of an effective combination of interventions for breaking up the transmission linkage of as-yet unrecognized multidrug-resistant *Acinetobacter* spp. is imperative, especially of those with pathogenic potential to patients via contact with contaminated hospital surfaces (13).

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Conflict of interest None to declare.

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