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Identification of Aminoglycoside-Resistant *Pseudomonas aeruginosa* Producing RmtG 16S rRNA Methyltransferase in a Cystic Fibrosis Patient

Gabriela Rodrigues Francisco,[a] Sandra Terezinha Rodrigues Nora,[a] Maria Fernanda Campagnari Bueno,[a] Luiz Vicente Ribeiro Ferreira da Silva Filho,[b] Doroti de Oliveira Garcia[a]

Center of Bacteriology, Instituto Adolfo Lutz, Sao Paulo, Brazil[a]; Pediatric Pulmonology Unit, Instituto da Criança, Faculdade de Medicina da Universidade de São Paulo, Sao Paulo, Brazil[b]

Pseudomonas aeruginosa is the most prevalent microorganism isolated from the respiratory tract of cystic fibrosis (CF) patients (1). Aminoglycosides are frequently used in the therapy of airway infection for CF patients, especially in the context of nebulized inhaled therapy. In the last decade, acquired 16S rRNA methyltransferases (16S-RMTases) have been described as a novel and high-level resistance mechanism against all aminoglycosides (2–4). RmtD was first described in a *P. aeruginosa* clinical isolate in Brazil and has subsequently been detected in multiple species of *Enterobacteriaceae* in Brazil, Argentina, and Chile (3, 5–9). We recently described the second 16S-RMTase, RmtG, which was coproduced with KPC and CTX-M β-lactamases among *K. pneumoniae* isolates identified in São Paulo State in Brazil between 2010 and 2011 (5).

In this study, we sought to identify 16S-RMTases in *P. aeruginosa* clinical isolates from CF patients. Clinical samples recovered from the respiratory tract of patients attending the outpatient clinic of the Instituto da Criança (University of São Paulo Medical School), collected during three periods from 2003 to 2004, 2006 to 2007, and in 2009, were sent to the Instituto Adolfo Lutz for culture and biochemical identification. A total of 580 *P. aeruginosa* isolates from 98 patients were identified. Of these, isolates from patients who had at least 5 isolates in at least two of the periods of time cited above were studied further; these included 129 *P. aeruginosa* isolates from 11 patients. Disk diffusion tests showed that nine *P. aeruginosa* isolates were completely resistant to all aminoglycosides (gentamicin, amikacin, and tobramycin). The MICs were determined by Etest, and the isolates were screened for the known 16S-RMTase genes by PCR as previously described (2, 5). As a result, seven serial isolates from patient 3 were found to be positive for *rmtG*, which was confirmed by DNA sequencing (Table 1). These isolates also presented resistance or diminished susceptibility to ciprofloxacin and levofloxacin and susceptibility to imipenem, meropenem, ceftazidime, cefepime, and polymyxin B (10). Two isolates presented intermediate susceptibility to ticarcillin-clavulanate (Table 1) (10). The *P. aeruginosa* isolates were subjected to pulsed-field gel electrophoresis (PFGE) using the restriction enzyme SpeI (New England Biolabs) (11) and to multilocus sequence typing (MLST) (12). The database http://pubmlst.org/paeruginosa was used to determine the sequence types (STs). Patient 3 carried *P. aeruginosa* isolates with the same PFGE profile A, with 3 subtypes, A1, A2, and A3, isolated between 2004 and 2007, and isolates obtained from patient 9 showed PFGE profile B. MLST revealed that all strains belonged to ST235, an international clone that has successfully spread worldwide. Several attempts to obtain transconjugants by mating or transformants by electropo-

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Address correspondence to Doroti de Oliveira Garcia, dogarcia@yahoo.com.

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TABLE 1 Antimicrobial susceptibility, PCR results for *rmtD* and *rmtG*, and PFGE and MLST profiles of nine *P. aeruginosa* isolates resistant to aminoglycosides obtained from cystic fibrosis patients

Isolate	Patient	Age	Mo/yr of isolation	MIC (μg/ml) of[a]:											PCR result for:		PFGE profile	MLST
				AK	GM	TM	TZ	PM	TLc	IP	MP	CI	LE	PO	*rmtD*	*rmtG*		
302-2	3	13	2/2004	>256	>256	>256	3	4	64/2	0.75	1.5	>32	>32	1	−	+	A	235
538	3	15	1/2007	>256	>256	>256	0.38	1.5	0.25/2	0.125	0.023	>32	>32	0.19	−	+	A	235
653-1	3	16	5/2007	>256	>256	>256	0.75	2	0.25/2	0.25	0.047	>32	>32	0.5	−	+	A	235
653-2	3	16	5/2007	>256	>256	>256	0.75	1.5	0.25/2	0.5	0.047	4	2	1	−	+	A1	235
783-1	3	16	8/2007	>256	>256	192	0.75	3	0.38/2	0.38	0.094	4	4	0.75	−	+	A2	235
783-2	3	16	8/2007	>256	>256	>256	2	4	64/2	0.75	1	>32	>32	0.75	−	+	A3	235
783-3	3	16	8/2007	>256	>256	>256	0.5	1	0.25/2	0.5	0.047	4	2	0.75	−	+	A2	235
19	9	8	6/2003	>256	>256	128	ND[b]	ND	ND	ND	ND	ND	ND	ND	−	−	B	ND
487	9	11	11/2006	>256	24	8	ND	ND	ND	ND	ND	ND	ND	ND	−	−	B	ND

[a] AK, amikacin; GM, gentamicin; TM, tobramycin; TZ, ceftazidime; PM, cefepime; TLc, ticarcillin-clavulanate; IP, imipenem; MP, meropenem; CI, ciprofloxacin; LE, levofloxacin; PO, polymyxin B.
[b] ND, not determined.

ration were unsuccessful. PFGE of S1 nuclease-treated genomic DNA (13), followed by hybridization using an *rmtG* probe, was also unsuccessful. The gene *rmtG* is therefore likely located in the chromosome. This is also the first description of 16S-RMTase production in a CF patient to our knowledge. Our case shows that acquired resistance can play a significant role in multiresistance of CF isolates.

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