Emergence of Qnr determinants in human \textit{Salmonella} isolates in Taiwan

Jiunn-Jong Wu$^1$, Wen-Chien Ko$^2$, Chien-Shun Chiou$^3$, Hung-Mo Chen$^4$, Li-Ron Wang$^{1,4}$ and Jing-Jou Yan$^{4*}$

$^1$Department of Medical Laboratory Science and Biotechnology, National Cheng Kung University Medical College, Tainan 70101, Taiwan; $^2$Department of Internal Medicine, National Cheng Kung University Medical College and Hospital, Tainan 70428, Taiwan; $^3$The Central Branch Office, Center for Disease Control, Taichung 40855, Taiwan; $^4$Department of Pathology, National Cheng Kung University Medical College and Hospital, Tainan 70428, Taiwan

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\textbf{Objectives}: The aim of this study was to determine the prevalence and characteristics of \textit{qnr}-carrying \textit{Salmonella} isolates from humans in southern Taiwan.

\textbf{Methods}: A total of 446 \textit{Salmonella} isolates collected between 2003 and 2006 were screened for \textit{qnrA}, \textit{qnrB} and \textit{qnrS} by PCR experiments. Genetic structures of \textit{qnr} were determined by PCR-based methods or direct sequencing of plasmid DNA.

\textbf{Results}: \textit{qnrB2} and \textit{qnrS1} were detected in two serovar Enteritidis isolates and two serovar Typhimurium isolates, respectively. One \textit{qnrS1}-positive isolate was found to produce the CMY-2 AmpC enzyme. \textit{qnrS1} was identified on a 10 kb plasmid, which exhibited $>99\%$ nucleotide sequence identity with plasmid TPqnrS-1a reported from the UK. \textit{qnrB2} was found in a complex \textit{sulI}-type class 1 integron on a $>100$ kb plasmid.

\textbf{Conclusions}: This study demonstrated the occurrence of \textit{qnrB2} and \textit{qnrS1} in \textit{Salmonella} for the first time in Taiwan and characterized their genetic structures.

Keywords: quinolones, cephalosporinases, plasmid-mediated quinolone resistance

\textbf{Introduction}

Qnr determinants, including three major groups, \textit{QnrA}, \textit{QnrB} and \textit{QnrS}, have been described in various countries in many enterobacterial species including \textit{Salmonella} since the first discovery of plasmid-mediated quinolone resistance determinant in 1998.$^{1,2}$ and they can confer decreased susceptibility to fluoroquinolones probably by protecting DNA gyrase from quinolone inhibition.$^3$ In Taiwan, \textit{Qnr} determinants have been identified in \textit{Enterobacter cloacae}, \textit{Escherichia coli} and \textit{Klebsiella pneumoniae} in a university hospital.$^8$ The aim of the present study was to assess whether \textit{qnr} genes have been disseminated in \textit{Salmonella} of human origin in Taiwan. The genetic structures of \textit{qnr} genes were also investigated.

\textbf{Materials and methods}

Between 2003 and 2006, 571 \textit{Salmonella} isolates from 446 patients were isolated and collected at National Cheng Kung University Hospital, and one isolate per patient was analysed. Records of the isolates were retrospectively reviewed and showed that $40.8\%$ (182 isolates) and $7.2\%$ (32 isolates) of them were non-susceptible to nalidixic acid and ciprofloxacin, respectively, based on the CLSI criteria for the disc diffusion method.$^9$ At the time of the study, 321 isolates collected between 2004 and 2006 had been serotyped at the Central Branch Office, Center for Disease Control, Taiwan, and $61.1\%$ of them belonged to four serovars: Typhimurium (25.5\%), Enteritidis (17.4\%), Stanley (10.9\%) and Choleraesuis (7.2\%). Isolates obtained in 2003 were not serotyped.

PCR detection of \textit{qnrA} and \textit{qnrS} was performed for all isolates by PCR with primers published previously.$^9$ PCR detection of \textit{qnrB} was performed with primer qnrB-CS-2A (5'-GCGGAAAAATGACAGAA-3') in combination with primer qnrB-CS-3B (5'-ACTCCGAATTTGAGACAGAA-3') or qnrB-CS-2B (5'-TTTGGCAAGGGCTCAAATGG-3') and primer qnrB-CS-3B (5'-TTTGGCAAGGGCTCAAATGG-3'). Among \textit{qnrB1} to \textit{qnrB19} alleles, only \textit{qnrB2}, \textit{qnrB4}, \textit{qnrB9} and \textit{qnrB14} could not be amplified with primers \textit{qnrB-CS-2A} and \textit{qnrB-CS-3B} because of the presence of mismatched nucleotides; primers \textit{qnrB-CS-2A} and \textit{qnrB-CS-2B} were designed for the four alleles. PCR products were purified with a
commercial kit (Roche Applied Science, Mannheim, Germany) and sequenced on both strands.

MICs of various antimicrobials for qnr-positive isolates were determined by using Etest strips (AB Biodisk, Solna, Sweden). The expression of β-lactamases was analysed by isoelectric focusing, and bla<sub>CMY-2</sub> was detected by PCR and sequencing with primers described previously.¹²

PFGE analysis of XbaI- or NdeI-digested genomic DNA was performed using a CHEF-DR 3 apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to the instruction manual, and banding patterns were compared with the BioNumerics software (Applied Maths, Kortrijk, Belgium).

qnr-positive plasmids were transferred from Salmonella isolates to streptomycin-resistant <i>E. coli</i> C600 by the liquid mating-out assay or introduced into <i>E. coli</i> HB101 by electroporation.¹³ Transconjugants were selected on tryptic soy agar plates containing streptomycin 512 mg/L (Sigma Chemical Co., St Louis, MO, USA) and nalidixic acid 16 mg/L (Sigma), and transformants were selected with nalidixic acid 16 mg/L.

The association of integrons with qnr was investigated by PCR using primers for qnr detection and class 1 integron-specific primers Int1-8A (5'-ACGAAAAATGCGGATGTTGCG-3') and qacE-2B (5'-GATTTTAATGCGGATGTTGCG-3'). Genetic structures of qnr genes were then determined by primer walking with PCR products or extracted plasmid DNA samples from transconjugants or transformants. Nucleotide and amino acid sequences were analysed and compared by use of the BLAST computer program (National Center for Biotechnology Information) and the FASTA program (Genetics Computer Group).

Nucleotide sequences for the qnrS1-positive plasmid pST728/06-2 and the qnrB2-containing integron were submitted to GenBank under accession numbers EU715253 and EU715254, respectively.

Results and discussion

PCR and nucleotide sequencing revealed the presence of qnrB2 and qnrS1 in 2 (0.4%) and 2 (0.4%) of the 446 Salmonella isolates, respectively. qnrA was not detected. Thus, the 4 qnr-positive isolates represented 2.2% of all isolates non-susceptible to nalidixic acid and none of the 32 isolates non-susceptible to ciprofloxacin. The two qnrB2-positive isolates were isolated in 2004 and 2005 and belonged to serotype Enteritidis. Both qnrS1-positive isolates belonged to serotype Typhimurium and were isolated in 2006. All qnr-positive isolates were associated with community-onset infections in patients from different counties, indicating the spread of qnrB and qnrS in the community in Taiwan. To the best of our knowledge, this is the first report of the emergence of qnr in Salmonella in Taiwan.

Both qnrB2-positive isolates revealed the same PFGE patterns (data not shown), suggesting clonal spread. They were susceptible to all antimicrobials tested, except nalidixic acid and trimethoprim/sulfamethoxazole (Table 1) based on CSLI criteria.¹⁰ Co-resistance to the two drugs was transferred to <i>E. coli</i> C600 by transconjugation experiments. The transferred plasmids were >100 kb in size.

Both qnrS1-positive isolates also shared the same PFGE patterns; however, they exhibited quite different resistance phenotypes (Table 1). One of them (isolate 728/06) revealed decreased susceptibilities to ciprofloxacin and cephalosporins and resistance to all drugs tested except trimethoprim/sulfamethoxazole and gentamicin, while the other isolate was susceptible to all drugs but nalidixic acid.¹⁰ PCR, nucleotide sequencing and isoelectric focusing revealed that the multidrug-resistant isolate produced the CMY-2 AmpC enzyme (pI = 9.0), which can confer non-susceptibilities to all β-lactams tested except cephalosporins. Plasmid analysis revealed that isolate 728/06 had a 10 kb plasmid and an ~80 kb plasmid, while the other had a 10 kb plasmid alone. Resistance to nalidixic acid cannot be transferred from each of the two isolates to <i>E. coli</i> C600 by conjugation experiments, but instead was transferred to <i>E. coli</i> HB101 by electroporation of the 10 kb plasmid, designated pST728/06-2, from isolate 728/06. Unlike its donor strain, the transformant was susceptible to all antimicrobials tested, except nalidixic acid. Previous studies revealed the association of qnr with the SHV-type extended-spectrum β-lactamases, the IMP-8 metallo-β-lactamase or the DHA-1 AmpC enzyme in Taiwan, and the association was in part attributed to the coexistence of qnr and the bla genes on plasmids.⁸,⁹ None of the qnr-positive isolates in this study was found to produce extended-spectrum β-lactamases or metallo-β-lactamases. The coexistence of qnrS1 and bla<sub>CMY-2</sub> in isolate 728/06 should be coincidental because they were located on different plasmids. The spread of qnr among ceftriaxone-resistant Salmonella isolates may become a therapeutic problem in Taiwan.

Plasmid pST728/06-2 was extracted from the transformant and sequenced on both strands by primer walking. The plasmid, with a size of 10 107 bp, shares the same genetic organization with the 10 066 bp plasmid TPqnrS-1a reported in a multi-resistant Salmonella enterica serovar Typhimurium DT193 strain from the UK (DQ885572),¹⁴ and the two plasmids exhibit 99% nucleotide sequence identity. The distribution of the very similar plasmids in different geographic regions is interesting, but the epidemiological link between them is not known.

A 2830 bp region flanking qnrS1 in plasmid pST728/06-2 was found to exhibit >99% nucleotide identity to that in plasmid pK245 from a <i>K. pneumoniae</i> strain (DQ449578) isolated previously at our hospital (Figure 1a) and that in plasmid pINF5 from an <i>S. enterica</i> serovar Infantis strain from Germany (AM234722).⁵,¹⁵ The region includes a truncated IS2 insertion sequence and an incomplete Tn5058-related resolvase gene upstream and downstream of qnrS1, respectively. Downstream of the 2830 bp region, a 2.1 kb region with high homology (99% nucleotide identity) among plasmids pST728/06-2, TPqnrS-1a, pK245 and pINF5 was found; however, the region in pST728/06-2, similar to that in TPqnrS-1a, was orientated in the opposite direction when compared with pK245 and pINF5. The regions upstream of the 2830 bp region containing qnrS1 do not show structural similarities among pST728/06-2, pK245 and pINF5. The region in pST728/06-2 contains three partially overlapping reading frames that code for the mobilization proteins MobA, MobB and MobC. The comparable region in pINF5 consists of bla<sub>TEM-1</sub>, trapR and trapA, and that in pK245 contains two open reading frames that encode a protein (459 amino acids) similar to peptidoglycan synthetase and the LAP-2 β-lactamase.

Primers Int1-8A and qnrB-CS-1A generated an ~7.4 kb fragment from <i>E. coli</i> transconjugant of isolate 936/05, suggesting that qnrB2 was in a class 1 integron; however, no amplification was detected by PCR with primers qnrB-CS-1B and qacE-2B. Thus, the sequence of the 3' part of the integron was determined by primer walking with extracted plasmid DNA from the transconjugant until mixed sequences were obtained. Sequence analysis revealed that qnrB2 was located in a complex sul1-type
Table 1. MICs of various antimicrobials for four qnr-positive Salmonella isolates and their E. coli transconjugants or transformant

<table>
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<th>FOX</th>
<th>CTX</th>
<th>FEP</th>
<th>NAL</th>
<th>CIP</th>
<th>CHL</th>
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<td>2</td>
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<td>0.094</td>
<td>32</td>
<td>0.19</td>
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</tr>
<tr>
<td>transconjugant</td>
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<td>4</td>
<td>2</td>
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<td>0.047</td>
<td>32</td>
<td>0.25</td>
<td>8</td>
<td>0.75</td>
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<tr>
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<td>0.125</td>
<td>0.094</td>
<td>32</td>
<td>0.19</td>
<td>4</td>
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<tr>
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<td>3</td>
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<td>0.032</td>
<td>32</td>
<td>0.19</td>
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<td>&gt;256</td>
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<td>0.25</td>
<td>4</td>
<td>0.5</td>
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*AMC, amoxicillin/clavulanate; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; FEP, ceftape; FOX, cefoxitin; GEN, gentamicin; NAL, nalidixic acid; SXT, trimethoprim/sulfamethoxazole.

Figure 1. Comparison of the structure of qnrS1 plasmid pST728/06-2 with those of pINF5 from S. enterica serovar Infantis and pK245 from K. pneumoniae (a), and comparison of the qnrB2-containing integrons between plasmid pSE936/05 and that from S. enterica serovar Keurmassar (b). The sequenced parts obtained in this study are bounded by thick vertical lines. The open arrows represent the reading frames and their direction of transcription. The attI recombination sites are represented by empty ovals. The numbers in the open arrows indicate the amino acid numbers of hypothetical proteins encoded by the reading frames.
The two integrons differ only by the first gene cassette. A second copy of orf513 was found downstream of the integron from the serovar Keurmassar strain. Although no amplification was detected by PCR with primer qnrB-CS-1B and orf513-specific primers (data not shown), the possibility of the presence of another orf513 downstream of the integron in our isolate cannot be excluded.

The present study demonstrated the occurrence of two qnr variants, qnrB2 and qnrS1, at a low prevalence rate in human Salmonella isolates in Taiwan. The widespread of such plasmid-mediated resistance determinants in Salmonella may become a serious threat to public health by facilitating the emergence of high-level resistance to fluoroquinolones, and thus constant surveillance is warranted.

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**Transparency declarations**

None to declare.

**References**


