C2-716

Plasmid Mediated Quinolone Resistance Mechanisms (PMQRs) in Clinical Enterobacteria from Argentina: A View from the Microbiological Practice

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ABSTRACT

PMQRs were already reported worldwide. To date, only *qnrB* and *aac(6´)-Ib-cr* were found in Argentina.
Aim: to analyze the occurrence and distribution of PMQRs in enterobacteria with decreased quinolone susceptibility (DQS).
Methods: 84 enterobacteria [38 *Klebsiella* spp. (KL); 20 *Escherichia coli* (EC); 12 *Salmonella* spp. (SA); 10 *Enterobacter* spp. (EN); other genera (4)] with DQS [nalidixic acid > 6 mm and ciprofloxacin (CIP) < 29 mm; CLSI disk diffusion method (DD)] were found during routine microbiological tasks at 26 hospitals [Buenos Aires (BA) and 10 Provinces, 2005 - 2008] and sent to INEI for characterization. Presence of *qnr* (*A*, *B* and *S*) was tested by standard PCR; *aac(6´)-Ib* was firstly screened in all isolates by not susceptibility to kanamycin (DD inhibition zones ≤ 17 mm) and then *aac(6´)-Ib* and *aac(6´)-Ib-cr* were differentiated in selected isolates by 2 selective PCRs (results were validated by DNA sequencing).
Results: 54/84 isolates (64%; 21 hospitals in BA and 9 Provinces; 2005 - 2008) had at least one PMQR (*qnrB*, *qnrS* and *aac(6´)-Ib-cr*, 46, 5 and 21 isolates, respectively); 33 isolates showed *qnr* (*B* or *S*) and 3 had *aac(6´)-Ib-cr*, as the unique PMQR while 18 isolates showed both mechanisms. The occurrence of PMQRs was high among the 4 major species (%): KL (58); EC (65); SA (100); EN (70). The proportion (%) of aac(6´)-Ib-cr was significantly higher in KL (39) and EN (50) than in EC (5) and SA (0) ($P \leq 0.010$, Fisher´s test) but there were not significant differences for *qnr*. Of 56 isolates with CIP susceptibility by CLSI breakpoint (≥ 21 mm), 30 (54%) had PMQRs: *qnrB* alone, 27 isolates (mostly EC and SA, 12 isolates each); *aac(6´)-Ib-cr* alone, 2 KL, and both mechanisms, 1 EN.
Conclusions: This is the first report on *qnrS* in Argentina where PMQRs are widely spread. In this work, CLSI CIP breakpoint could not detect more than 50% of PMQR producing isolates.

INTRODUCTION

PMQRs have been reported worldwide. To date, only *qnrB* and *aac(6')-Ib-cr* were found in Argentina. However, their abundance and diversity among clinical enterobacteria routinely recovered at the hospital setting have never been studied in our country.

qnrB-10 and *aac(6´)-Ib-cr* (Antimicrob Agents Chemother 51:4466-70, 2007)

qnrB-9-like (Antimicrob Agents Chemother 53:1665-6, 2009)]

AIM

To analyze the occurrence and distribution of PMQRs [*qnrA*, *B* and *S*; and *aac(6´)-Ib-cr*] in enterobacteria with decreased quinolone susceptibility routinely recovered at the hospital laboratory of microbiology.

MATERIAL & METHODS

CLINICAL ISOLATES

submitted to the Reference Laboratory due to decreased quinolone susceptibility.

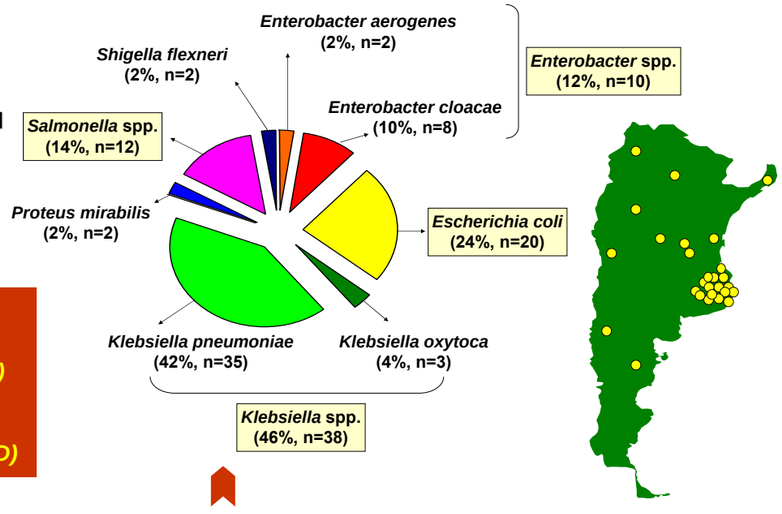
SELECTION CRITERIA

NALIDIXIC ACID > 6 mm (DD)

AND

CIPROFLOXACIN < 29 mm (DD)

 # 84 ENTEROBACTERIAL ISOLATES



26 Hospitals from Buenos Aires and 10 Provinces.
JAN 2005 - DEC 2008 (4 years)

SUSCEPTIBILITY TESTING

Antimicrobial susceptibility profiles were obtained by the disk diffusion method (DD, CLSI).

MOLECULAR ASSAYS

PCR for *qnr* (*A*, *B* and *S*) and *aac(6')-Ib* genes, and DNA sequencing were done by standard methods.

aac(6')-Ib-cr: SCREENING AND CHARACTERIZATION

The presence of an *aac(6')-Ib* allele was firstly screened in all isolates by not susceptibility to kanamycin (DD inhibition zones ≤ 17 mm).

Then *aac(6´)-Ib* and *aac(6´)-Ib-cr* were differentiated in selected isolates by 2 allele-specific PCRs:

aac(6')-Ib gene (GB#: DQ310703)

```
ATG ──────────── 276 bp ──────────── TAC
GTTGCTCTTGGAAGCGGGGACGGATGGTGGGAAGAAGAAACCGATCCAGGAGTACG
   GCTCTTGGAAGCGGGGACGAAT
   GCTCTTGGAAGCGGGGACGAAA

CGG ──────────── 162 bp ──────────── GGG
GTTTGAGAGGCAAGGTACCGTAACCACCCCAGATGGTCCAGCCGTGTACATGGTTC
                                GACGGTCCAGCCGTGTACATGGTTC
                                TACGGTCCAGCCGTGTACATGGTTC

AAACACGCCAGGCATTCGAGCGAACACGCAGTGATGCCTAA
```

Primer aac-WT-F
Primer aac-cr-ARG-F

1st *aac(6')-Ib-cr* mut.: TGG (W) → AGG (R)

2nd *aac(6')-Ib-cr* mut.: GAT (D) → TAT (Y)

Primer aac-WT-R
Primer aac-cr-MUT-R

Primer WT-F / WT-R = wild-type; ARG-F / MUT-R = double mutant, allele found in isolates from Argentina

The results were validated by DNA sequencing.

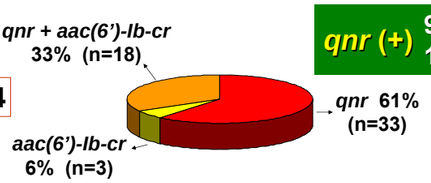
RESULTS

DISTRIBUTION AND OCURRENCE OF PMQRs

64% (54/84) ISOLATES HAD AT LEAST 1 PMQR

21 hospitals from Buenos Aires and 9 Provinces; 2005-2008

N = 54

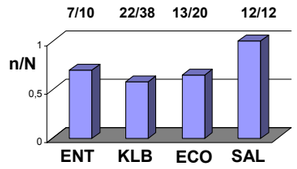


***qnr* (+)** 90% (46/51): *qnrB* 10% (5/51): *qnrS*

All isolates were *qnrA* NEGATIVE

Ocurrence of PMQRs among the 4 major species: ratio n/N, Nº with PMQR/total in each specie

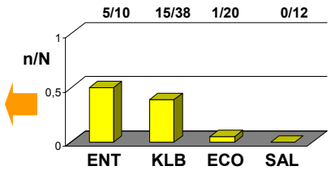
PMQRs



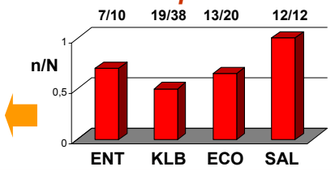
The occurrence of PMQRs was high among the 4 major species

The ratio of *aac(6')-Ib-cr* was significantly higher in *Klebsiella* spp. (KLB) and *Enterobacter* spp. (ENT) than in *E. coli* (ECO) and *Salmonella* spp. (SAL) (P ≤ 0.010, Fisher's Test)

aac(6')-Ib-cr



qnr



There were not significant differences for *qnr*

Screening for *aac(6')-Ib* alleles
Kanamycin (NoS) ≤ 17 mm (DD)

68% (57/84) → PCR → (+) N = 34 / (-) N = 23

By allele-specific PCR
6% (2/34): *aac(6')-Ib-cr*
38% (13/34): *aac(6')-Ib*
41% (14/34): both alleles
15% (5/34): both negative → 1st *aac-cr* mut. CGG (no AGG)

By DNA sequencing
21% (7/34): *aac(6')-Ib-cr*
38% (13/34): *aac(6')-Ib*
41% (14/34): both alleles

We designed a new primer for the "international" allele (CGG):
MUT-F,
GCTCTTGGAAGCGGGGACGAAC

Allele-specific PCR
100% SPECIFIC
100% SENSITIVE

SUSCEPTIBILITY TESTING

A – Distribution of antibiotic DD inhibition zones (mm), CLSI

N: 84 isolates	# NAL		# CIP		# LEV		# KAN		# AKN	
	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX	MIN	MAX
	8	26	6	29	13	33	6	25	11	25
MEDIAN	15		23		24		13		21	

NAL: Nalidixic Acid; CIP: Ciprofloxacin; LEV: Levofloxacin; KAN: Kanamycin; AKN: Kanamaycin

B – CLSI breakpoints interpretation

aCLSI	# NAL		# CIP		# LEV		# KAN		# AKN	
	N	%	N	%	N	%	N	%	N	%
S	20	24	56	67	80	95	27	32	67	80
I	31	37	11	13	3	4	14	17	10	12
R	33	39	17	20	1	1	43	51	7	8
TOTAL	84	100	84	100	84	100	84	100	84	100

NAL: Nalidixic Acid; CIP: Ciprofloxacin; LEV: Levofloxacin; KAN: Kanamycin; AKN: Kanamaycin

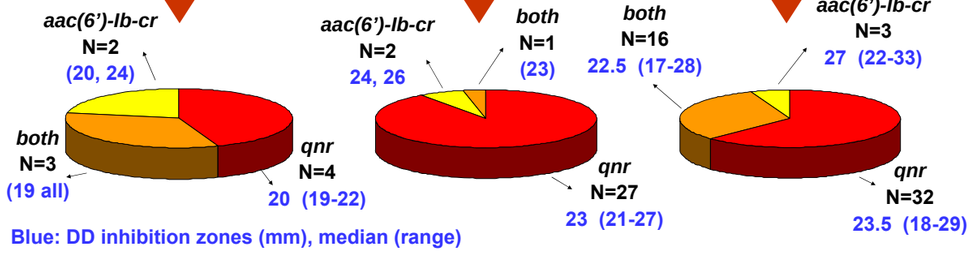
aZone Diameter Breakponits (mm)
NAL: S/I/R, ≥19 / 18–14 / ≤13
CIP: S/I/R, ≥21 / 20–16 / ≤15
LEV: S/I/R, ≥17 / 16–14 / ≤13
KAN: S/I/R; ≥18 / 17–14 / ≤13
AKN: S/I/R; ≥17 / 16–15 / ≤14

45% (9/20) isolates SUSCEPTIBLE to NAL HAD PMQRs

54% (30/56) isolates SUSCEPTIBLE to CIP HAD PMQRs

64% (51/80) isolates SUSCEPTIBLE to LEV HAD PMQRs

C – Distribution of PMQRs in susceptible isolates (DD CLSI breakpoints)



Blue: DD inhibition zones (mm), median (range)

Molecular characterization of a *qnrB* determinant → *qnrB-19*

Previous report:
qnrB10 was located in complex (IS*CR1*) class 1 integrons (*K. pneumoniae*, *Citrobacter freundii*, *E. cloacae*). Quiroga et al, AACh 51: 4466-70, 2007

Here, we selected a *Salmonella* isolate (M7849) to characterize the *qnrB* determinant and its genetic platform

Plasmid extraction + sequencing (DNA walking)

➢ *qnrB19* was located in a small plasmid which was 100% identical to pSG115 (unpublished, GB# FN428572).

➢ *qnrB19* was not associated to IS*CR1*.

CONCLUDING REMARKS

✓ PMQRs are widely spread among clinical enterobacteria from Argentina.

✓ This is the first report on *qnrS* in Argentina.

✓ *qnr* had similar distribution among species but *aac(6')-Ib-cr* was mainly associated to *Klebsiella* spp. and *Enterobacter* spp.

✓ *qnrB* harbored in at least 2 different genetic platforms.

✓ CLSI quinolones breakpoints could not detect more than 50% of PMQRs-producing isolates.