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 Tsuruhat, 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 µg/mL), levofloxacin (8 µg/mL), ceftazidime (>16 µg/mL), cefepime (>32 µg/mL), ceftriaxone (>32 µg/mL), and meropenem (16 µg/mL), but it was susceptible to piperacillin-tazobactam (16 µg/mL) and minocycline (<0.5 µg/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-
mented with dipicolinic acid, indicating production of MBL. An expanded inhibition zone was also detected with disks containing meropenem supplemented with cefoxitin, 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 ß-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 \( \text{bla}_{\text{IMP}-1} \) within a class 1 integron located on a Tn402-like transposon. Between the 5'-conserved segment and the tin module, the gene cassettes included \( \text{orfE}, \text{aac(6')}-\text{III}, \text{aacA7}, \text{bla}_{\text{IMP}-1}, \text{aacA7}, \text{aac(6')}-\text{II}, \) and \( \text{qacE}2 \) (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-ß-lactamase genes and primer walking

| Primer | Sequence (5' → 3') | References |
|--------|------------------|------------|
| NDM-F  | GCC CAA TAT TAT GCA CCC GG | This study |
| NDM-R  | CGG AAT GGC TCA CGA TC | This study |
| SPM-NF | TGC GGG AGC ATT GTC TG | This study |
| SPM-NR | TTC CAC CGG TGC CGT CAA 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 AAT 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 GTG CTG AA | 7 |
| GIM-R  | AAC TTC CAA CTT TGC CAT GC | 7 |
| INT1-5CS | GCC ATG CCA GCA GAC | 8 |
| intt1-1F | ACA TGG TGG TAG ATC ATC TGC G | 9 |
| attl-R  | CCT TGT TTT AGG GCG ACT GC | This study |
| aac6-F1 | GCT CCG TGA GAT GCT GTT CA | This study |
| aac7-R  | GAA GCA GCG TAC TGG ACA AA | This study |
| IMP-1R  | CCT TTA ACC GCC TGC TCT AAT G | 10 |
| IMP14   | AGG GTT 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 TTT GCT ATT TTT GCC CG | This study |
| TnIR-2  | GTT GCG ACG TAT TGC GCA | This study |
| TnIQ-1  | TGG GTT TTC GAC TTT TGC GC | This study |
| TnIQ-2  | GAC CAG AAT AGC TTT GCC TG | This study |
| TnIQ-1M | TGG GTT TTC GAC TGC TGC AG | This study |
| TnIB-1  | GGA GAA 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 T | 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 \( \text{bla}_{\text{IMP}-1} \), 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*<sub>IMP-1</sub> gene linked to a mobile element might spread among *Delftia* or other bacterial species.

ACKNOWLEDGEMENTS

None.

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.

RESEARCH FUNDING

None.

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