Original Article

Plasmid-mediated Quinolone Resistance Determinants in Urinary Isolates of *Escherichia coli* and *Klebsiella pneumoniae* in a Large Singapore Hospital

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Abstract

Introduction: At the time of the study, 3 plasmid-borne *qnr* determinants (*qnrA*, *qnrB* and *qnrS*) and 1 plasmid-borne aminoglycoside-modifying enzyme determinant that confers quinolone resistance (*aac(6')-Ib-cr*) had been described in the literature. Materials and Methods: We studied the prevalence of the 3 *qnr* determinants in a total of 117 nalidixic acid-resistant urinary isolates of *Klebsiella pneumoniae* (61 isolates) and *Escherichia coli* (56 isolates) using multiplex polymerase chain reaction (PCR). Further, a subset of the original strains (comprising 14 *E. coli* and 38 *K. pneumoniae*) showing reduced susceptibility to the aminoglycosides underwent PCR for *aac(6')-Ib*, followed by restriction digestion with *Bts*CI to detect the variant *aac(6')-Ib-cr*. Results: Twenty-eight of 61 (45.9%) *Klebsiella* isolates were found to possess at least 1 *qnr* determinant. Only 1/56 (1.8%) *E. coli* isolates were found to possess a *qnr* determinant. Two of the *Klebsiella* isolates possessed 2 *qnr* determinants each (*qnrB* and *qnrS*). The predominant determinant was *qnrB* (19 isolates). There were 11 isolates harbouring *qnrS*, and only 1 with *qnrA*. 1/14 (7.1%) *E. coli* and 35/38 *K. pneumoniae* (92.1%) were found to possess *aac(6')-Ib-cr*. There was pairwise association between each of *qnr*, *aac(6')-Ib-cr* and the presence of an extended-spectrum beta-lactamase. Conclusions: A high prevalence of plasmid-mediated quinolone resistance determinants [i.e., *qnrS*, *qnrB* and *aac(6')-Ib-cr*] was found in quinolone-resistant *K. pneumoniae* isolated in a large hospital in Singapore.

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Introduction

Plasmid-mediated quinolone resistance is a recently recognised phenomenon. The initial report of this transferable mode of resistance was in 1998 in Birmingham, Alabama, where the first *qnr* (quinolone-resistance) determinant was described on a conjugative plasmid in a *Klebsiella* isolate.[1] The *qnr* determinant codes for a 218 amino acid member of the pentapeptide repeat family of proteins, which are thought to protect against DNA gyrase inhibition. While *qnr* raised the minimal inhibitory concentrations (MICs) of quinolones in all strains, it was not sufficient to yield frank quinolone resistance without an ancillary mechanism like an accompanying outer membrane porin (OMP) loss.[2]

Subsequently, other workers described finding similar determinants in enterobacterial strains from Asia,[3-8] Europe,[9-13] Australia,[14] and the United States of America.[15] In all, a total of 3 distinct *qnr* determinants had been described prior to the date of the study, designated *qnrA*, *qnrS* , *qnrB* in order of discovery.

Following the discovery of *qnr*, a novel mechanism of transferable quinolone resistance was reported. The *-cr* variant of the aminoglycoside acetyltransferase enzyme AAC(6')-Ib conferred reduced susceptibility to ciprofloxacin by *N*-acetylation of its piperazinyl amine.[16]

Ours is a 1500-bedded acute tertiary care hospital. Quinolones are the second most commonly prescribed class of antimicrobials in the hospital with a usage of 40.73 DDD/100 patient days in 2006.[17] Moreover, resistance to quinolones is alarmingly common with nearly half of all *Klebsiella pneumoniae* and more than a third of *E. coli* testing resistant to the quinolones. We therefore embarked on a study to define the prevalence of the known mechanisms of transferable quinolone resistance among the nalidixic acid-resistant urinary isolates of *E. coli* and *Klebsiella* in our hospital.

Materials and Methods

Sequential isolates of *E. coli* and *K. pneumoniae* collected over 3 months (from August to October 2005) from urine

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cultures that tested resistant or intermediate to nalidixic acid by CLSI criteria) were archived and used for the study.[18] During this period, a total of 891 *E. coli* [of which 498 (55.9%) showed reduced susceptibility to nalidixic acid] and 483 *K. pneumoniae* [of which 297 (61.5%) showed reduced susceptibility to nalidixic acid] were isolated from urine specimens processed in our laboratory.

From the archived isolates, a consecutive selection of 56 isolates of *E. coli* and 61 isolates of *K. pneumoniae* were selected for the study. There were 3 patients who had specimens yielding 2 isolates each. These were distinct organisms, namely 1 *E. coli* and 1 *K. pneumoniae* in each case. Hence, there were no duplicate isolates tested.

MICs were determined to nalidixic acid and ciprofloxacin using Etest strips (AB Biodisk, Sweden) according to the recommendations of the manufacturer. Testing for extended-spectrum beta-lactamase (ESBLs) was performed using a CLSI recommended method.[18]

The *qnr* genes were assessed by means of a multiplex polymerase chain reaction (PCR) for the simultaneous detection of *qnrA, qnrB* and *qnrS* in a single reaction.[15] A representative selection of PCR amplicons (including 2 instances each of *qnrA, qnrB* and *qnrS*) were sequenced by dye-terminator chemistry using the respective primer pairs.

A subset of the original 117 strains was then selected for the detection of the variant aminoglycoside modifying enzyme determinant *aac(6')-Ib-cr.* Fourteen quinolone-resistant *E. coli* and 38 quinolone-resistant *K. pneumoniae* which showed reduced susceptibility to either or both of our routinely tested aminoglycosides, amikacin and gentamicin, were tested for *aac(6')-Ib* by PCR.[19] The amplicons were digested with *Bts*CI (an isoschizomer of *Bst*F5I).[19] The absence of restriction, suggesting the presence of the *-cr* variant encoding quinolone acetylating activity, was indicated by the persistence of a solitary 482bp band. Two representative amplicons were sequenced to confirm the *-cr* variant. Statistical analyses were carried out using the SPSS software version 14.0 for Windows.

Results and Discussion

All of the strains had high MICs of nalidixic acid (64-≥256 μg mL^{-1}). Twenty eight (45.9%) of the 61 *K. pneumoniae* and only 1 (1.8%) of the 56 *E. coli* were found to contain contain *qnr* type determinants. The breakdown of the determinants detected was as follows; *qnrA* : 1 isolate (*K. pneumoniae*), *qnrB* : 19 isolates (*K. pneumoniae*), *qnrS*: 11 isolates (10 *K. pneumoniae* and 1 *E. coli*). Two of the *Klebsiella* isolates possessed 2 *qnr* determinants each (*qnrB* and *qnrS*). Five of the 29 (17.2%) *qnr* positive strains showed low level resistance to ciprofloxacin (MICs of 4 to 8 μg mL^{-1}). In contrast, only 2 of the 88 (2.3%) *qnr* negative isolates had lower level resistance with MICs of 4 μg mL^{-1}. The remainder of the strains gave ciprofloxacin MICs of ≥32 μg mL^{-1}. Surprisingly, there was a significant negative association between the presence of *qnr* and the level of quinolone resistance (Kendall τ-c= -0.110, P = 0.043). An explanation for this finding is not immediately apparent. It is possible that strains with overt quinolone resistance (due to gyrase mutations and drug efflux, for instance) may have less selective pressure to acquire and/or keep *qnr*-bearing plasmids.

The prevalence of *qnr* amongst our nalidixic acid-resistant *Klebsiella* (45.9%) is far higher than the *qnr* prevalence (11.1%) in *Klebsiella* found in another study by Wang et al using isolates from Shanghai, China, that used selection

Table 1. Prevalence of *qnr* Determinant

qnr positive (n = 29)				*qnr* negative (n = 88)			
ESBL positive (n = 22)		ESBL negative (n = 7)		ESBL positive (n = 40)		ESBL negative (n = 48)	
E. coli	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*
0	22	1	6	14	26	41	7

Table 2. Prevalence of *aac-6'-Ib-cr* Determinant

aac-6'-Ib-cr positive (n = 36)				*aac-6'-Ib-cr* negative (n = 16)			
ESBL positive (n = 33)		ESBL negative (n = 3)		ESBL positive (n = 5)		ESBL negative (n = 11)	
E. coli	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*
0	33	1	2	2	3	11	0

criteria similar to ours.[3] In our survey, the most prevalent determinant was *qnrB*; *qnrS* was the second most prevalent and *qnrA* was the least prevalent with only 1 strain (1/29 = 3.4%) harbouring the determinant. A recent survey by Wu et al[8] among *Enterobacter cloacae* in a Taiwanese hospital found a distribution very similar to our own (predominance of *qnrB*, followed by *qnrS* and a minority of *qnrA*).

Of the 52 strains selected for detection of *aac(6')-Ib-cr*, 35 of 38 *K. pneumoniae* (92.1%) and 1 of 14 *E. coli* (7.1%) were positive for *aac(6')-Ib*. The amplicons were not susceptible to restriction digestion by *Bts*CI, implying that all 36 strains possessed the variant *aac(6')-Ib-cr*. This is in contrast to a study in the United States which found *aac(6')-Ib-cr* more common in *E. coli* than in *Klebsiella*. However, strain selection criteria were somewhat different (in the latter study inclusion was based on ceftazidime resistance without considering aminoglycoside resistance).[19] Of the 36 strains positive for *aac(6')-Ib-cr*, 2 (5.6%) showed low level resistance (MIC = 4 μg mL^{-1}) to ciprofloxacin. Amongst the 16 strains that were negative for the aminoglycoside resistance trait, 1 (6.3%) had a ciprofloxacin MIC of 4 μg mL^{-1}. No association was found between the presence of *aac(6')-Ib-cr* and quantitative quinolone resistance (Kendall τ-c= 0.006, $P = 0.923$). This may indicate either a lack of power (small sample size) or the greater relative importance of other determinants in quinolone resistance.

Among the *aac(6')-Ib-cr* positive strains, there were 16 (44.4%) which were also *qnr* positive. Of the 16 strains that tested negative for the aminoglycoside modifying enzyme, there were 2 *qnr* positive strains (12.5%). Association between the 2 determinants was significant at the 5% level (16/36 vs. 2/16, 2-tailed Fisher's exact test, $P = 0.031$). The combination of *qnr* and *aac(6')-Ib-cr* had no discernible additive effect on quinolone susceptibility.

Twenty-two of the 29 (75.9%) *qnr* positive strains possessed an ESBL by phenotypic testing. This is in contrast to 40 of the remaining 88 (45.5%) *qnr* negative strains which possessed an ESBL. (22/29 vs. 40/88; 2-tailed Fisher's exact test $P = 0.005$). Thirty-three of the 36 (91.7%) strains positive for *aac(6')-Ib-cr* also possessed an ESBL, in contrast to 5 ESBL-producers out of the 16 (31.3%) that tested negative for the aminoglycoside modifying enzyme (33/36 vs. 5/16; 2-tailed Fisher's exact test $P < 0.001$). The association between ESBL-producers and *qnr* has been well documented but is less well described with *aac(6')-Ib-cr*.[2,4] It cannot be definitively determined from our data whether any of the *qnr, aac(6')-Ib-cr* or ESBL-determinants were co-located on the same plasmid, but the pairwise association between the determinants is consistent with the possibility.

A summary of the prevalence of the determinants and their association with ESBLs is presented in Tables 1 and 2.

While most of the 29 *qnr* positive strains came from our hospital inpatients, 9 were derived from other sources (3 from our outpatient clinics and specialty outpatient centres, 3 from a Community Hospital, 2 from different government run clinics and 1 from a volunteer-staffed Nursing Home). The presence of these determinants in the outpatient and chronic-care settings is worrisome, because of the potential for spread of the plasmid in a background of high oral quinolone usage. Further work should be carried out to track the evolution of these determinants in our local and regional population.

It should be noted that 2 new *qnr* determinants (*qnrC* and *qnrD*), and a novel plasmid-mediated quinolone efflux pump (QepA) have been described since the completion of our study.[20-23]

The GenBank/EMBL/DDBJ accession numbers for the partial gene sequences of *qnrB*, *qnrS* and *qnrA* in *K. pneumoniae* and *E. coli* are EF421178-83. The GenBank/EMBL/DDBJ accession numbers for the partial gene sequences of *aac(6')-Ib-cr* in *K. pneumoniae* are EF542812-3.

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