Plasmid-mediated quinolone resistance determinants in quinolone-resistant *Escherichia coli* isolated from patients with bacteremia in a university hospital in Taiwan, 2001–2015

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The aim of this study was to characterize fluoroquinolone (FQ)-resistant *Escherichia coli* isolates from bacteremia in Taiwan in 2001–2015. During the study period, 248 (21.2%) of 1171 isolates were identified as levofloxacin-resistant. The results of phylogenetic group analysis showed that 38.7% of the FQ-resistant isolates belonged to phylogenetic group B2, 23.4% to group B1, 22.6% to group A, 14.9% to group D, and 0.4% belonged to group F. FQ-resistant isolates were highly susceptible to cefepime (91.5%), imipenem (96.0%), meropenem (98.8%), amikacin (98.0%), and fosfomycin (99.6%), as determined by the agar dilution method. β-lactamases, including *bla*<sub>TEM</sub> (66.1%), *bla*<sub>CMY-2</sub> (16.5%), *bla*<sub>CTX-M</sub> (5.2%), *bla*<sub>SHV-2</sub> (1.6%), and *bla*<sub>SHV-12</sub> (1.6%), were found in FQ-resistant isolates. The results of PCR and direct sequencing showed that 37 isolates (14.9%) harbored plasmid-mediated quinolone resistance (PMQR) genes. *qnrB2*, *qnrB4*, *qnrS1*, coexistence of *qnrB4* and *qnrS1*, *oqxAB*, and *aac(6′)-Ib-cr* were found in 1, 4, 4, 1, 15, and 14 isolates, respectively. PMQR genes were successfully transferred for 11 (29.7%) of the 37 PMQR-harboring isolates by conjugation to *E. coli* C600. These findings indicate that *qnr* genes remained rare in *E. coli* but demonstrate the potential spread of *oqxAB* and *aac(6′)-Ib-cr* in Taiwan.

Fluoroquinolones (FQs) are potent and broad-spectrum agents extensively used to treat a wide range of Gram-positive/negative bacterial infections by inhibiting the activity of both DNA gyrase (GyrA and GyrB) and the topoisomerase IV enzymes (ParC and ParE)¹. Unfortunately, despite prescribing guidelines that now recommend reserving FQ use, over the last decade, worldwide spread of FQ-resistant organisms has reduced their therapeutic effectiveness and emerged as an important threat to global health².

Organisms resistant to FQs can occur via several mechanisms, including intrinsic mutations under selection pressure or harboring transferable plasmid-mediated quinolone resistance (PMQR) determinants². The most common mechanism of high-level FQ resistance is due to mutation in one or more of the genes that encode the
Table 1. Distribution of phylogenetic group, PMQR genes, and β-lactamase genes in 248 FQ-resistant bacteremia E. coli isolates. ‡qnr alleles (qnrA, qnrC, qnrD, qnrVC) and qepA were not found in any of the detected isolates. §Isolate 1315 harbored only qoxA but not qoxB.

| Characteristic | No. (%) of isolates |
|---------------|---------------------|
| Phylogenetic group, No. (%) | 2001–2003 45 (19.4) | 2004–2006 49 (20.4) | 2007–2009 41 (19.2) | 2009–2012 59 (24.3) | 2013–2015 54 (22.2) | Total 248 (21.2) |
| A              | 10 (22.2)           | 14 (28.6)           | 11 (26.8)           | 14 (23.7)           | 7 (13.0)           | 56 (22.6)       |
| B1             | 16 (35.6)           | 14 (28.6)           | 10 (24.4)           | 8 (13.6)            | 10 (18.5)          | 58 (23.4)       |
| B2             | 7 (15.5)            | 7 (14.2)            | 18 (44.0)           | 29 (49.1)           | 35 (64.8)          | 96 (38.7)       |
| D              | 12 (26.7)           | 14 (28.6)           | 1 (2.4)             | 8 (13.6)            | 2 (3.7)            | 37 (14.9)       |
| F              | 0                   | 0                   | 1 (2.4)             | 0                   | 0                  | 1 (0.4)         |
| PMQR genes, No. (%) |                         |                      |                        |                      |                    |                 |
| qnrB           | —                   | 1 (qnrB2)           | 1 (qnrB4)           | —                   | —                   | 3               |
| qnrS           | 1 (qnrS1)           | —                   | —                   | 1 (qnrS1)           | 2 (qnrS1)          | 4               |
| qnrB + qnrS    | —                   | —                   | —                   | 1 (qnrB4, qnrS1)    |                    | 1               |
| qepAB          | 2                   | 6                   | 2§                  | 4                   | 1                  | 15              |
| aac(6’)-Ib-cr  | —                   | 4                   | 2                   | 5                   | 1                  | 12              |
| qnrB + aac(6’)-Ib-cr | —               | —                   | 1 (qnrB4)           | —                   | 1 (qnrB4)          | 2               |
| β-lactamase genes, No. (%) |                    |                      |                        |                      |                    |                 |
| blaTEM         | 28                  | 21                  | 21                  | 27                  | 28                  | 125             |
| blaCTX-M       | 0                   | 0                   | 0                   | 0                   | 7                   | 7               |
| blaCMY         | 0                   | 4                   | 1                   | 8                   | 1                   | 14              |
| blaTEM/blaCTX-M | 1                  | 1                   | 1                   | 0                   | 1                   | 3               |
| blaTEM/blaOXA  | 0                   | 0                   | 1                   | 0                   | 0                   | 1               |
| blaTEM/blaCMY  | 1                   | 7                   | 9                   | 6                   | 9                   | 32              |
| blaTEM/blaOXA  | 0                   | 1                   | 0                   | 1                   | 0                   | 2               |
| blaCTX-M/blaCMY | 0                 | 0                   | 0                   | 0                   | 1                   | 1               |
| blaOXA/blaCMY  | 0                   | 0                   | 0                   | 1                   | 0                   | 1               |
| blaTEM/blaOXA/blaCMY | 0          | 1                   | 0                   | 0                   | 0                   | 1               |

Results

Long-term surveillance and antimicrobial susceptibility of FQ-resistant E. coli. During the study period, 2001–2015, we randomly selected 1,171 E. coli isolates from patients with bacteremia, of which 248 (21.2%) were identified as levofloxacin-resistant by using the disk diffusion method (Table 1). The trend in the prevalence of FQ-resistant invasive isolates remained stable during the 15-year surveillance (19.2–24.3%) (Table 1). The phylogenetic analysis revealed five groups (A, B1, B2, D, and F) in 248 FQ-resistant isolates. Ninety-six (38.7%) of the FQ-resistant isolates belonged to phylogenetic group B2. Phylogenetic group B1 was the second most common, representing in 23.4% of the isolates, followed by group A (22.6%), group D (14.9%), and group F (0.4%) (Table 1). The dramatically increasing ratio of phylogenetic group B2 among FQ-resistant isolates was revealed during the study period (Table 1).
Table 2. In vitro activity of 15 antimicrobial agents against 248 FQ-resistant bacteremia E. coli isolates. MIC<sub>50/90</sub>: minimum inhibitory concentration for 50% and 90% of the isolates, respectively; S, susceptible; I, intermediate resistant; R, resistant. *One isolate was resistant to tigecycline, and all isolates were susceptible to colistin. †Antimicrobial susceptibilities of ampicillin, ampicillin-sulbactam, and gentamicin were determined by the disk diffusion method.

| Antibiotic<sup>a</sup> | MIC (μg/mL) | % Susceptibility |
|----------------------|-------------|-----------------|
|                       | Range       | MIC<sub>50</sub> | MIC<sub>90</sub> | S | I | R |
| Ampicillin<sup>b</sup> | —           | —               | —               | — | — | — |
| Ampicillin-sulbactam<sup>b</sup> | —           | —               | —               | 42.0 | 17.0 | 41.0 |
| Cefazidime            | <0.03–256   | 1               | 256             | 63.7 | 1.6 | 34.7 |
| Cefepime              | <0.03–256   | 0.12            | 4               | 91.5 | 3.2 | 5.3  |
| Cefotaxime            | <0.03–256   | 0.25            | 64              | 59.3 | 1.6 | 39.1 |
| Cefoxitin             | 0.12–256    | 8               | 256             | 54.0 | 1.6 | 44.4 |
| Imipenem              | 0.12–256    | 0.25            | 0.5             | 96.0 | 2.8 | 1.2  |
| Meropenem             | <0.03–128   | <0.03           | 0.06            | 98.8 | 0   | 1.2  |
| Amikacin              | 1–256       | 4               | 8               | 98.0 | 0.4 | 1.6  |
| Gentamicin<sup>b</sup> | —           | —               | —               | 54.0 | 3.6 | 42.4 |
| Kanamycin             | 2–256       | 16              | >256            | 68.1 | 5.6 | 26.3 |
| Tetracycline          | 1–256       | 128             | 256             | 26.2 | 0.4 | 73.4 |
| Fosfomycin            | 0.25–256    | 1               | 2               | 99.6 | 0   | 0.4  |
| Ciprofloxacin         | 0.12–256    | 32              | 128             | 1.6  | 0   | 98.4 |
| Levofloxacin          | 4–128       | 16              | 64              | 3.6  | 0   | 96.4 |

The susceptibilities of the 248 FQ-resistant isolates to 15 antimicrobial agents are shown in Table 2. All isolates were resistant to levofloxacin and ciprofloxacin, as determined by the agar dilution method. However, the entire collection was highly susceptible to cefepime (91.5%), imipenem (96.0%), meropenem (98.8%), amikacin (98.0%), and fosfomycin (99.6%) (Table 2). One isolate showed resistance to tigecycline, and all isolates were susceptible to colistin. Moreover, a total of 89 (35.9%) and 223 (89.9%) isolates were defined to be ESBL-producers (98.0%), and fosfomycin (99.6%) (Table 2). One isolate showed resistance to tigecycline, and all isolates were susceptible to colistin. Moreover, a total of 89 (35.9%) and 223 (89.9%) isolates were defined to be ESBL-producers and multidrug resistant (MDR) strains, respectively. The trends of resistance of FQ-resistant invasive isolates to 11 selected antimicrobial agents were generally stable during this 15-year surveillance (Fig. 1). The prevalence of antimicrobial resistance to tetracycline decreased from 86.7% to 55.6% during this period (Fig. 1).

Characterization of antimicrobial resistance genes. The numbers of β-lactamase- and PMQR-producers among the 248 FQ-resistant isolates are shown in Table 1. The results showed that the dominant β-lactamase was bla<sub>TEM</sub> (66.5%), followed by bla<sub>CMY</sub> (19.0%), bla<sub>CTX-M</sub> (4.8%), bla<sub>BLAH</sub> (1.6%), and bla<sub>ACTIV</sub> (1.6%) in FQ-resistant E. coli isolates (Table 1). Sequence analysis revealed that 6 bla<sub>CTX-M-14</sub>, 3 bla<sub>CTX-M-17</sub>, 2 bla<sub>CTX-M-15</sub>, 1 bla<sub>CTX-M-12</sub>, and 1 bla<sub>CTX-M-55</sub> genes were identified among 12 isolates producing β-lactamase (ESBLs) (isolate 1902 harbored bla<sub>CTX-M-14</sub> and bla<sub>CTX-M-15</sub>). Only the bla<sub>ACTIV</sub> ESBL was found in 4 bla<sub>TEM</sub>-producers. In addition, all bla<sub>ACTIV</sub> and bla<sub>ACTIV</sub> genes were identified as bla<sub>ACTIV</sub> and bla<sub>ACTIV</sub>, respectively.

The prevalence of PMQR genes, including qnr alleles, aac<sup>6′</sup>-Ib-cr, qepA, and oxqAB were determined by PCR and direct sequencing, and the results showed that 37 FQ-resistant isolates (14.9%) harbored at least one PMQR gene (Table 1). qnrB2, qnrB4, qnrS1, and the coexistence of qnrB4 and qnrS1 were found in 1, 4, 4, and 1 isolates, respectively (Table 1). oxqAB and aac<sup>6′</sup>-Ib-cr genes were identified in 15 (isolate 1315 harbored only oxqA but not oxqB) and 14 isolates (2 isolates also harbored qnrB4), respectively (Table 1). qnr alleles, including qnrA, qnrC, qnrD, qnrVC, and qepA, were not found in any of the detected isolates. This survey also showed a trend of increase in the prevalence of aac<sup>6′</sup>-Ib-cr and oxqAB among FQ-resistant isolates between 2004–2006 and 2010–2012 (Table 1). Among 10 qnr-producers, bla<sub>TEM</sub>, bla<sub>OH</sub>, and bla<sub>CMY</sub> were found in 3, 4, and 2 isolates, respectively. However, no
### Table 3. Phylogenic group, MICs, PMQR genes and QRDR mutations of 37 E. coli isolates harboring PMQR genes.

| Isolate | Year | Phylogenic group | MIC (µg/mL) | PMQR genes | QRDR mutationsa |
|---------|------|------------------|-------------|-------------|-----------------|
|         |      |                  | CIP | LVX | Gyra | ParC |
| 534     | 2001 | B1               | 64  | 32  | oqxAB | S83L, D87N | S80I |
| 613     | 2002 | A                | 4   | 8   | qrsSt | S83L           | A81P |
| 680     | 2002 | A                | 128 | 64  | oqxAB | S83L, D87N | S80I, A108V |
| 905     | 2005 | B1               | 64  | 64  | oqxAB | S83L, D87N | S80I |
| 906     | 2005 | B1               | 64  | 32  | oqxAB | S83L, D87N | S80I |
| 946     | 2005 | A                | 256 | 64  | oqxAB | S83L, D87N | S80I |
| 966     | 2005 | B1               | 128 | 32  | oqxAB | S83L, D87N | S80I |
| 970     | 2005 | B1               | 64  | 32  | oqxAB | S83L, D87N | S80I |
| 977     | 2005 | A                | 16  | 8   | oqxAB | S83L, D87N | S80I |
| 1019    | 2006 | A                | 1   | 4   | qrsB2 | ...3 | S129A, S134G, A141V, L151M |
| 1029    | 2006 | A                | 128 | 64  | aac(6′)-Ib-cr | S83L, D87N | S80I, E84V |
| 1045    | 2006 | D                | 128 | 16  | aac(6′)-Ib-cr | S83L, D87N | S80I |
| 1050    | 2006 | A                | 16  | 32  | qrsB4 | S83Y | G78C, S129A, S134G, A141V, L151M |
| 1077    | 2006 | D                | 128 | 8   | aac(6′)-Ib-cr | S83L, D87N | S80I |
| 1078    | 2006 | D                | 256 | 16  | aac(6′)-Ib-cr | S83L, D87N | S80I |
| 1206    | 2007 | B1               | 128 | 64  | oqxAB | S83L, D87N | S80I |
| 1262    | 2008 | B2               | 128 | 16  | aac(6′)-Ib-cr | S83L, D87N | S80I, E84V |
| 1270    | 2008 | B2               | 128 | 16  | aac(6′)-Ib-cr | S83L, D87N | S80I, E84V |
| 1315    | 2009 | B1               | 8   | 8   | oqxA | S83L, D87N | S80I |
| 1377    | 2009 | B1               | 128 | 64  | qrsB4, aac(6′)-Ib-cr | S83L, D87N | S80I |
| 1426    | 2010 | B2               | 256 | 128 | qrsB4, qrsSt | S83L, D87N | S80I, E84V |
| 1465    | 2010 | A                | 128 | 32  | oqxAB | S83L, D87N | S80I |
| 1480    | 2010 | B1               | 64  | 32  | oqxAB | S83L, D87N | S80I |
| 1504    | 2011 | A                | 128 | 32  | aac(6′)-Ib-cr | S83L, D87N | S80I |
| 1510    | 2011 | A                | 128 | 32  | aac(6′)-Ib-cr | S83L, D87N | S80I |
| 1516    | 2011 | B1               | 16  | 16  | qrsSt | S83L           | S80I |
| 1540    | 2011 | B2               | 256 | 16  | aac(6′)-Ib-cr | S83L, D87N | S80I, E84V |
| 1558    | 2011 | B1               | 32  | 16  | oqxAB | S83L, D87N | S80I |
| 1604    | 2012 | B1               | 128 | 64  | oqxAB | S83L, D87N | S80I |
| 1619    | 2012 | B2               | 64  | 16  | aac(6′)-Ib-cr | S83L, D87N | S80I, E84V |
| 1649    | 2012 | D                | 128 | 64  | qrsB4 | S83L, D87N | S80I |
| 1705    | 2012 | A                | >256 | 64  | aac(6′)-Ib-cr | S83L, D87N | A108T |
| 1706    | 2013 | B1               | 16  | 8   | qrsSt | S83L           | ...3 |
| 1763    | 2013 | A                | 32  | 64  | qrsSt | S83L           | ...3 |
| 1878    | 2014 | B1               | 16  | 16  | oqxAB | S83L, D87N | S80I |
| 1902    | 2015 | B2               | 256 | 32  | aac(6′)-Ib-cr | S83L, D87N | S80I, E84V |
| 1962    | 2015 | A                | 128 | 32  | qrsB4, aac(6′)-Ib-cr | S83L, D87N | S80I |

bla_{SHV-12} or bla_{K-12}, were detected in oqxAB- or aac(6′)-Ib-cr-producers. In contrast, bla_{CMY-2} was found in 7 oqxAB-producers (7/15, 46.7%) and 6 aac(6′)-Ib-cr-producers (6/14, 42.9%), respectively.

**Characterization of QRDR mutations in PMQR-harboring isolates.** Thirty-seven PMQR-harboring E. coli isolates were distributed into each of the four main phylogroups: A, 12 isolates (32.4%); B1, 15 isolates (40.5%); B2, 6 isolates (16.2%); and D, 4 isolates (10.9%) (Table 3). Chromosomal QRDR mutations were determined by PCR and direct sequencing, and the results showed that only 1 isolate (1019) and 2 (isolate 1706 and 1763) isolates contained wild-type Gyra and ParC, respectively (Table 3). The most common point mutations in PMQR-harboring isolates were Gyra S83L/D87N (31 isolates, 83.8%) and S83L (4 isolates, 10.8%), and those in ParC were S80I (23 isolates, 62.2%) and S80I/E84V (6 isolates, 16.2%) (Table 3).

**PMQR gene transfer and plasmid analysis.** E. coli isolates harboring PMQR genes were further analyzed by conjugation tests to determine whether there was horizontal plasmid spread in Taiwan. Transfer of PMQR genes by conjugation to recipient cells of E. coli C600 was successful for 11 (29.7%) of the 37 selected isolates (2, 4, 1, 2, and 2 parental isolates harbored qrsB, qrsS, qrsB/qrsS, oqxAB, and aac(6′)-Ib-cr/qrsB,
| Group and Isolate | MIC (µg/mL) of antimicrobial agent | Presence or absence of PMQR genes | Plasmid characterization |
|-------------------|----------------------------------|----------------------------------|-------------------------|
|                   | AMP | CAZ | CTX | FOX | TET | KN | FOS | CIP | LVX | TMP | qnrS | qnrB | aac(3’)-Ib-cr | qepAB | β-lactamase | No. Size (~Kb) | Replicon(s) |
| Clinical isolates (donors) |     |     |     |     |     |     |     |     |     |     |     |     |               |       |            |               |          |
| 534               |      | 0.25 | 0.06 | 4   | 2   | 4   | 0.5 | 32  | 32  | 1   |      |      |      |               |       |            |               |          |
| 613               |      | 0.25 | 0.06 | 4   | 128 | 8   | 1   | 4   | 8   | 0.25|      |      |      | + (S1)       |       |            |               |          |
| 906               |      | 0.25 | 0.06 | 4   | 32  | 256 | 128 | 8   | 1   | 8   | 8   | >256|      |      |               |       |            |               |          |
| 1019              |      | 0.25 | 0.06 | 4   | 2   | 16  | 256 | 8   | >256| 32  | 1    | 2   | >256| + (B2)       |       |            |               |          |
| 1377              |      | 0.25 | 0.06 | 4   | 128 | 256 | 128 | 16  | 16  | >256| 1    | 6     | >256| + (B4)       |       |            |               |          |
| 1426              |      | 0.25 | 0.06 | 4   | 32  | >256| 4   | >256| 1   | 256 | 256 | >256| + (S1)       |       |            |               |          |
| 1516              |      | 0.25 | 0.06 | 4   | 128 | 256 | 1   | 64  | 1   | 16  | >256|      |      | + (S1)       |       |            |               |          |
| 1649              |      | 0.25 | 0.06 | 4   | 8   | >256| 2   | >256| 1   | 64  | 64  | >256| + (B4)       |       |            |               |          |
| 1706              |      | 0.25 | 0.06 | 4   | 2   | 128 | 8   | >256| 1   | 32  | 64  | >256|      |      | + (S1)       |       |            |               |          |
| 1763              |      | 0.25 | 0.06 | 4   | 128 | >256| 1   | 128 | 32  | >256| + (B4)       |       |            |               |          |
| 1962              |      | 0.25 | 0.06 | 4   | 16  | >256| 16  | 1   | 128 | 32  | >256| + (B4)       |       |            |               |          |

Recipient

| Group and Isolate | MIC (µg/mL) of antimicrobial agent | Presence or absence of PMQR genes | Plasmid characterization |
|-------------------|----------------------------------|----------------------------------|-------------------------|
| C600              | 0.25 | 0.06 | 2   | 2   | 8   | 2   |      | 0.03| 0.06| 0.25|      |      |      |               |       |            |               |          |

Table 4. MICs, antimicrobial resistance genes and plasmid profiles of *E. coli* isolates used in conjugation experiments. Abbreviations: AMP, ampicillin; CAZ, ceftazidime; CTX, cefotaxime; FOX, cefoxitin; TET, tetracycline; KN, kanamycin; FOS, fosfomycin; CIP, ciprofloxacin; LVX, levofloxacin; TMP, trimethoprim.

| aac(3’)-Ib-cr | qnrB | qnrS | oqxAB | β-lactamase |
|---------------|------|------|-------|------------|
|               |      |      |       |            |

respectively) (Table 4). Plasmid numbers and sizes present in parental isolates and transconjugants were verified according to the method of Kado and Liu, and the results showed that 14 transconjugants (except 1962-3) contained only a single plasmid with a size over 50 kb (Table 4). The antimicrobial resistance genes in transconjugants were further verified by PCR, and the results showed that the *aac(3’)-Ib-cr* and *qnrB* genes were located on the same plasmid in 1377-3. Two and three transconjugants harboring different plasmid profiles were selected from parental isolates 1426 and 1962, respectively (Table 4). No co-transference of *qnrB* and *qnrS* were found in 30 randomly selected transconjugants from isolate 1426. In contrast, transference of *aac(3’)-Ib-cr*, *qnrB*, and *aac(3’)-Ib-cr/qnrB* from isolate 1962 was found in 6 (20%), 4 (13.3%), and 20 (66.7%) of transconjugants. Co-transference of *bla*<sub>C Sheila 1</sub> and *qnrB* to recipient cells was found in 3 of 5 *qnrB*-producers (isolates 1377, 1426, and 1962) (Table 4). No *bla*<sub>CMV 2</sub> was detected in *qepAB* or *aac(3’)-Ib-cr*-harboring transconjugants. Transconjugant
1649-2 showed resistance to ampicillin and cefoxitin with an un-identified β-lactamase gene. In addition, transconjugants 1377-3, 1706-2, and 1962-2 showed increased MICs to tetracycline. The results indicated the co-transference of the tetracycline resistance gene with PMQR determinants. Moreover, 6 of 11 transconjugants showed high resistance to trimethoprim (MIC > 256 μg/mL) (Table 4). PCR-based replicon typing results revealed that IncN, IncFII, and IncHII were identified in 4, 4, and 2 PMQR-plasmids of transconjugants harboring only a single plasmid. However, 3 plasmids (613-3, 1377-3 and 1426-4) were nontypable by PCR-based replicon typing (Table 4).

**Discussion**

In this study, we present the characteristics of 248 FQ-resistant bacteremia isolates of *E. coli* from Taiwan, 2001–2015. Among them, 37 isolates harbored at least one PMQR gene. *oxxAB* and *aac(6′)-Ib-cr* genes were most prevalent among PMQR-producers. In addition, horizontal transmission of PMQR genes is often accompanied by transmission of genes conferring resistance to other antimicrobial agents.

Antimicrobial resistance in Gram-negative bacteria is on the rise worldwide, particularly in *E. coli*, which constitutes a majority of invasive Gram-negative isolates. Wong *et al.* showed that ciprofloxacin resistance in *E. coli* isolated from bacteremia in Canada peaked in 2006 at 40% and subsequently stabilized at 29% in 2011, corresponding to decreasing ciprofloxacin usage after 2007. In this study, we showed the prevalence of FQ-resistant invasive *E. coli* isolates is lower compared with Canada (Table 1). In addition, the prevalence of FQ resistance in bacteremia-causing *E. coli* was lower than urinary-tract-related *E. coli* in Taiwan (21.2% vs. 32%)11. Moreover, the entire collection was highly susceptible to cefepime, imipenem, meropenem, amikacin, and fosfomycin (Table 2). Fosfomycin is found active against *Enterobacteriaceae*, particularly *E. coli*, regardless of source (urinary tract infections or bacteremia), ciprofloxacin resistance, and ESBL production12–14. In addition, fosfomycin is recommended as one of the first-line agents for treatment of urinary tract infections (UTIs) in the latest guidelines endorsed by the Infectious Diseases Society of America and the European Society for Clinical Microbiology and Infectious Diseases15. As a result, the clinical usefulness of fosfomycin, as a first-line treatment agents of bacteremia *E. coli* infections, should be evaluated further, especially in regions where ciprofloxacin resistance rates are high.

The phylogenetic group B2 was the most common pathogenic *E. coli* in many countries, and group A and group B1 were usually isolated as commensals16,17. Massot *et al.* showed a parallel and linked increase in the frequency of the B2 group strains (from 9.4% in 1980 to 22.7% in 2000 and 34.0% in 2010) and of virulence factors18. Here, we showed 38.7% of the FQ-resistant bacteremia groups B2, followed by group B1 (23.4%), group A (22.6%), group D (14.9%), and group F (0.4%) (Table 1). Moreover, based on the 15-year epidemiologic analysis, we further showed that the increasing trend of group B2 among bacteremia *E. coli* isolates (Table 1). Phylogenetic group B2 dominates the bacteremia *E. coli* isolates during the period 2007–2015, but group B1 was most prevalent among bacteremia *E. coli* isolates during the period 2001–2006 (Table 1). As a result, the longitudinal collection of clinical isolates provides the opportunity to characterize the dynamics of the epidemiologic trend and evolution in infectious pathogens over long periods.

Zho *et al.* showed that *qnr, aac(6′)-Ib-cr, qepA, and oxxAB* were found in 2.7%, 24.5%, 11.9% and 6.3% of ciprofloxacin-resistant *E. coli* isolates in China, respectively19. Yang *et al.* showed that PMQR genes were detected in 59 of 80 (73.8%) ciprofloxacin-nonsusceptible bacteremia *E. coli* isolates from Korea20. In this study, we revealed the prevalence of PMQR genes among FQ-resistant *E. coli* in Taiwan (14.9%) was relatively lower than in China (37.3%)20 or in Korea (73.8%)21. In addition, the dominant PMQR genes among FQ-resistant *E. coli* in Taiwan is *oxxAB* (40.5%), followed by *aac(6′)-Ib-cr* (37.8%), and *qnr* alleles (27.0%). No *qepA* producer was found in this study. Although PMQR genes provide a low level of FQ resistance, they have been reported to favor the selection of additional chromosome-encoded resistance mechanisms21. Moreover, all of the PMQR-positive isolates had QRDR mutations (Table 3). These results suggest that along with high-level resistance mediated by QRDR mutations, selection pressure from FQs was absent, and in this case PMQR genes may be lost21. It is possible that evolution by natural selection may explain the higher level of FQ resistance and the relatively lower prevalence of PMQR genes in FQ-resistant invasive *E. coli* from Taiwan. As a result, continual epidemiologic surveillance of PMQR genes is necessary to evaluate whether there are specific plasmids disseminated in Taiwan.

Previous studies showed the most common point mutations in ciprofloxacin-resistant *E. coli* isolates from China were *GyrA* S83L/D87N (263 isolates, 87.1%) and S83L (21 isolates, 7.0%), and those in *ParC* were S80I (233 isolates, 77.2%) and S80I-E84V (35 isolates, 11.6%)19. Our results regarding the distribution of QRDR mutations among FQ-resistant *E. coli* isolates were consistent with previous studies (Table 3). Isolate 1019 showed low-level FQ resistance presented S129A/S134G/A141V/L151M substitutions in ParC in the absence of *GyrA* substitutions raised the possibility that these mutations were not associated with FQ resistance. However, the direct evidence to demonstrate the association of specific QRDR mutations with FQ susceptibility is still limited and thus worth investigating.

A striking association between *blaDHA-1* and *qnrB4* was reported in Korea and Taiwan22,23, and this tight association was also observed in our study (Table 4). The co-transference of the *blaDHA-1* and *qnrB4* genes was identified by conjugation assay (3/4, 75%) (Table 4). In contrast, although 7 *oxxAB*’-’ (7/15) and 6 *aac(6′)-Ib-cr* producers (6/14) also carried *blaCMY-2*, the results of the conjugation assay showed that no *blaCMY-2* was located on *oxxAB- or aac(6′)-Ib-cr*-containing plasmids (Table 4). To our knowledge, this is the first description of the high co-occurrence of *blaCMY-2* in *oxxAB* or *aac(6′)-Ib-cr*-producing *E. coli*.

Highly transferable PMQR genes were observed in this study (11/37, 29.7%) (Table 4). Additional phenotypically expressed resistances were co-transferred with PMQR genes by 12 plasmids (92.3%, except 613-3), resulting in diverse resistance patterns (Table 4). Overall, the most frequently co-transferred resistances were to ampicillin (69.2%), trimethoprim (42.6%), cefazidime (38.5%), cefotaxime (30.8%), ceftoxin (30.8%), kanamycin (30.8%), and tetracycline (23.1%) (MICs > 4-fold change) (Table 4). This indicated the high co-existence of antimicrobial resistance genes on the PMQR-plasmids.

In summary, plasmid profiling of *E. coli* isolates exhibiting the co-existence of both PMQR genes and other antimicrobial resistance genes on a single plasmid shows how they contribute to the rapid spread and increase
in bacterial resistance, which is important to public health. The plasmid backgrounds of the PMQR genes were variable, ruling out the hypothesis for the spread of specific plasmids in Taiwan, however, continual epidemiologic surveillance and monitoring antimicrobial prescriptions and consumption would decrease the prevalence of FQ-resistant organisms and PMQR spread.

**Methods**

**Sampling and isolation of *E. coli***. Bacteremia *E. coli* isolates were recovered in National Cheng Kung University hospital, 2001 to 2015. The Ethics Committee approved that no formal ethical approval was needed to use these clinically obtained materials, because the isolates were remnants from patient samples, and the data were analyzed anonymously. A total of 1,171 non-duplicate clinical isolates were randomly selected and stored at −80 °C in Luria-Bertani (LB) broth containing 20% glycerol (v/v) until used. *E. coli* was identified in the clinical laboratory by colony morphology, Gram stain, biochemical tests, and the Vitek system (bioMérieux, Marcy l’Etoile, France) according to the manufacturer's recommendations. Susceptibility to levofloxacin for *E. coli* isolates was determined by the disk diffusion method (5 μg/disc, BD BBL™ Sensi-Disc™, Sparks, MD, USA) on Mueller-Hinton (MH) agar (Bio-Rad, Marne la Coquette, France) based on the CLSI guidelines. A total of 248 levofloxacin-nonsusceptible bacteremia *E. coli* isolates were identified for further analysis.

**Antimicrobial susceptibility testing.** Antimicrobial susceptibilities to ampicillin, ampicillin-sulbactam, gentamicin, colistin, and tigecycline (BD BBL™ Sensi-Disc™) were determined by the disk diffusion method on Mueller-Hinton agar. MICs of selected antimicrobial agents (from Sigma-Aldrich: amikacin, cephalime, cefotaxime, cefazidime, ciprofloxacin, fosfomycin, kanamycin, levofloxacin; from USP Standards: cefotixin, imipenem, meropenem) were determined by the agar dilution method in accordance with CLSI guidelines. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. The interpretation of resistance to these antimicrobial agents was determined according to the recommendations of the CLSI. Tigecycline and colistin susceptibilities were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and previous study, respectively, MDR *E. coli* was defined as isolates that were resistant to at least 3 classes of the tested antimicrobial agents.

**Characterization of antimicrobial resistance genes.** All 248 FQ-resistant *E. coli* isolates were further screened for selected β-lactamas (blaTEM, blaSHV, blaCTX-M, blaVIM, and blaGES-1) and PMQR genes (qnr alleles, oqxAB, qepA, and aac(6′)Ib-cr) by PCR amplification with specific primers (Supplementary Table S1). DNA sequencing was further carried out on 124 β-lactamase (except blaTEM) and PMQR genes, and the DNA sequences and deduced amino acid sequences were compared with genes in the GenBank database (http://www.ncbi.nlm.nih.gov/genbank/) to confirm the subtypes of antimicrobial resistance genes.

**Screening for mutations in quinolone resistance-determining regions.** GyrA and ParC QRDRs of 37 isolates harboring PMQR genes were examined by amplifying and sequencing gyrA (490 bp) and parC (470 bp) genes using primers (Supplementary Table S1) described by Zhao et al. Amplimers were sequenced and amino acid mutations were determined using the control strain *E. coli* K-12 (NZ_AKBV01000001.1) as a reference.

**Determination of the phylogenetic origin of *E. coli* isolates.** Phylogenetic grouping of *E. coli* isolates was performed using a previously published method. Primers used are described in Supplementary Table S1. The PCR-amplified products were separated by electrophoresis on 1.8% agarose gels, stained with ethidium bromide, and assigned to one of the seven phylogenetic groups A, B1, B2, C, D, E and F.

**Conjugation experiments and plasmid analysis.** The liquid mating-out assay was carried out to transfer PMQR genes from 37 FQ-resistant *E. coli* isolates to rifampicin-resistant *E. coli* C600 as described previously. Transconjugants were selected on LB plates containing 256 μg/mL rifampicin (Sigma) and 0.06 μg/mL ciprofloxacin. The plasmids were extracted as described previously, followed by electrophoresis in a 0.6% agarose gel at 50 V for 3 h and compared by co-electrophoresis with plasmids of known sizes from *Salmonella* OU7526 and a GeneRuler™ DNA ladder (Fermentas, Burlington, ON, Canada) to predict the plasmid sizes. Plasmids were typed by PCR-based replicon typing according to the previous study.

**References**

1. Drlica, K., Haas, H., Kerns, R., Malik, M., Mustaev, A. & Zhao, X. Quinolones: action and resistance updated. *Curr Top Med Chem.* 9, 981–998 (2009).
2. Redgrave, L. S., Sutton, S. B., Webber, M. A. & Piddock, L. J. Fluoroquinolone resistance: mechanisms, impact on bacteria, and role in evolutional success. *Trends Microbiol.* 22, 438–445 (2014).
3. Kishi, R. & Takei, M. Relationship between the expression of ompF and quinolone resistance in *Escherichia coli*. *J Infect Chemother.* 15, 361–366 (2009).
4. Sato, T. et al. Fluoroquinolone resistance mechanisms in an *Escherichia coli* isolate, HUE1, without quinolone resistance-determining region mutations. *Front Microbiol.* 4, 12 (2013).
5. Xiong, X., Bromley, E. H., Oelschlaeger, P., Woolfson, D. N. & Spencer, J. Structural insights into quinolone antibiotic resistance mediated by pentapeptide repeat proteins: conserved surface loops direct the activity of a Qnr protein from a gram-negative bacterium. *Nucleic Acids Res.* 39, 3917–3927 (2011).
6. Robicsek, A. et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med.* 12, 83–88 (2006).
7. Wong, M. H., Chan, E. W. & Chen, S. Evolution and dissemination of OqxAB-like efflux pumps, an emerging quinolone resistance determinant among members of *Enterobacteriaceae*. *Antimicrob Agents Chemother.* 59, 3290–3297 (2015).
8. Yamane, K. et al. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrob Agents Chemother.* 51, 3354–3360 (2007).
9. Kado, C. I. & Luk, S. T. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol. 145, 1365–1373 (1981).
10. Wong, P. H. et al. Antimicrobial co-resistance patterns of gram-negative bacilli isolated from bloodstream infections: a longitudinal epidemiological study from 2002-2011. BMC Infect Dis. 14, 393 (2014).
11. Wang, M. C. et al. Bacterial characteristics and glycemic control in diabetic patients with Escherichia coli urinary tract infection. J Microbiol Immunol Infect. 46, 24–29 (2013).
12. Sastry, S. et al. Clinical Appraisal of Fosfomycin in the Era of Antimicrobial Resistance. Antimicrobial Agents Chemotherapy. 59, 7355–7361 (2015).
13. Falagas, M. E., Kastoris, A. C., Kapaskelis, A. M. & Karageorgopoulos, D. E. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. Lancet Infect Dis. 10, 43–50 (2010).
14. Ko, K. S. et al. In vitro activity of fosfomycin against ciprofloxacin-resistant or extended-spectrum beta-lactamase-producing Escherichia coli isolated from urine and blood. Diagn Microbiol Infect Dis. 58, 111–115 (2007).
15. Gupta, K. et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 52, e103–e120 (2011).
16. Juang, F. et al. Phylogenetic and genomic diversity of human bacteremic Escherichia coli strains. BMC Genomics 9, 560 (2008).
17. Brohman, A. et al. Epidemiology of extended-spectrum beta-lactamase-producing Escherichia coli in Sweden 2007-2011. Clin Microbiol Infect. 20, O344–O352 (2014).
18. Massot, M. et al. Phylogenetic, virulence and antibiotic resistance characteristics of commensal strain populations of Escherichia coli from community subjects in the Paris area in 2010 and evolution over 30 years. Microbiology (in press).
19. Zhao, L. et al. Molecular epidemiology and genetic diversity of fluoroquinolone-resistant Escherichia coli isolates from patients with community-onset infections in 30 Chinese county hospitals. J Clin Microbiol. 53, 766–770 (2015).
20. Yang, H. Y., Nam, Y. S. & Lee, H. J. Prevalence of plasmid-mediated quinolone resistance genes among ciprofloxacin-nonsusceptible Escherichia coli and Klebsiella pneumoniae isolated from blood cultures in Korea. Can J Infect Dis Med Microbiol. 25, 163–169 (2014).
21. Wang, M. et al. Plasmid-mediated quinolone resistance in clinical isolates of Escherichia coli from Shanghai, China. Antimicrob Agents Chemother. 47, 2242–2248 (2003).
22. Pai, H., Heo, M. R. & Choi, T. Y. Association of QnrB determinants and production of extended-spectrum beta-lactamases or plasmid-mediated AmpC beta-lactamases in clinical isolates of Klebsiella pneumoniae. Antimicrob Agents Chemother. 51, 366–368 (2007).
23. Wu, J. J., Ko, W. C. Wu, H. M. & Yan, J. J. Prevalence of Qnr determinants among bloodstream isolates of Escherichia coli and Klebsiella pneumoniae in a Taiwanese hospital, 1999-2005. J Antimicrob Chemother. 61, 1234–1239 (2008).
24. Institute. C. a. L. S. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (Clinical and Laboratory Standards Institute, Wayne, PA, 2009).
25. Institute, C. a. L. S. Performance standards for antimicrobial susceptibility testing (Clinical and Laboratory Standards Institute, Wayne, PA, 2015).
26. Institute, The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters (EUCAST, 2016).
27. Jones, R. N., Anderegg, T. R. & Swenson, J. M. Quality control guidelines for testing gram-negative control strains with polymyxin B and colistin (polymyxin E) by standardized methods. J Clin Microbiol. 43, 925–927 (2005).
28. Magiorakos, A. P. et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 18, 268–281 (2012).
29. Clermont, O., Christenson, J. K., Denamur, E. & Gordon, D. M. The Clermont Escherichia coli phyo-type method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep. 5, 58–65 (2013).
30. Kao, C. Y. et al. Molecular characterization of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella spp. isolates in Mongolia. J Microbiol Immunol Infect (in press).
31. Carattoli, A. et al. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63, 219–228 (2005).

Acknowledgements
We thank Robert Jonas for helpful comments on this manuscript. This study was supported by grant MOST 104-2320-B-006-018-MY3 from the Ministry of Science and Technology, R.O.C. and by grant NCKUH-10301002 from the National Cheng Kung University Hospital, Tainan, Taiwan.

Author Contributions
C.-Y.K. designed the study. C.-Y.K., H.-M.W., W.-H.L., C.-C.T., M.-C.W. and C.-H.T. were responsible for E. coli isolation, antimicrobial susceptibility test, genotyping of the antimicrobial resistance genes and drafted the manuscript. C.-Y.K., J.-J.Y. and J.-J.W. contributed ideas and edited the manuscript. All authors read, commented on, and approved the final manuscript.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Kao, C.-Y. et al. Plasmid-mediated quinolone resistance determinants in quinolone-resistant Escherichia coli isolated from patients with bacteremia in a university hospital in Taiwan, 2001–2015. Sci. Rep. 6, 32281; doi: 10.1038/srep32281 (2016).

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