Surveillance of 16S rRNA Methylases in Enterobacteria from Argentina: Description of a New Allele, *rmtD2*

A-079

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INTRODUCTION

The most common mechanism of resistance to aminoglycosides is the enzymatic modification of the drug. Since few years ago, a new enzyme-mediated mechanism of target modification (methylation of 16S rRNA in positions A1405 or G1408) was described in clinical pathogens, producing high-level resistance to all available aminoglycosides used for systemic therapy. Until now, six 16S rRNA methyltransferases genes have been identified: *armA* and *rmtB* are most widespread and have been found in East Asia, Europe and North America; *rmtA* and *rmtC* have been reported from Japan and Australia; *rmtD* has been described in Gram-negative isolates from South America; *npmA* has been characterized from a clinical *Escherichia coli* isolate from Japan. Some of these genes have been found in association with transposons or transposon-like elements. These transposons have been demonstrated to be functional for ArmA and RmtC, suggesting that transposase-mediated recombination events are likely responsible for the acquisition and dissemination of these genes.
Although data on the prevalence of aminoglycoside resistance mediated by 16S methylation among gram-negative bacilli is still scarce, recent data suggest the global emergence of this mechanism.

AIM

The aim of this work was to perform a nationwide survey of aminoglycoside resistance mediated by 16S methylation among clinical enterobacteria from Argentina.

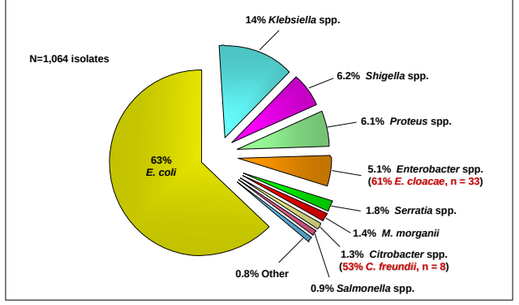
MATERIALS & METHODS

Clinical isolates and antimicrobial susceptibility testing. A total of 1,064 consecutive, non-duplicate enterobacterial isolates (Fig. 1) were collected from 66 hospitals belonging to the WHONET-Argentina Resistance Surveillance Network (April 2007, 5-days period). Initial screening of methylase-conferring phenotype was performed by disc diffusion method for amikacin and gentamicin (inhibition zones ≤ 10 mm) (CLSI, 2007). MICs were determined by Etest (AB Biodisk).

Molecular assays. Presence of methylase genes was tested by PCR using standard conditions. Additional set of primers was used for sequencing. Genomic DNA was purified employing commercial kits. DNA library from *Enterobacter cloacae* Q-4010 was generated in *E. coli* Top10 using the pACYC184 vector. Selection of recombinant strains was achieved in Mueller-Hinton agar supplemented with chloramphenicol (34 µg/ml) and kanamycin (30 µg/ml). DNA sequencing was performed using primers binding the cloning vector, the *rmtD* gene and sequence based primers (DNA walking) under the BigDye terminator methodology. Agar mating method was used to transfer high-level aminoglycoside resistance from positive clinical isolates to sodium azide-resistant *E. coli* J53 or rifampin-resistant *E. coli* ER1793 recipients. Amikacin (AKN, 50 µg/ml) and gentamicin (GEN, 50 µg/ml) plus sodium azide (100 µg/ml) or rifampin (300 µg/ml), respectively, were used to select for transconjugants.

RESULTS

Fig. 1. Distribution (by genera) of clinical enterobacteria included in this work.

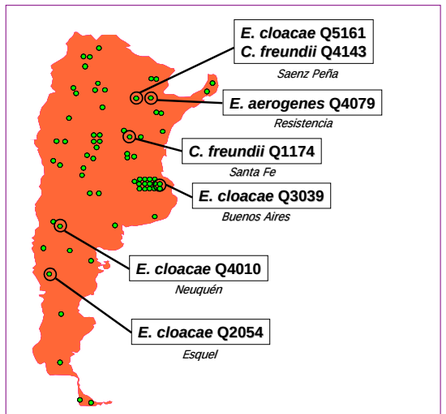


Selection criteria: AKN ≤ 10 mm + GEN ≤ 10 mm

Results of antibiotic susceptibility testing for clinical isolates (n = 12)

Strain	Spp	Disc diffusion (mm)								MIC (E-test, µg/ml)					PCR methylase genes					
		AKN	GEN	NAL	CIP	CTX	AMC	TET	CMP	TOB	KAN	GEN	NET	AKN	*armA*	*rmtA*	*rmtB*	*rmtC*	*rmtD*	*npmA*
Q-1174	*cfr*	6	6	6	6	6	8	6	6	≥1024	≥256	≥1024	≥256	≥256	-	-	-	-	+	-
Q-2054	*ecl*	6	6	6	6	6	6	6	6	≥1024	≥256	256	≥256	≥256	-	-	-	-	+	-
Q-3039	*ecl*	6	6	6	6	6	6	6	6	≥1024	≥256	≥1024	≥256	≥256	-	-	-	-	+	-
Q-4010	*ecl*	6	6	6	6	6	6	6	6	≥1024	≥256	≥1024	≥256	≥256	-	-	-	-	+	-
Q-4079	*eae*	6	6	16	26	10	10	17	6	256	≥256	256	≥256	≥256	-	-	-	-	+	-
Q-4143	*cfr*	6	6	6	6	6	6	6	11	768	≥256	≥1024	≥256	128	-	-	-	-	+	-
Q-5161	*ecl*	6	6	6	6	6	6	6	6	≥1024	≥256	256	≥256	≥256	-	-	-	-	+	-
Q-1097	*mmo*	6/19	6	6	6	10	16	NR	NR	32	≥256	96	128	6	-	-	-	-	-	-
Q-1217	*pmi*	8	6	6	6	10	15	NR	NR	-	-	-	-	-	-	-	-	-	-	-
Q-1218	*se-*	7	6	6	11	6	6	NR	NR	-	-	-	-	-	-	-	-	-	-	-
Q-2113	*sma*	7	6	6	15	6	6	NR	NR	96	≥256	512	≥256	96	-	-	-	-	-	-
Q-5212	*se-*	12	6	29	39	8	6	NR	NR	-	-	-	-	-	-	-	-	-	-	-

References: *cfr, C. freundii*; *ecl, E. cloacae*; *eae, E. aerogenes*; *mmo, M. morganii*; *pmi, P. mirabilis*; *sma, S. marcescens*; *se-, Serratia* spp.; AKN, amikacin; GEN, gentamicin; TOB, tobramycin; KAN, kanamycin; NET, netilmycin; NAL, nalidixic acid; CIP, ciprofloxacin; CTX, cefotaxime; AMC, amoxicilin-clavulanic acid; TET, tetracycline; CMP, chloranfenicol.



Geographic distribution of the *rmtD2* positive isolates. Green dots represent different cities involved in this surveillance, members of WHONET-Argentina resistance network.

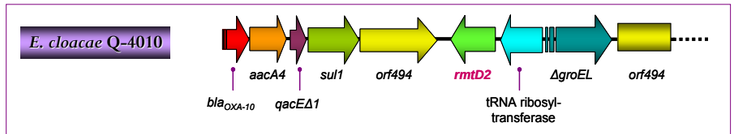
- A unique gene was found in these 7 isolates. This gene showed 97.3% of nucleotide identity (20 nucleotides of difference) and 96.4% of amino acid identity (9 residues of difference) with *rmtD*.

- The new allele was named *rmtD2* as recommended by Doi et al (AAC, **52**:2287, 2008).

Methylase	AA Identity (%)						
	ArmA	NpmA	RmtA	RmtB	RmtC	RmtD	RmtD2
ArmA		9.3	31.0	31.0	28.7	25.0	**25.5**
NpmA			11.9	6.2	11.8	13.6	**13.6**
RmtA				81.7	27.4	39.9	**39.6**
RmtB					29.6	41.3	**40.9**
RmtC						26.4	**44.2**
RmtD							**96.4**

- *E. coli* transconjugant strains highly resistant to aminoglycosides were selected after conjugative assays using all the seven *rmtD2* isolates as donors.

- Flanking regions of *rmtD2* gene showed high sequence similarity with the one described for *rmtD* gene in *Pseudomonas aeruginosa* from Brazil.



Schematic representation of *rmtD2* flanking regions.

SUMMARY & CONCLUSION

- A new allele of the methytransferase RmtD was detected in enterobacteria from Argentina.
- The *rmtD2* gene had a broad geographical distribution across the country.
- The new allele could be transferred to *E. coli* by conjugation.
- The genetic structure of *rmtD2* flanking region was similar to the described for *rmtD* gene in *P. aeruginosa* from Brazil.
- *rmtD2* gene was only found in *Enterobacter* and *Citrobacter* isolates (prevalence rates of 9.3% and 13.3%, respectively). This result suggests a possible reservoir of *rmtD2* in these genera.
- This presumption and the broad distribution of this gene deserve monitoring by continuous surveillance.