Journal of Global Antimicrobial Resistance 14 (2018) 273–274

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Letter to the Editor

Clinical cases of VIM-producing *Pseudomonas mendocina* from two burned patients

Sir,

Since its first description in 1970 by Palleroni et al. from water and soil samples collected in the province of Mendoza in Argentina [1], few cases of human infection by *Pseudomonas mendocina* have been reported. The majority of reported cases are associated with endocarditis, lumbar spondylodiscitis and sepsis [2,3]. VIM enzymes are one of the most widespread metallo-β-lactamases (MBLs), which are commonly associated with class 1 integrons or even plasmids, contributing to the global spread of this resistance mechanism. Although *P. mendocina* has the ability to form biofilms and to carry class 1 integrons containing a carbapenemase gene such as bla_{IMP-8} [4], the isolation of carbapenemase-producing *P. mendocina* clinical isolates has not yet been described in the literature.

Here we report two cases of VIM-producing *P. mendocina* (Pm53 and Pm173) infections in patients in Argentina. The isolates were recovered from two male alcoholic patients (aged 56 years and 36 years) who were both hospitalised due to severe burns. *Pseudomonas mendocina* was recovered from several surgical debridements of burn wounds after 15 days of hospital admission for both patients. The patient in case 1 presented with severe burns of the lower limbs, developed grave right peripheral vascular insufficiency requiring supracondylar amputation, and later developed hypernatremia, grade IV dyspnoea requiring mechanical ventilatory support, hypotension, signs of peripheral hypoperfusion and multiple organ failure resulting in the patient's death. In case 2, after several surgical toilettes and receiving several skin grafts, the patient progressed favourably and ultimately was discharged. Ampicillin sulbactam/colistin was used to treat case 1, whilst colistin for was used for case 2.

The isolates were identified by conventional biochemical tests as well as the VITEK®2 system (bioMérieux, Marcy-l'Étoile, France) and were confirmed by amplification and subsequent sequencing of the *rpoB* gene. Antimicrobial susceptibility testing was performed using the VITEK®2 system and minimum inhibitory concentrations (MICs) were determined by Etest (bioMérieux). The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines. The MIC results for carbapenems for isolates Pm53 and Pm173, respectively, were as follows: imipenem, 8 μg/mL and 8 μg/mL; and meropenem, 4 μg/mL and 32 μg/mL. To test for the presence of MBL, the disk diffusion assay and double-disk assay using an ethylene diamine tetra-acetic acid/sodium mercaptoacetic acid (EDTA/SMA) disk (1900/750 μg per disk) (Laboratorios Britania, Buenos Aires, Argentina) and an imipenem disk, placed 15 mm from each other, were performed. This assay showed synergism between the carbapenem and EDTA/SMA disks, suggesting the presence of a putative MBL. Taking into account these results, PCR amplification was performed for the most widespread MBL genes (bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{SPM}). Total DNA extraction and PCR were performed according to the manufacturer's instructions (Promega, Madison, WI). Positive results were obtained for amplification of the bla_{VIM} gene and sequence analysis of the positive amplification showed 99% and 100% identity with bla_{VIM-2} for Pm53 and Pm173, respectively. In addition, a positive result for the *intI1* gene was obtained in both strains and PCR cartography to characterise the integron was performed. PCRs were sent for sequencing (Eton Biosciences Inc., San Diego, CA) and sequence analysis revealed the presence of two different class 1 integron arrays among the isolates: *intI1*–$bla_{VIM-like}$–*qacEΔ1* in strain Pm53; and *intI1*–*gcuD*–bla_{VIM-2}–*gcuD*–*qacEΔ1* in strain Pm173 (Fig. 1) (GenBank accession nos. **MH612381** and **MH612380**). The strains were not clonally related as seen by degenerate oligonucleotide-primed PCR [5] (data not shown).

Conjugation assays were performed as described previously [6]. Briefly, cultures of strain Pm53 or Pm173 and sodium azide-resistant *Escherichia coli* J53 were mixed (1:10 donor:recipient ratio) and were incubated for 18 h at 30 °C. Positive transconjugant cells were selected with sodium azide (150 μg/mL) and ampicillin (100 μg/mL). Positive results were obtained at a high frequency (6.2×10^{-1} per donor cell), suggesting a plasmid location of the enzyme.

In conclusion, we believe that the distribution of these enzymes in species of non-fermenting Gram-negative bacilli not previously described in the literature needs to be addressed to gain a better understanding of their distribution and their role in the horizontal gene transfer.

Funding

JF has received a SOAR-ELEVAR Scholar Fellowship from Latina/o Graduate Students from the US Department of Education. JF and

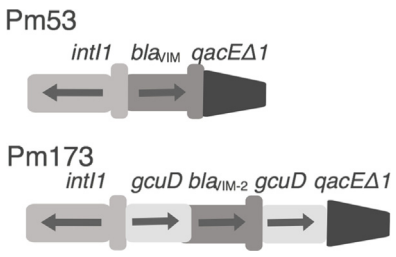

Fig. 1. Schematic map of class 1 integrons found in *Pseudomonas mendocina* isolates Pm53 and Pm173 (arrows indicate the translational orientation).

https://doi.org/10.1016/j.jgar.2018.08.002
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MH were supported by grant MHIRT 2T37MD001368 from the National Institute on Minority Health and Health Disparities, National Institute of Health. SM has received a Doctoral Fellowship from CONICET.

Competing interests

None declared.

Ethical approval

Not required.

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Received 2 July 2018

Available online 14 August 2018