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Risk factors and clinical characteristics of patients with *qnr*-positive *Klebsiella pneumoniae* bacteraemia

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Objectives: Plasmid-mediated quinolone resistance (PMQR) caused by *qnr* genes has been known for 15 years. Information about global distribution and prevalence of *qnr* genes is abundant, but clinical information concerning infections produced by these isolates and risk factors for their acquisition is limited.

Methods: *Klebsiella pneumoniae* blood isolates ($n=227$) from a 1 year prospective cohort of patients in Taiwan were studied. MICs of quinolones were determined for all isolates, and multiplex PCR for the presence of PMQR genes and DNA gyrase mutations was applied to all 24 isolates with ciprofloxacin MICs $\geq$0.12 mg/L and a control group of 72 isolates with MICs $\leq$0.06 mg/L.

Results: All *qnr* isolates were in the group with ciprofloxacin MICs $\geq$0.12 mg/L, constituting 9.4% of tested isolates and 3.9% (*qnrB* 2.6% and *qnrS* 1.3%) of total isolates. *aac(6′)-Ib-cr* and *qepA* were not found. Risk factors for *qnr* included nosocomial infection, bedridden status, surgery within 3 months, non-K1/K2 serotypes and prior antimicrobial use. Ciprofloxacin MIC $\geq$0.12 mg/L was associated with prior quinolone use; in contrast, prior cephalosporin use was more closely linked to the presence of *qnr*. Fourteen-day mortality was similar in patients infected with *qnr*-positive versus *qnr*-negative isolates, but there was a trend for increased in-hospital mortality in patients infected with *qnr*-positive isolates.

Conclusions: In *K. pneumoniae* blood isolates collected at a hospital in Taiwan, the overall prevalence of *qnr* genes was 3.9%. Prior quinolone use was linked to increased ciprofloxacin MIC, but not with the prevalence of *qnr*, which was most strongly linked to exposure to other antimicrobials, especially cephalosporins.

Keywords: *K. pneumoniae*, PMQR, resistance

Introduction

Quinolones have been used clinically for four decades, and resistance to them has increased over time, mostly due to mutations altering DNA gyrase and the regulation of expression of efflux pumps.[1] Plasmid-mediated quinolone resistance (PMQR) caused by *qnr* was first reported in 1998,[2] and further studies showed that various *qnr* genes were distributed in multiple species of Enterobacteriaceae in many countries, although with differing patterns.[3] Furthermore, other mechanisms of PMQR, such as a quinolone-modifying enzyme, encoded by *aac(6′)-Ib-cr*, and plasmid-encoded efflux pumps, including those encoded by *qepA* and *oqxAB*, were subsequently found.[3–6]

In spite of the abundant information on the global distribution and prevalence of *qnr* and other PMQR genes, clinical information, including the risk factors for acquiring *qnr*-harbouring isolates, are limited.[7,8] Some studies have demonstrated high percentages of co-existence of extended-spectrum β-lactamases (ESBLs) among these isolates.[8,9] In order to delineate the risk factors for *qnr* and its clinical presentations, we used a collection of *Klebsiella pneumoniae* isolates from a previous 1 year prospective cohort of patients with bacteraemia.[10] MICs for all isolates of several quinolones were determined, and an extensive survey for PMQR genes and gyrase mutations was performed.

Methods

Demographic information on patients, bacterial isolates and determination of MICs

A prospective study of patients with *K. pneumoniae* bloodstream infection was performed from 1 January to 31 December 2007 at the Far Eastern Memorial Hospital, a tertiary 1050 bed hospital in northern Taiwan. Identification of *K. pneumoniae* was based on colony morphology and traditional biochemical reactions.[11] Each patient was included only once and patients aged <18 years were excluded. The following data were recorded for each

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patient: age, sex, underlying illness, location of care in the hospital, serum albumin and creatinine levels, past antibiotic use within 1 year and severity of bacteraemia assessed by the Pittsburgh bacteraemia score.[12] Malignancy in inactive status was not included as an underlying illness. MICs for all available bacterial isolates of ciprofloxacin, levofloxacin, moxifloxacin, nemonoxacin and gatifloxacin were determined by the broth microdilution method.[13] *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Susceptibilities of antimicrobial agents were performed with the disc diffusion method according to the CLSI.[14] ESBL phenotype was confirmed by double disc methods. Bacterial serotypes K1 and K2 were determined by *cps* genotyping.[15]

PCR and sequencing for possible mechanisms for *K. pneumoniae* isolates with elevated MICs

Since PMQR genes are associated with a ciprofloxacin MIC ≥0.12 mg/L,[3] all isolates with ciprofloxacin MICs >0.06 mg/L were selected for screening by PCRs for quinolone resistance genes, including *gyrA*, *parC*, *qnrA*, *qnrB*, *qnrC*, *qnrS*, *aac(6′)-Ib* and *qepA*. Seventy-two isolates with MICs ≤0.06 mg/L were selected randomly as controls (the cases were listed by time of bacteraemia onset, and every third case was picked as a control, with three controls for each case with reduced quinolone susceptibility). In brief, colonies were suspended in 50 μL of water in a microcentrifuge tube and boiled to prepare DNA templates for PCR. Pairs of primers to amplify internal fragments were designed from sequences in the NCBI web site (http://www.ncbi.nlm.nih.gov/) and from previous research (Table 1).[16] Screening of the PMQR determinants was carried out by two sets of multiplex PCR amplification, one for *qnrA*, *qnrB*, *qnrC* and *qnrS* and the other for *aac(6′)-Ib* and *qepA*. The presence of *oqxAB* was not studied because most *K. pneumoniae* carry a chromosomal copy of the gene.[17]

In each multiplex PCR, all of the primers were added to the template DNA and PCR SuperMix high-fidelity polymerase (Invitrogen, Carlsbad, CA, USA). Clinical isolates that had previously been confirmed to carry the *qnr* genes, *aac(6′)-Ib* and *qepA* were used as positive controls. The PCR conditions used for *qnr* were as follows: initial denaturation at 94°C for 2 min, followed by 32 cycles of amplification at 94°C for 45 s, annealing at 50°C for 45 s and extension at 72°C for 60 s. The final extension step was at 72°C for 3 min. The PCR conditions used for *qepA* and *aac(6′)-Ib-cr* were as follows: initial denaturation at 94°C for 2 min, followed by 34 cycles of amplification at 94°C for 45 s, annealing at 57°C for 45 s and extension at 68°C for 60 s. The final extension step was at 68°C for 10 min. Amplification products were identified by their sizes after electrophoresis on 1.8% agarose gels at 130 V for 30 min and staining with ethidium bromide. Positive results for *qnr* genes were confirmed by direct sequencing of PCR products. All PCR products positive for *aac(6′)-Ib* were further analysed by digestion with BtsCI (New England Biolabs, Ipswich, MA, USA) to identify *aac(6′)-Ib-cr*, which lacks the BtsCI restriction site present in the wild-type gene.[16] The wild-type *aac(6′)-Ib* PCR product yielded 210 and 272 bp fragments after restriction.

PCR amplifications of the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* were carried out using the primers shown in Table 1. Purified PCR products were sequenced on both strands, and then QRDR DNA sequences were compared with the sequences of *K. pneumoniae* (GenBank accession numbers were AF052258 for *gyrA* and AF303641 for *parC*).

Table 1. Primers used in this study

Gene	Primer	Sequence (5′→3′)	Size of the amplified product (bp)
qnrA	qnrAF	ATTTCTCACGCCAGGATTTG	516
	qnrAR	GATCGGCAAAGGTTAGGTCA	
qnrB	qnrBF	GATCGTGAAAGCCAGAAAGG	476
	qnrBR2	ATGAGCAACGATGCCTGGTA	
qnrC	qnrCF	GGGTTGTACATTTATTGAATCG	307
	qnrCR	CACCTACCCATTTATTTTCA	
qnrS	qnrSF	GCAAGTTCATTGAACAGGGT	428
	qnrSR	TCTAAACCGTCGAGTTCGGCG	
aac(6′)-Ib	aacIbF	TTGCGATGCTCTATGAGTGGCTA	482
	aacIbR	CTCGAATGCCTGGCGTGTTT	
qepA	qepAF	AACTGCTTGAGCCCGTAGAT	596
	qepAR	GTCTACGCCATGGACCTCAC	
gyrA	gyrAF	AAATCTGCCCGTGTCGTTGGT	344
	gyrAR	GCCATACCTACGGCGATACC	
parC	parCF	CTGAATGCCAGCGCCAAATT	168
	parCR	GCGAACGATTTCGGATCGTC	

These primers were adapted from the protocol of Kim *et al*.[16]

Statistical analysis

The clinical characteristics of patients with ciprofloxacin MICs ≥0.12 mg/L were compared with those of patients with ciprofloxacin MICs ≤0.06 mg/L. These groups were chosen because strains with PMQR or gyrase mutation generally have ciprofloxacin MICs ≥0.12 mg/L. A similar comparison was performed between isolates with and without *qnr*. The Mann–Whitney *U* method was used for continuous variables and the χ^2 test or Fisher's exact test for categorical variables. Means and standard deviations were calculated for continuous variables. Percentages were used for categorical variables. Binary logistic regression was performed for risk factors for *qnr*. Data were analysed with SPSS software for Windows (Release 15.0; SPSS, Chicago, IL, USA).

Results

Among 231 patients with *K. pneumoniae* bacteraemia from 1 January to 31 December 2007, 227 isolates were still available. MICs of five quinolones for these isolates were determined (Table 2). Overall, ciprofloxacin and gemifloxacin had the lowest MICs, and the MICs of nemonoxacin were highest. Comparison between patients with ciprofloxacin MICs ≥0.12 mg/L ($n=24$) and MICs ≤0.06 mg/L ($n=203$) was performed (Table 3). In the MIC ≥0.12 mg/L group, patients were significantly older and had higher percentages of bedridden status, receiving surgery within 3 months and quinolone exposure within the previous year. All four isolates with an ESBL phenotype confirmed by the double disc method were in the MIC ≥0.12 mg/L group. The Pittsburgh bacteraemia scores, sites of infection, mortality rates, bacterial serotypes and exposure to antibiotics other than quinolones were not statistically different between the two groups.

Table 2. MICs (mg/L) of five quinolones for 227 *K. pneumoniae* isolates

	Range	MIC_{50}	MIC_{90}
Ciprofloxacin	0.015 to ≥32	0.06	0.12
Levofloxacin	0.03 to ≥32	0.12	0.25
Nemonoxacin	0.03 to ≥32	0.25	1
Gemifloxacin	0.03 to ≥32	0.06	0.12
Moxifloxacin	0.03 to ≥32	0.12	0.5

Table 3. Demographic information on 227 patients with *K. pneumoniae* bacteraemia based on ciprofloxacin MIC

	MIC ≥0.12 mg/L (*n*=24), *n* (%) or mean ± SD	MIC ≤0.06 mg/L (*n*=203), *n* (%) or mean ± SD	*P* value
Patient location (place where bacteraemia was detected)			0.07
emergency department	9 (37.5)	126 (62.1)	
hospital-acquired infection			
ward	10 (41.7)	50 (24.6)	
intensive care unit	5 (20.8)	27 (13.3)	
Age (years)	**68.4 ± 3.1**	**60.7 ± 1.1**	**0.02**
Sex (male)	12 (50.0)	135 (66.5)	0.11
Pittsburgh bacteraemia score	4.5 ± 1.0	4.2 ± 0.3	0.77
Time to positivity of culture (hours)[a]	13.7 ± 2.7	13.1 ± 1.1	0.99
Underlying illness/condition			
diabetes mellitus	11 (45.8)	77 (37.9)	0.45
cirrhosis	1 (4.2)	32 (15.8)	0.13
end-stage renal disease	2 (8.3)	8 (3.9)	0.29
stroke	5 (20.8)	23 (11.3)	0.18
chronic heart disease	3 (12.5)	42 (20.7)	0.43
bedridden status	**10 (41.7)**	**25 (12.3)**	**<0.001**
active malignancy	5 (20.8)	34 (16.7)	0.62
surgery within 3 months	**10 (41.7)**	**47 (23.2)**	**0.048**
Infection focus			
primary bacteraemia	5 (20.8)	57 (28.1)	0.45
pneumonia	7 (29.2)	34 (16.7)	0.14
urinary tract infection	5 (20.8)	31 (15.3)	0.48
biliary tract infection	3 (12.5)	28 (13.8)	1.00
liver abscess	2 (8.3)	28 (13.8)	0.75
intra-abdominal infection	1 (4.2)	13 (6.4)	1.00
catheter-related bacteraemia	1 (4.2)	5 (2.5)	0.49
soft tissue infection	0 (0)	3 (1.5)	1.00
meningitis	0 (0)	3 (1.5)	1.00
other	0 (0)	1 (0.5)	1.00
Outcome			
14 day mortality	6 (25.0)	44 (21.7)	0.71
in-hospital mortality	10 (41.7)	63 (31.0)	0.29
Bacterial serotype			
K1	2 (8.3)	39 (19.2)	0.27
K2	1 (4.2)	36 (17.7)	0.39
non-K1/K2	21 (87.5)	128 (63.1)	0.11
ESBL phenotype	**4 (16.7)**	**0 (0)**	**<0.001**
Quinolone use within 1 year	**6 (25.0)**	**13 (6.4)**	**0.002**
Quinolone use within 270 days	**6 (25.0)**	**12 (5.9)**	**0.001**
Quinolone use within 180 days	**6 (25.0)**	**10 (4.9)**	**<0.001**
Quinolone use within 90 days	**6 (25.0)**	**6 (3.0)**	**<0.001**
Other antibiotic use within 1 year	12 (50.0)	81 (39.9)	0.34
Other antibiotic use within 270 days	12 (50.0)	78 (38.4)	0.27
Other antibiotic use within 180 days	10 (41.7)	74 (36.5)	0.62
Other antibiotic use within 90 days	9 (37.5)	66 (32.5)	0.62

Categories with *P* values <0.05 appear in bold.

[a]Time to positivity of culture was acquired using the BACTEC 9240 automated detection blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA), which defines time to positivity as the time from the start of incubation to the start of the alert signal indicating positive blood culture.

Table 4. Summary of quinolone resistance mutations and *qnr* genes found in isolates with ciprofloxacin MICs ≥0.12 mg/L

No.	Ciprofloxacin MIC (mg/L)	ESBL	*qnrB*	*qnrS*	*gyrA* mutation	*parC* mutation
50	0.12	no	no	no	nil	nil
61	0.12	no	no	no	nil	nil
125	0.12	no	no	no	nil	nil
172	0.12	no	no	no	nil	nil
68	0.25	no	no	no	nil	nil
93	0.25	no	no	no	nil	nil
134	0.25	no	no	no	nil	nil
46	0.25	no	yes	no	nil	nil
55	0.5	no	no	no	nil	nil
23	0.5	no	yes	no	nil	nil
34	0.5	no	no	no	TCC→TAC, Ser83→Tyr	nil
191	1	no	no	no	nil	nil
189	1	no	no	yes	nil	nil
154	1	no	no	no	TCC→TAC, Ser83→Tyr	nil
208	4	no	yes	no	TCC→TAC, Ser83→Tyr	nil
103	16	no	no	yes	TCC→TTC, Ser83→Phe	nil
128	16	no	yes	no	TCC→TTC, Ser83→Phe	nil
44	16	yes	no	no	TCC→TTC, Ser83→Phe	nil
35	32	no	no	no	TCC→ATC, Ser83→Phe	AGC→ATC, Ser80→Ile
9	64	yes	no	yes	TCC→TTC, Ser83→Phe	nil
45	64	no	yes	no	TCC→ATC, Ser83→Phe	GAA→AAA, Glu84→Lys
21	64	yes	no	no	TCC→ATC, Ser83→Phe	AGC→ATC, Ser80→Ile
173	64	no	no	no	TCC→ATC, Ser83→Phe	AGC→ATC, Ser80→Ile
104	>128	yes	yes	no	TCC→ATC, Ser83→Phe	AGC→ATC, Ser80→Ile

Multiplex PCR for PMQR genes was performed in 96 isolates (24 with ciprofloxacin MICs ≥0.12 mg/L and 72 with ciprofloxacin MICs ≤0.06 mg/L). Nine isolates were found to carry *qnr*, including six *qnrB* and three *qnrS*; all of them were in the MIC ≥0.12 mg/L group. No *qnrA* or *qnrC* was identified. Thus, we detected *qnr* genes in 9.4% of the 96 isolates tested directly. Since all *qnr*-positive isolates were found in the higher MIC group, the lowest value of which is the lowest known MIC attributable to *qnr* genes alone and all members of which were tested, it is reasonable to infer that the untested members of the lower MIC group were also negative for *qnr* genes, thus making the overall prevalence of *qnr* genes (9/227) 3.9%. Two isolates were found to carry *aac(6′)-Ib*, but neither of them was *aac(6′)-Ib-cr*. No isolate was positive for *qepA*. PCR amplifications of QRDRs (*gyrA* and *parC*) were performed for the 24 isolates with ciprofloxacin MICs ≥0.12 mg/L, and half of them carried gyrase mutations. Seven isolates had a *gyrA* mutation alone, and five isolates had *gyrA* and *parC* mutations together. No *parC* mutation alone was detected. The ciprofloxacin MICs and molecular mechanisms of quinolone resistance of these isolates are summarized in Table 4.

Clinical information for patients with isolates carrying *qnr* was compared with that for patients without *qnr* (Table 5). Patients with *qnr*-positive isolates had a higher percentage of nosocomial infections, bedridden status and surgery within 3 months. No K1 or K2 isolates carried *qnr*. The percentages of ciprofloxacin MIC ≥1 mg/L, ESBL phenotype and gyrase mutation were higher in the *qnr* group. Prior exposure to antimicrobials other than quinolones was significantly higher in the *qnr* group, for any timepoints within 1 year. In contrast, in spite of a trend of increased quinolone exposure in the *qnr* group, only quinolone use within 90 days was statistically different between the two groups. The relationships of prior antimicrobial exposure and *qnr* were further analysed with multivariate analysis. After adjusting for age, sex, patient location, bedridden status, surgery within 3 months and bacterial serotypes, there was a trend for non-quinolone antimicrobial exposure linked to the presence of *qnr* that did not quite reach statistical significance (OR 8.64, 95% CI 0.83–90.18, $P=0.07$). In contrast, under the same conditions, quinolone exposure showed no significant association or trend with the presence of *qnr* (OR 2.54, 95% CI 0.39–16.79, $P=0.33$).

To evaluate the differences between gyrase mutations and isolates carrying *qnr*, we compared the 24 isolates with ciprofloxacin MICs ≥0.12 mg/L (Table 6). Gyrase mutation was significantly related to ciprofloxacin MIC ≥1 mg/L and associated with a higher in-hospital mortality rate ($P=0.04$); in contrast, *qnr* resistance was associated with higher prior antimicrobial exposure of non-quinolones, particularly cephalosporins. Prior quinolone exposure was not statistically significant. Gyrase mutation or *qnr* was each associated with cefazolin, gentamicin and ciprofloxacin resistance determined by disc methods, but ceftriaxone and aztreonam resistances were only significantly higher in isolates with gyrase mutations. There were six isolates carrying both *qnr* and gyrase mutations. Compared with the remaining 18 isolates, these isolates were associated with a higher in-hospital mortality rate (83% versus 28%, $P=0.05$). The mortality rates were 0% for *qnr* alone (0/3), 22% for isolates without known resistance genes (2/9), 50% for gyrase mutation alone (3/6) and 83% for both *qnr* and gyrase mutations (5/6).

Table 5. Comparison of 96 *K. pneumoniae* isolates with ciprofloxacin MICs ≥0.12 mg/L, with and without *qnr*

	Positive for *qnr* (*n*=9), *n* (%) or mean ± SD	Negative for *qnr* (*n*=87), *n* (%) or mean ± SD	*P* value
Patient location (place where bacteraemia was detected)			**0.01**
emergency department	**1 (11.1)**	**50 (57.5)**	
hospital-acquired infection			
ward	**5 (55.6)**	**30 (34.5)**	
intensive care unit	**3 (33.3)**	**7 (8.0)**	
Age (years)	65.9 ± 3.9	60.5 ± 1.8	0.11
Sex (male)	5 (55.6)	54 (62.1)	0.73
Pittsburgh bacteraemia score	4.3 ± 1.4	3.7 ± 0.5	0.65
Time to positivity of culture (hours)	9.6 ± 1.1	15.5 ± 2.4	0.17
Underlying illness/condition			
diabetes mellitus	3 (33.3)	39 (44.8)	0.73
cirrhosis	1 (11.1)	11 (12.6)	1.00
end-stage renal disease	1 (11.1)	3 (3.4)	0.33
stroke	3 (33.3)	9 (10.3)	0.08
chronic heart disease	1 (11.1)	11 (12.6)	1.00
bedridden status	**4 (44.4)**	**11 (12.6)**	**0.03**
active malignancy	3 (33.3)	14 (16.1)	0.20
surgery within 3 months[a]	**7 (77.8)**	**18 (20.7)**	**0.001**
Infection focus			
primary bacteraemia	2 (22.2)	22 (25.3)	1.00
biliary tract infection	0 (0)	19 (21.8)	0.20
urinary tract infection	3 (33.3)	10 (11.5)	0.10
pneumonia	3 (33.3)	10 (11.5)	0.10
liver abscess	0 (0)	14 (16.1)	0.35
intra-abdominal infection	0 (0)	8 (9.2)	1.00
catheter-related bacteraemia	1 (11.1)	3 (3.4)	0.33
soft tissue infection	0 (0)	2 (2.3)	1.00
Outcome			
14 day mortality	2 (22.2)	18 (20.7)	0.10
in-hospital mortality	5 (55.5)	26 (29.9)	0.14
Bacterial serotype			
K1	0 (0)	18 (20.7)	0.20
K2	0 (0)	14 (16.1)	0.35
non-K1/K2	**9 (100.0)**	**55 (63.2)**	**0.03**
Ciprofloxacin MIC ≥1 mg/L	**6 (66.7)**	**5 (5.7)**	**<0.001**
ESBL phenotype	**2 (22.2)**	**2 (2.3)**	**0.04**
Gyrase mutation	**6 (66.7)**	**6 (6.9)**	**<0.001**
Quinolone use within 1 year	3 (33.3)	8 (9.2)	0.07
Quinolone use within 270 days	3 (33.3)	8 (9.2)	0.07
Quinolone use within 180 days	3 (33.3)	8 (9.2)	0.07
Quinolone use within 90 days	**3 (33.3)**	**5 (5.7)**	**0.03**
Other antibiotic use within 1 year	**8 (88.9)**	**29 (33.3)**	**0.002**
Other antibiotic use within 270 days	**8 (88.9)**	**28 (32.2)**	**0.001**
Other antibiotic use within 180 days	**8 (88.9)**	**25 (28.7)**	**0.001**
Other antibiotic use within 90 days	**7 (77.8)**	**21 (24.1)**	**0.002**

Categories with *P* values <0.05 appear in bold.
[a]In the *qnr* group, surgery within 3 months included two urological interventions, two tracheostomies, one neurological surgery, one port-A removal and one wound debridement.

Table 6. Comparison of antimicrobial exposures and susceptibilities of 24 *K. pneumoniae* isolates with ciprofloxacin MICs ≥0.12 mg/L with or without *qnr* or gyrase mutation

	Positive for *qnr* (*n*=9), *n* (%)	Negative for *qnr* (*n*=15), *n* (%)	*P* value	Positive for gyrase mutation (*n*=12), *n* (%)	Negative for gyrase mutation (*n*=12), *n* (%)	*P* value
Ciprofloxacin MIC ≥1 mg/L	7 (77.8)	6 (40.0)	0.11	**11 (91.7)**	**2 (16.7)**	**0.001**
14 day mortality	2 (22.2)	3 (20.0)	1.00	3 (25.0)	2 (16.7)	1.00
In-hospital mortality	5 (55.6)	5 (33.3)	0.40	**8 (66.7)**	**2 (16.7)**	**0.04**
Quinolone use within 1 year	3 (33.3)	3 (20.0)	0.64	4 (33.3)	2 (16.7)	0.64
Quinolone use within 270 days	3 (33.3)	3 (20.0)	0.64	4 (33.3)	2 (16.7)	0.64
Quinolone use within 180 days	3 (33.3)	3 (20.0)	0.64	4 (33.3)	2 (16.7)	0.64
Quinolone use within 90 days	3 (33.3)	3 (20.0)	0.64	4 (33.3)	2 (16.7)	0.64
ESBL phenotype	2 (22.2)	2 (13.3)	0.62	4 (33.3)	0 (0)	0.09
Other antibiotic use within 1 year	**8 (88.9)**	**4 (26.7)**	**0.009**	7 (58.3)	5 (41.7)	0.41
Other antibiotic use within 270 days	**8 (88.9)**	**4 (26.7)**	**0.009**	7 (58.3)	5 (41.7)	0.68
Other antibiotic use within 180 days	**8 (88.9)**	**2 (13.3)**	**<0.001**	5 (41.7)	5 (41.7)	1.00
Other antibiotic use within 90 days	**7 (77.8)**	**2 (13.3)**	**0.003**	5 (41.7)	4 (33.3)	1.00
Antimicrobial exposed other than quinolones						
cephalosporin	**6 (66.7)**	**4 (26.7)**	**0.04**	6 (50.0)	4 (33.3)	0.68
penicillin	2 (22.2)	0 (0)	0.13	1 (8.3)	1 (8.3)	1.00
aminoglycoside	1 (11.1)	0 (0)	0.38	0 (0)	1 (8.3)	1.00
carbapenem	1 (11.1)	1 (6.7)	1.00	2 (16.7)	0 (0)	0.48
glycopeptide	1 (11.1)	1 (6.7)	1.00	2 (16.7)	0 (0)	0.48
fluconazole	2 (22.2)	1 (6.7)	0.53	3 (25.0)	0 (0)	0.22
Antimicrobial resistance (disc diffusion method)						
cefazolin	**6 (66.7)**	**4 (26.7)**	**0.04**	**8 (66.7)**	**2 (16.7)**	**0.04**
ceftriaxone	3 (33.3)	2 (13.3)	0.33	**5 (41.7)**	**0 (0)**	**0.04**
cefepime	2 (22.2)	2 (13.3)	0.62	4 (33.3)	0 (0)	0.09
gentamicin	**6 (66.7)**	**4 (26.7)**	**0.04**	**9 (75.0)**	**1 (8.3)**	**0.003**
amikacin	2 (22.2)	1 (6.7)	0.53	3 (25.0)	0 (0)	0.22
ampicillin/sulbactam	6 (66.7)	6 (40.0)	0.40	8 (66.7)	4 (33.3)	0.22
piperacillin/tazobactam	4 (44.4)	3 (20.0)	0.36	6 (50.0)	1 (8.3)	0.07
ciprofloxacin	**8 (88.9)**	**4 (26.7)**	**0.009**	**10 (83.3)**	**2 (16.7)**	**0.003**
aztreonam	3 (33.3)	3 (20.0)	0.64	**6 (50.0)**	**0 (0)**	**0.01**
trimethoprim/sulfamethoxazole	7 (77.8)	10 (66.7)	0.67	11 (91.7)	6 (50.0)	0.07
ertapenem	0 (0)	0 (0)	—	0 (0)	0 (0)	—

Categories with *P* values <0.05 appear in bold.

Discussion

Prior quinolone use is considered one of the major driving forces of quinolone resistance.[18–22] In this study, we show that in a cohort of patients with *K. pneumoniae* bacteraemia, elevated ciprofloxacin MIC was correlated with prior quinolone exposure. Moreover, when we focused on isolates with higher MICs due to gyrase mutation or the presence of *qnr*, we were surprised to find that the presence of *qnr* is strongly correlated with cephalosporin and other non-quinolone antimicrobial exposure. Although the occurrence of *qnr* genes was relatively low in the population studied, their association with prior exposure to non-quinolone antimicrobials within 90 days to 1 year remained statistically strong (Table 5). In contrast, a weaker association of *qnr* with prior quinolone exposure was only seen at 90 days. The relative strengths of these associations, however, may have been affected by the almost 4-fold greater exposure to non-quinolones than quinolones (39% versus 11%) in the group analysed. In Taiwan, antimicrobials including quinolones can only be obtained by prescription. In a report analysing risk factors for *qnr* in Enterobacteriaceae, Chong *et al*.[8] found that prior aminoglycoside and trimethoprim/sulfamethoxazole use, but not quinolone use, was associated with the presence of *qnr*. Both observations could be related to the frequent occurrence of *qnr* on plasmids containing multiple other resistance genes, including those for ESBLs that provide resistance to cephalosporins.[8,9]

The prevalence rates of *qnrB* and *qnrS* in this study were both somewhat lower (2.6% and 1.3%, respectively) than those in a previous report (5.9% and 2.8%, respectively) among *K. pneumoniae* blood isolates in Taiwan.[9] In that report, the prevalence of *qnrB4* isolates increased from 0% in 1999 to 7.6% in 2005, probably due to horizontal transfer of *qnr* genes.[9] In one Korean 9 year study[15] and another study in Israel,[23] there were also trends of increasing *qnr* among Enterobacteriaceae over time. Community-acquired

infection constituted more than half of the cases in our study, and none of the K1 or K2 isolates, which were considered more likely as community strains in previous study,[24] was found to carry *qnr*. This circumstance might explain the low prevalence of *qnr* in this cohort and support the idea that *qnr* is selected in patients with healthcare exposure and the use of antimicrobials.

There has been limited clinical information about patients with *qnr*-harbouring isolates. In a comparison of *qnr*-positive *K. pneumoniae* and *Enterobacter* spp. with *qnr*-negative isolates,[8] malignancy was a risk factor for *qnr*, and the use of any antibiotics within 30 days and ESBL production were more common in the *qnr*-positive group. In our study, the risk factors for *qnr* included nosocomial infection, bedridden status, surgery within 3 months, non-K1/K2 serotypes and prior antimicrobial use. The differences between the two studies may be explained, at least in part, by the different populations studied, as community-acquired infection accounted for only 15.4% of the earlier cohort, in contrast to community *K. pneumoniae* infection, which is endemic in Taiwan.[24] Both studies, however, showed that prior antimicrobial use was an important risk factor.

In patients infected with *qnr*-positive isolates, the 14 day mortality rate was similar to that for *qnr*-negative isolates, and the in-hospital mortality rate was somewhat higher but not significantly different, consistent with an earlier report.[8] In contrast, in-hospital mortality was significantly higher in the gyrase mutation group. In this study, only 20 patients received empirical quinolone (ciprofloxacin, levofloxacin or moxifloxacin) therapy, and 7 patients received quinolone as definitive therapy. Among patients who received empirical quinolone therapy, only two had ciprofloxacin MICs $\geq$0.12 mg/L (MICs of 64 and 0.5 mg/L), and both of them died in spite of combination therapy with quinolone and β-lactam (one with pneumonia and one with primary bacteraemia). In the same group, five patients died in spite of having infections caused by isolates with low ciprofloxacin MIC ($\leq$0.06 mg/L). All patients who received quinolone as definitive therapy survived. Thus, the correlation of gyrase mutation and mortality would likely reflect the underlying conditions of these patients.

There are several limitations of this study. First, it was a 1 year study from a single hospital in an area where community-onset *K. pneumoniae* infection was endemic. Geographical differences in *K. pneumoniae* infection can be anticipated.[24] Second, the information on prior antimicrobial exposure was obtained from medical records at our hospital. Thus, we cannot exclude the possibility that some patients might have received offsite antimicrobial exposure prior to the bacteraemic episode. Nonetheless, we included all antimicrobials used within 1 year to maximize the probable effects of prior antimicrobial exposure. Third, we screened only *qnrA*, *qnrB*, *qnrC* and *qnrS*. *qnrD* was not included in the screen, but it has not previously been reported in *K. pneumoniae*.

In conclusion, in this 1 year study the overall prevalence of *qnr* genes was 3.9%. Risk factors for *qnr* alleles included nosocomial infections, bedridden status, surgery within 3 months, non-K1/K2 serotypes and prior antimicrobial use. Most notably, prior cephalosporin use was more closely linked to the presence of *qnr* than to prior quinolone use, suggesting that other resistance genes that broaden the resistance phenotypes of *qnr*-containing strains may have a greater effect on *qnr* prevalence than the low-level quinolone resistance that *qnr* itself provides.

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Transparency declarations

None to declare.

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