



First Identification of IMP-1 Metallo- β -Lactamase in *Delftia tsuruhatensis* Strain CRS1243 Isolated From a Clinical Specimen

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Dear Editor,

Delftia tsuruhatensis is a gram-negative bacillus that was first isolated from activated sludge collected from a domestic wastewater treatment plant in Tsuruhata, Japan [1]. The bacteria are motile, slightly curved, and short rods. The species is closely related to and often misidentified as *Delftia acidovorans* (formerly *Comamonas acidovorans*) in biochemical tests because of their shared characteristics [1, 2]. However, the original *D. tsuruhatensis* isolate cannot utilize D-mannitol, whereas most *D. acidovorans* strains can [3]. We present the first report of an IMP-1 metallo- β -lactamase (MBL)-producing *D. tsuruhatensis* strain that was isolated from a clinical specimen. The Institutional Review Board of the CHA Bundang Medical Center, Seongnam, Korea, approved this study (approval number: 2020-04-011-001) and waived the need for informed consent.

D. tsuruhatensis is typically susceptible to carbapenem [2, 4]; however, we isolated a carbapenem-resistant strain from a 65-yr-old man diagnosed as having stomach cancer who underwent total gastrectomy. On post-operative day 16, bacterial culture was performed because of onset of fever. A *Delftia* species (strain CRS1243) was isolated from the surgical drainage fluid.

The isolate was identified as *D. acidovorans* with 99% probability using Vitek 2 GN cards (bioMérieux, Marcy-l'Étoile, France) and with a score of 2.40 using the MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA). However, as the isolated bacteria were unable to metabolize D-mannitol, we performed 16S rRNA gene sequencing and identified the strain as *D. tsuruhatensis* (100% identity with GenBank accession number HQ731453.1).

The isolate was suspected to produce carbapenemases based on routine antimicrobial susceptibility testing using Vitek AST N212 cards (bioMérieux). Antimicrobial susceptibility was determined according to the CLSI Minimum Inhibitory Concentration (MIC) Interpretive Standards for other non-*Enterobacteriaceae* using the broth microdilution method [5, 6]. The isolate was not susceptible to amikacin (MIC > 64 μ g/mL), levofloxacin (8 μ g/mL), ceftazidime (> 16 μ g/mL), cefepime (> 32 μ g/mL), ceftriaxone (> 32 μ g/mL), and meropenem (16 μ g/mL), but it was susceptible to piperacillin-tazobactam (16 μ g/mL) and minocycline (\leq 0.5 μ g/mL). The modified Hodge test result was positive, and in carbapenemase inhibition testing (Rosco Diagnostica A/S, Taastrup, Denmark), we observed enlarged inhibition zones when using disks containing meropenem supple-

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mented with dipicolinic acid, indicating production of MBL. An expanded inhibition zone was also detected with disks containing meropenem supplemented with cloxacillin, but not with those containing boronic acid. Moreover, the cefoxitin-Hodge test result was negative. Based on these results, we concluded that this isolate was negative for AmpC β-lactamase activity.

Multiplex PCR was performed to detect the specific MBL. The specific primer pairs for the detection of the New Delhi MBL and São Paulo MBL were designed in this study and those for the detection of Verona integron-encoded MBL, imipenemase (IMP), Seoul IMP, and German IMP were selected from a previous report (Table 1). The sequence of the bacterial integron carrying the MBL-encoding gene was identified using primer walking (Table 1). We found that *D. tsuruhatensis* CRS1243 harbored a gene encoding *bla_{IMP-1}* within a class 1 integron located on a Tn402-like transposon. Between the 5'-conserved segment and the *t_{ni}* module, the gene cassettes included *orfE*, *aac(6')-31-like*, *orfE*, *orfE*, *aac(6')-II*, *aacA7*, *bla_{IMP-1}*, *aacA7*, *aac(6')-II*, and *qacE2* (Fig. 1). The nucleotide sequence of the class 1 integron has been deposited in GenBank under accession number KC170993. While a class 3 integron has been previously identified in a *D. tsuruhatensis* strain, the gene cassettes were not evaluated functionally [12]. To determine the location of the class 1 integron, we performed plasmid extraction and conjugation [13]. However, we could not identify the band corresponding to the plasmid DNA, and the carbapenem resistance was not transferred to sodium azide-resistant *Escherichia coli* J53.

Cases of human infection with *D. tsuruhatensis* are rare [2]. In 2011, *D. tsuruhatensis* was identified as the etiologic agent of a human catheter-related infection; since then, it has also been associated with other human infections [2]. Recently, Fenollar, *et al.* [4] identified *D. tsuruhatensis* as an emerging opportunistic pathogen that should be considered as a cause of infection in patients with underlying disease and those using intravascular devices. The *D. tsuruhatensis* strain identified in this study was isolated twice in five days from pure cultures from surgical drainage fluid. Treatment with ciprofloxacin, an empirical antibi-

Table 1. Primers used for the PCR analysis of metallo-β-lactamase genes and primer walking

Primer	Sequence (5'→3')	References
NDM-F1	GCC CAA TAT TAT GCA CCC GG	This study
NDM-R	CGG AAT GGC TCA TCA CGA TC	This study
SPM-NF	TGC GGG AGC GCC ATT GTC TG	This study
SPM-NR	TTC CAC CCG TGC CGT CCA AA	This study
VIM-F	GAT GGT GTT TGG TCG CAT A	7
VIM-R	CGA ATG CGC AGC ACC AG	7
IMP-F	GGA ATA GAG TGG CTT AAY TCT C	7
IMP-R	CCA AAC YAC TAS GTT ATC T	7
SIM-F	TAC AAG GGA TTC GGC ATC G	7
SIM-R	TAA TGG CCT GTT CCC ATG TG	7
GIM-F	TCG ACA CAC CTT GGT CTG AA	7
GIM-R	AAC TTC CAA CTT TGC CAT GC	7
INT1-5CS	GGC ATC CAA GCA GCA AGC	8
int1-1F	ACA TGC GTG TAA ATC ATC GTC G	9
attI-R	CIT TGT TTT AGG GCG ACT GC	This study
aac6-F1	GCT CGT TGA GAT GCT GTT CA	This study
aacA7-R	GAA GCA GCG TAC TTG AGC AA	This study
IMP-1R	CCT TTA ACC GCC TGC TCT AAT G	10
IMP14	AGG CGT GCT GCT GCA ACG ACT TGT	11
qacEd1-R	TGA GCC CCA TAC CTA CAA AGC	9
qacE2-R	ATT TGA GTG TCA GCG ACA GG	This study
TniR-1	GTG TTC GGT ATT TTT GCC GC	This study
TniR-2	GTA ATC CCG AGT TCT TCG CA	This study
TniQ-1	TGT GGT TTC GAC TTC TTC GC	This study
TniQ-2	GAC CAG AAT AGC TTT GCC TG	This study
TniQ-1M	TGT GGT TTC GAC TGC TAC GC	This study
TniB-1	GGA AAT GGA GCA ACT GGC T	This study
TniB-2	TTT CCA ACT GGT CAT CGG AG	This study
TniB-1M	AGA AAT GGA ACA ACT GGC G	This study
TniA-1	TAG AGC GCT GGC TCA CAT T	This study
TniA-2	GGA TGT GGT CGA TGA CAA AG	This study

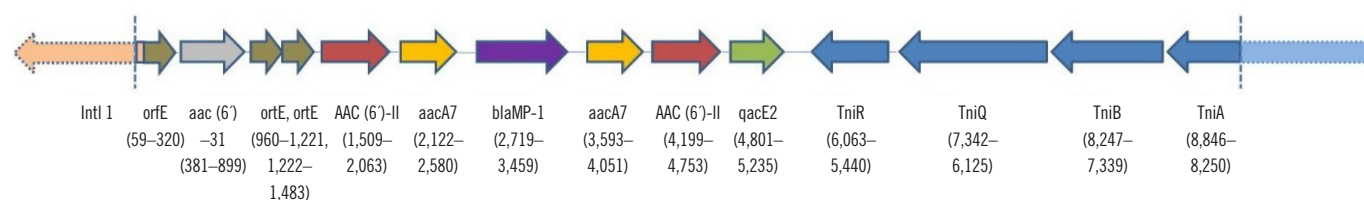


Fig. 1. Schematic representation of the 8,846-bp partial DNA sequence of the class 1 integron containing *bla_{IMP-1}* within a Tn402-like module in *Delftia tsuruhatensis* CRS1243.

otic was started; however, piperacillin-tazobactam was administered after confirmation of the antibiotic susceptibility results. The patient's fever subsided one day after initiation of the treatment.

To the best of our knowledge, this is the first report of IMP-1 MBL production from a *D. tsuruhatensis* strain. Our findings suggest that clinical microbiologists need to be aware of *D. tsuruhatensis* as a potential cause of opportunistic infections. We note that the *bla*_{IMP-1} gene linked to a mobile element might spread among *Delftia* or other bacterial species.

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AUTHOR CONTRIBUTIONS

Hong SG conceptualized and designed the study and coordinated the drafting of the manuscript. Hong SG performed the microbiologic test and PCR work. Hong SG, Cho SM, and Lee Y performed data analysis and wrote the manuscript. Song W, Yong D, Jeong SH, Lee K, and Chong Y supervised the study, reviewed and commented on the manuscript, and approved the final draft. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

1. Shigematsu T, Yumihara K, Ueda Y, Numaguchi M, Morimura S, Kida K. *Delftia tsuruhatensis* sp. nov., a terephthalate-assimilating bacterium isolated from activated sludge. *Int J Syst Evol Microbiol* 2003;53:1479-83.
2. Preiswerk B, Ullrich S, Speich R, Bloemberg GV, Hombach M. Case report human infection with *Delftia tsuruhatensis* isolated from a central venous catheter. *J Med Microbiol* 2011;60:246-8.
3. Jorgensen NO, Brandt KK, Nybroe O, Hansen M. *Delftia lacustris* sp. nov., a peptidoglycan-degrading bacterium from fresh water, and emended description of *Delftia tsuruhatensis* as a peptidoglycan-degrading bacterium. *Int J Syst Evol Microbiol* 2009;59:2195-9.
4. Ranc A, Dubourg G, Fournier PE, Raoult D, Fenollar F. *Delftia tsuruhatensis*, an emergent opportunistic healthcare-associated pathogen. *Emerg Infect Dis* 2018;24:594-6.
5. CLSI. Performance standards for antimicrobial susceptibility testing; approved standard. CLSI M100-S28. Wayne, PA: Clinical and Laboratory Standards Institute. 2018.
6. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. CLSI M7-A11. Wayne, PA: Clinical and Laboratory Standards Institute. 2018.
7. Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *J Antimicrob Chemother* 2007;59:321-2.
8. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant *Salmonella enterica* Typhimurium DT104. *FEMS Microbiol Lett* 1997;157:177-81.
9. Zhao WH, Hu ZQ. IMP-type metallo- β -lactamases in Gram-negative bacilli: distribution, phylogeny, and association with integrons. *Crit Rev Microbiol* 2011;37:214-26.
10. Jeong SH, Bae IK, Park KO, An YJ, Sohn SG, Jang SJ, Sung KH, Yang KS, Lee K, Young D, Lee SH. Outbreaks of imipenem-resistant *Acinetobacter baumannii* producing carbapenemases in Korea. *J Microbiol* 2006;44:423-31.
11. Chu YW, Afzal-Shah M, Houang ET, Palepou MI, Lyon DJ, Woodford N, Livermore DM. IMP-4, a novel metallo-beta-lactamase from nosocomial *Acinetobacter* spp. collected in Hong Kong between 1994 and 1998. *Antimicrob Agents Chemother* 2001;45:710-4.
12. Xu H, Davies J, Miao V. Molecular characterization of class 3 integrons from *Delftia* spp. *J Bacteriol* 2007;189:6276-83.
13. Walsh TR, Weeks J, Livermore DM, Toleman MA. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 2011;11:355-62.