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Molecular Characterization of Imipenem-Resistant, Meropenem-Susceptible *Pseudomonas aeruginosa* with *bla*_{VIM-2} Phenotype: Potential for Dissemination

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Pseudomonas aeruginosa is a nosocomial pathogen that causes a wide range of infections, especially in patients admitted to intensive care units. It has the ability to develop resistance during therapy, which can greatly affect clinical outcome. Carbapenems are the active agents used for the treatment of multidrug-resistant *P. aeruginosa*. Due to the inappropriate use of carbapenems, carbapenem-resistant pathogens have emerged and have become a serious problem worldwide. Carbapenemase-producing bacteria have become a global problem due to the rapid dissemination and limited therapeutic options. *bla*_{NDM} carbapenemase was first identified in INDIA and found to spread rapidly (1). Other carbapenemases such as *bla*_{IMP}, *bla*_{VIM}, *bla*_{KPC} are reported less frequently than *bla*_{NDM}. *bla*_{VIM} carbapenemase was first identified in Italy (2), after which it rapidly spread to other countries.

This report highlights the isolation of *P. aeruginosa* (PA3841), a clinical isolate with the unusual carbapenem-susceptibility profile with an imipenem-resistant and meropenem-susceptible (IRMS) phenotype. This pathogen was isolated from a 61-year-old

woman who was admitted for the evaluation of angina pectoris.

The isolated *P. aeruginosa* was subjected to various phenotypic and molecular methods for the characterization of its resistance mechanisms. Minimum inhibitory concentrations (MICs) of imipenem, meropenem, and doripenem were determined by the Etest method and the results were interpreted according to CLSI guidelines (3). The isolate was subjected to Carba NP testing (4), a modified Hodge test (3), and examination of imipenem with EDTA and cloxacillin treatment for synergism (5). The isolate was then assessed for carbapenemase genes by multiplex PCR for the detection of *bla*_{IMP}- (6), *bla*_{VIM}- (7), *bla*_{NDM}- (8), *bla*_{KPC}- (9), and *bla*_{Oxa-48}-like genes; PCR for class I and class II integrons; Sanger sequencing of *oprD* (10); and relative quantification of *oprD* mRNA by real-time quantitative PCR (RT-qPCR) (11).

The antimicrobial susceptibility profile of PA3841 identified that the isolate was resistant to ceftazidime, levofloxacin, tobramycin, amikacin, cefoperazone/sulbactam, imipenem, cefepime, netilmicin, and aztreonam. PA3841 was moderately susceptible to piperacillin/tazobactam, and fully susceptible to meropenem and colistin. The MICs of imipenem, meropenem, and doripenem were 8 µg/ml, 2 µg/ml, and 1 µg/ml, respectively. Carba NP testing was positive, indicating that the isolate was a carbapenemase producer. While the modified Hodge test was negative, EDTA disk synergism with imipenem was positive, indicating that the isolate was a metallo-β-lactamase producer. Multiplex PCR for

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*bla*_{IMP}, *bla*_{VIM}, *bla*_{NDM}, *bla*_{KPC}, and *bla*_{Oxa-48} like genes indicated that PA3841 is positive for *bla*_{VIM}. Sequencing revealed it was the *bla*_{VIM-2} variant. Thus, the phenotypic results correlated with the molecular detection of *bla*_{VIM} by PCR. In addition, integron PCR for *bla*_{VIM} revealed that the gene was carried on a class I integron. *oprD* loss is recognized as the resistance mechanism responsible for the presentation of the IRMS phenotype. Relative levels of *oprD* transcripts in PA3841 determined by RT-qPCR were normalized to the expression in *P. aeruginosa* ATCC 27853, which was set to 1. *oprD* mRNA expression was found to be 0.27 compared to that in *P. aeruginosa* ATCC 27853, indicating that *oprD* expression was down-regulated in PA3841.

*bla*_{VIM} expression results in higher hydrolysis activity for imipenem than for meropenem. PA3841 exhibited both *oprD*-mediated resistance and *bla*_{VIM} expression. This genotype may have occurred due to the low level of *bla*_{VIM}, and/or remaining meropenem susceptibility. Sanger sequencing indicated that the variant was *bla*_{VIM-2}. Therefore, we hypothesize that the resistance mechanisms responsible for this phenotype could be a result of porin loss. Even though *bla*_{VIM-2} was not found to play a significant role in PA3841, there is a high risk of rapid dissemination by integron transfer within clinical settings. This is the first report of isolation of a *bla*_{VIM-2}-expressing IRMS *P. aeruginosa* in India. Laboratories need to be aware of this in order to carefully monitor and identify these unusual phenotype, while ensuring the implementation of appropriate control measures to prevent further spread.

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Conflict of interest None to declare.

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