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Letter to the Editor

Isolation of *Pseudomonas aeruginosa* Coproducing Metallo-β-Lactamase SPM-1 and 16S rRNA Methylase RmtD1 in an Urban River[▽]

Pseudomonas aeruginosa (strain 48-1997A) producing São Paulo metallo-beta-lactamase (MBL)-1 (SPM-1) was described for the first time in 1997 and came from a 4-year-old leukemic girl in São Paulo, Brazil (15). Since then, SPM-1-producing *P. aeruginosa* has become endemic in Brazilian hospitals, being recurrently associated with outbreaks of nosocomial infection (8, 11, 12). More recently, the first reports of the MBL SPM-1 were described in Europe (14) and western Asia (GenBank accession no. HM370523.1), denoting the potential for SPM-1 to become endemic worldwide (6). Unfortunately, carbapenem-resistant *P. aeruginosa* isolates carrying the SPM-1 enzyme have exhibited additional resistance to aminoglycosides, which has been mediated by a novel 16S rRNA methylase defined as RmtD (3), rendering ineffective a potent double-coverage regimen of a carbapenem plus an aminoglycoside and contributing to the emergence of panresistant phenotypes (3, 4). In this letter, we report the first environmentally isolated *Pseudomonas aeruginosa* carrying the $bla_{\text{SPM-1}}$ and *rmtD1* genes, providing additional data on the epidemiology of these genetic determinants of resistance.

In August of 2010, during a surveillance study established to monitor the occurrence of antimicrobial resistance in Gram-negative bacteria from urban rivers in southeastern Brazil, a panresistant *P. aeruginosa* isolate (TIES-04900) was recovered from the Tietê River. Tietê is one of the main rivers of the region, which runs across São Paulo state from east to west for about 1,100 km. The water sample was collected from a dam located downstream of many important cities, including São Paulo, the largest and most populous metropolitan area in Brazil.

P. aeruginosa strain TIES-04900 was resistant to imipenem, meropenem, ertapenem, ceftazidime, cefotaxime, cefepime, piperacillin-tazobactam, amikacin, tobramycin, gentamicin, ciprofloxacin, levofloxacin, and trimethoprim-sulfamethoxazole; remaining susceptible only to aztreonam and polymyxin B (Table 1). MBL production was confirmed by using an MBL Etest strip. Based on the antimicrobial susceptibility pattern and genetic backgrounds of multidrug-resistant *P. aeruginosa* strains reported from Brazil (4, 8), antibiotic resistance genes were studied by PCR and sequencing, confirming the presence of the $bla_{\text{SPM-1}}$, $bla_{\text{OXA-56}}$, *rmtD1*, *aacA4*, *aadA7*, *sul1*, and *dhfr* genes (GenBank accession numbers HQ876773 to HQ876777). This resistance genotype has been previously reported in clinical isolates of *P. aeruginosa* (3) and most likely has contributed to the endemicity of this panresistant phenotype in Brazilian hospitals (3, 4, 6, 8, 11, 12). In fact, enterobacterial repetitive intergenic consensus-PCR fingerprint analysis (Dice similarity coefficient and unweighted-pair group method using average linkages clustering), undertaken to ascertain the relatedness of TIES-04900 to clinical SPM-1- and RmtD-1-positive *P. aeruginosa* isolates (3, 15), showed that environmental *P. aeruginosa* TIES-04900 was clonally related to human *P. aeruginosa* strains PA0905 (95.2% similarity) and 48-1997A (90% similarity), previously identified in São Paulo (3, 15).

In summary, we report the presence of SPM-1- and RmtD-1-coproducing *P. aeruginosa* in an urban river, showing that strains harboring the $bla_{\text{SPM-1}}$ and *rmtD1* genes seem not to be restricted to the hospital setting, a fact that emphasizes the importance of surveying environmental strains that might act as a source and/or reservoir of resistance genes with clinical relevance (1, 5, 7, 13) and have the potential for dissemination in communities through river environments (1, 10).

TABLE 1. MICs of antibiotics for environmental *Pseudomonas aeruginosa* strain TIES-04900 carrying the $bla_{\text{SPM-1}}$, $bla_{\text{OXA-56}}$, *rmtD1*, *aacA4*, *aadA7*, *sul1*, and *dhfr* genes[a]

Antibiotic	MIC (μg/ml) for *P. aeruginosa* TIES-04900
Imipenem	>256[b,c]
Imipenem + EDTA	2[d]
Meropenem	>32[c]
Ertapenem	>32[c]
Ceftazidime	>256[b,c]
Cefotaxime	>256[b,c]
Cefepime	>256[c]
Piperacillin-tazobactam	>256[c]
Aztreonam	8[b,c]
Amikacin	>256[b,c]
Gentamicin	>256[b,c]
Ciprofloxacin	>32[b,c]
Levofloxacin	>32[c]
Trimethoprim-sulfamethoxazole	>32[c]
Tigecycline	16[c]
Polymyxin B	2[b,c]

[a] *P. aeruginosa* isolate TIES-04900 was identified by using the Vitek 2 automated instrument ID system and conventional biochemical tests (9). The presence of $bla_{\text{CTX-M}}$-, bla_{SHV}-, bla_{GES}-, bla_{PER}-, bla_{OXA}-, bla_{TEM}-, bla_{VIM}-, bla_{IMP}-, and bla_{SPM}-like β-lactamase genes; *aac(6′)*-, *aadA1*-, and *aadB*-like aminoglycoside transferase genes; *rmtD*-like 16S rRNA methylase genes; and the *sul1* and *dhfr* genes was studied by PCR and sequencing.
[b] MICs were determined by agar dilution (2).
[c] MICs were determined using the Etest strip (AB Biodisk, Solna, Sweden) (2).
[d] MBL production was evaluated using the Etest MBL strip (16).

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