Plasmid-mediated quinolone resistance among Enterobacteriaceae in Australia; Results of the AGAR Studies (2008-2010)

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INTRODUCTION

Quinolone resistance is most commonly due to mutations in DNA gyrase and topoisomerase IV. More recently plasmid-mediated quinolone resistance (PMQR) has emerged. PMQR may be due to the presence of *qnr* genes, *aac(6')-Ib-cr*, encoding for a variant aminoglycoside acetyltransferase enzyme; or *qepA*, which encodes for an efflux pump. The aim of this study was to examine a collection of *Enterobacteriaceae* from the AGAR studies for evidence of PMQR.

METHODS

Isolates

Clinically significant, non-duplicate isolates of *E. coli* (n=70), *Klebsiella* spp. (n=20) and *Enterobacter* spp. (n=10) were collected by each participating laboratory from outpatients with urinary tract infections (2008, 2010), or from patients hospitalised more than 48 hours (2009).

Susceptibility testing

MICs were determined using Vitek 2 (BioMerieux) AST-N083 (2008-2009) and AST-N149 cards. MICs were interpreted according to current CLSI M100 guidelines[1]. Isolates with ciprofloxacin MIC ≥ 0.5 mg/L were referred to a central laboratory for molecular testing.

Plasmid-mediated quinolone resistance

All isolates received were screened for the presence of plasmid-mediated quinolone resistance (PMQR) genes using real-time PCR (LightCycler LC480, Roche) and published primers. *aac(6')-Ib-cr* genotyping was performed by simultaneous high resolution melting analysis of an unlabelled probe and full length amplicon[2].

ESBL resistance gene detection

All isolates were also screened for the presence of the bla_{TEM}, and bla_{SHV} genes using previously reported oligonucleotide primers. A multiplex real-time TaqMan PCR[3] was used to detect CTX-M-type genes. Strains were probed for plasmid-borne AmpC enzymes using the method described by Pérez-Pérez et al[4].

RESULTS

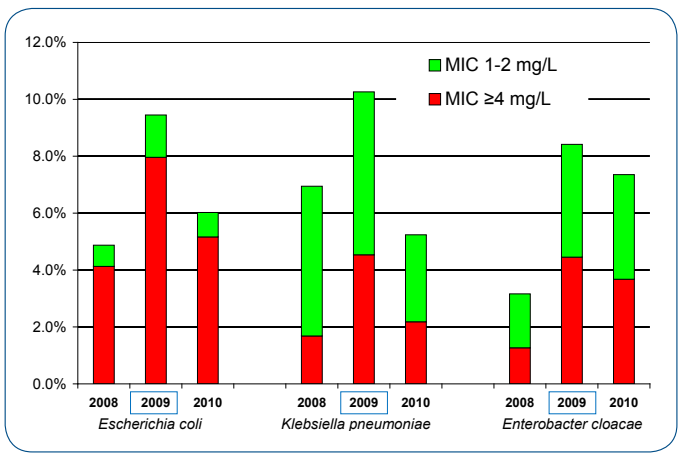
- Ciprofloxacin resistance among *E. coli* from outpatients with UTI increased from 4.9% in 2008 to 6.0% in 2010 (Figure 1, Table 1)
- Resistance was more prevalent among isolates collected from hospitalised patients in 2009 (approx. 10%).
- Over 94% of isolates with MIC ≥ 0.5 mg/L were received for molecular screening.
- PMQR genes were found in 13-14% of *E. coli* from outpatients with UTI, increasing to 23% among hospitalised patients.
- *Klebsiella* spp. harboring PMQR genes were common (>60%) among hospitalised inpatients.
- Over 80% of PMQR genes detected among *E. coli* were *aac(6')-Ib-cr* variant (Figures 2 and 3)
- *qnr* genes dominated among *Klebsiella* spp., with *qnrS* the dominant type.
- *qepA* was only detected in four *E. coli* isolates, all from different institutions, in different states, and from all years.
- Only *qnr* genes (predominantly *qnrA*) were detected in *Enterobacter* spp., however, 30% were found in association with *aac(6')-Ib-cr* variant.
- There was a strong correlation between PMQR and ESBL production; 75% of PMQR containing isolates contained an ESBL. CTX-M types were dominant among both *E. coli* (72%) and *K. pneumoniae* (54%).

Table 1. Ciprofloxacin MICs and association with PMQR genes and ESBL production by survey year

Organism	Year[a]	Percent MIC ≥ 1	PMQR[b]	Ciprofloxacin MIC (mg/L) 0.5	1	2	≥ 4	Total	ESBL[c]
Escherichia coli	2008 (n=2,155)	4.9%	-	23	9	1	66	99	16 (16%)
			+		1	1	14	16 (14%)	13 (81%)
	2009 (n=1746)	9.5%	-	21	19	2	97	139	42 (30%)
			+	1	3	1	37	42 (23%)	31 (74%)
	2010 (n=2092)	6.0%	-	64	13	4	82	163	25 (15%)
			+		1		24	25 (13%)	18 (72%)
Klebsiella pneumoniae	2008 (n=475)	6.9%	-	6	13	2	3	24	6 (25%)
			+		6	3	5	14 (37%)	7 (50%)
	2009 (n=419)	10.3%	-	2	7	2	6	17	5 (29%)
			+	3	7	8	12	30 (63%)	27 (90%)
	2010 (n=458)	5.2%	-	13	11		9	33	6 (18%)
			+	2	2	1	1	6 (15%)	5 (83%)

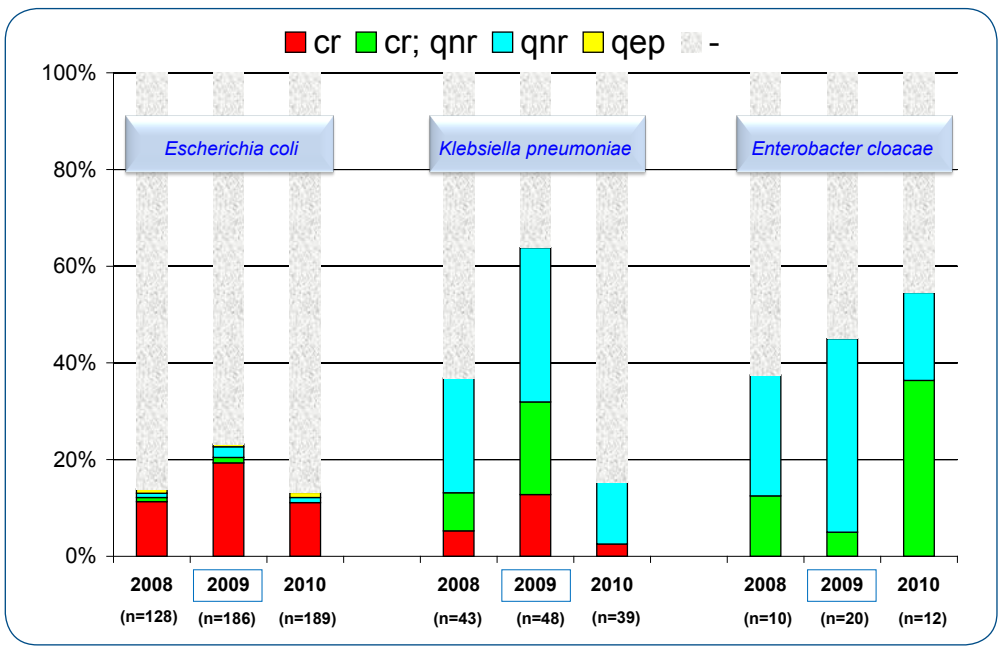
[a] Year (number tested); 2008, 2010 community UTI; 2009, hospital inpatients
[b] PMQR, Plasmid-mediated quinolone resistance gene, detected (+) or not detected (-)
[c] ESBL (TEM, SHV, CTX-M types) or plasmid-borne AmpC confirmed

Figure 2. Ciprofloxacin resistance vs study year[a]



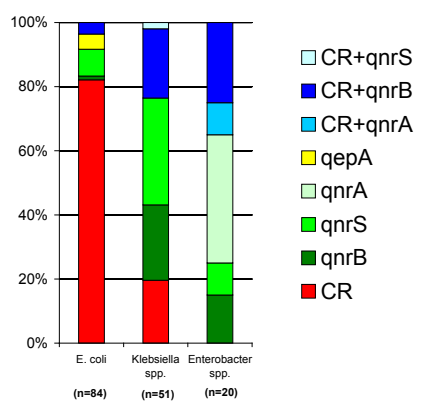
2008, 2010; outpatients with UTI; 2009; hospital inpatients

Figure 2. Proportion of PMQR genes vs year[a]



[a] 2008, 2010; outpatients with UTI; 2009; hospital inpatients (blue box). CR; *aac(6')-Ib-CR* variant

Figure 3. PMQR types/subtpye among bacterial species[a]



[a] CR; *aac(6')-Ib-CR* variant

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References

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CONCLUSIONS

- Although ciprofloxacin resistance is low in Australia, it is increasing
- Plasmid-mediated quinolone resistance is common, especially among hospitalised patients with *Klebsiella* spp.
- *aac(6')-Ib-cr* variant is the dominant PMQR among *E. coli*
- PMQR if often associated with ESBL enzymes

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