which should be cited to refer to this work.

Emergence of the 16S rRNA Methylase RmtG in an Extended-Spectrum-β-Lactamase-Producing and Colistin-Resistant *Klebsiella pneumoniae* Isolate in Chile

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While carbapenemases are disseminating worldwide in *Enterobacteriaceae*, and particularly in *Escherichia* and *Klebsiella pneumoniae* (1), therapeutic options increasingly rely on very few choices (2). Although colistin and tigecycline are now considered last-resort antibiotics for treating infections caused by carbapenemase producers, aminoglycosides may still be considered valuable treatment options, with amikacin remaining quite often active (3). While broad-spectrum β-lactamases such as extended-spectrum β-lactamases (ESBLs) or carbapenemases are spreading rapidly worldwide, the emergence of acquired 16S rRNA methylases has been also noticed (4). Those enzymes do confer high-level resistance to almost all aminoglycosides. The most frequent 16S rRNA methylases in *Enterobacteriaceae* are ArmA, RmtB to RmtF, and NpmA (4, 5). Those enzymes exhibit significant amino acid heterogeneity, sharing similar amino acid motifs but being overall distantly related. Nevertheless, they all confer the same type of resistance, whereas NpmA confers additional resistance to neomycin and apramycin (5).

Here, we report on a 22-year-old male patient who was admitted at the intensive care unit of the Puerto Montt Hospital located in Santiago, Chile, on 30 June 2012 for a traumatic subdural hematoma of the occipitotemporal region. He underwent surgery and was mechanically ventilated. Ceftriaxone and metronidazole were prescribed for 7 days (during which time cultures remained negative) as empirical treatment for pneumonia. Ten days after his admission, bronchial aspirate samples grew ceftriaxone-resistant *K. pneumoniae*, while an isolate with the same resistant pattern was recovered from his central venous catheter 3 days later. Therefore, ceftriaxone treatment was discontinued and switched to meropenem, and the patient was finally discharged after a good clinical response. Susceptibility testing performed and interpreted according to the updated CLSI guidelines (6) showed that it was resistant at a high level to all aminoglycosides (MICs of >256 μg/ml for amikacin, gentamicin, and tobramycin) and to most broad-spectrum β-lactams (MIC of cefotaxime and cefepime at 256 μg/ml, MIC of ceftazidime at 8 μg/ml). It remained susceptible to carbapenems (MICs of imipenem and meropenem at 0.5 and 1 μg/ml, respectively), and the Carba NP test remained negative (7). In addition, isolate 921 was resistant to tetracycline, chloramphenicol, sulfonamides, and to all tested fluoroquinolones. It remained susceptible to tigecycline (MIC of 1 μg/ml) according to EUCAST breakpoints (8), but the MIC of colistin was high as determined by Etest (4 μg/ml). According to the phenotypic test results (synergy between aztreonam and clavulanate), *K. pneumoniae* isolate 921 produced an ESBL.

PCR and sequencing performed as described previously (9) revealed that *K. pneumoniae* isolate 921 harbored the $bla_{\text{CTX-M-2}}$ ESBL gene, in addition to the $bla_{\text{TEM-1}}$ and $bla_{\text{SHV-11}}$ narrow-spectrum β-lactamase genes. Since isolate 921 was resistant at a high level to all aminoglycosides, screening for 16S RNA methylase genes was performed as reported (10). It failed to identify any known 16S rRNA methylase-encoding gene. Therefore, a shotgun cloning experiment was attempted as described previously (11), and selection of recombinant *Escherichia coli* strains was based on amikacin (30 μg/ml) and gentamicin (30 μg/ml). Recombinant strains expressing resistance to all aminoglycosides were obtained, suggesting the expression of a 16S rRNA methylase. Sequencing of the recombinant plasmid identified a putative open reading frame encoding a protein sharing 58% amino acid identity with the 16S rRNA methylase RmtD. However, it has a perfect identity with the newly described RmtG which was published while this study was in progress (12). The *rmtG* gene encoding high-level resistance to all aminoglycosides has been identified in a series of five *K. pneumoniae* isolates from Brazil, four coproducing the carbapenemase KPC-2, and all coproducing an ESBL of the CTX-M type (CTX-M-2, CTX-M-15, or CTX-M-59) (12).

Mating-out assays were performed as described previously (11) but remained unsuccessful. Therefore, an electrotransformation experiment was performed using a selection based on amikacin and gentamicin or on cefotaxime (30 μg/ml). It allowed us to obtain an *E. coli* transformant expressing RmtG, exhibiting resistance to all aminoglycosides, to sulfonamides, and to tetracycline but remaining susceptible to all β-lactams, and another type of *E. coli* transformant expressing CTX-M-2 and TEM-1. The *rmtG*-positive transformant harbored a single 80-kb plasmid that was of IncA/C type as identified by PCR-based replicon typing (13). The $bla_{\text{CTX-M-2}}$-positive transformant harbored a single 170-kb plasmid that was not typeable.

Multilocus sequence typing was performed as described previously (14), and results were analyzed by eBURST (http://pubmlst.org). It showed that isolate 921 belonged to the ST11 sequence type, whereas the sequence types of the RmtG-positive *K. pneumoniae* isolates from Brazil were 340, 442, and 1046 (12). However, in the same study, Bueno et al. identified several ST11 isolates expressing the RmtD2 methylase (12).

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Of note, the patient did not receive any aminoglycoside-based treatment before or after his admission. Therefore, selection of this aminoglycoside-resistant isolate remains intriguing. It may be speculated that clones or plasmids carrying the *rmtG* gene are disseminating in South American countries such as Chile and Brazil (12). Occurrence of multidrug-resistant strains is extremely worrisome, and there is an urgent need to implement adequate screening strategies in order to control their spread. This study underlines that a focus on emerging antibiotic resistance mechanisms in Gram negatives shall be made not only for broad-spectrum β-lactams but also for broad-spectrum aminoglycosides, since both types of resistance will contribute to the emergence of pan-drug resistance.

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