Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary *Escherichia coli* isolates

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A total of 1878 non-duplicate clinical *Escherichia coli* isolates (comprising 1711 urinary isolates and 167 blood-culture isolates), which were collected from multiple centres in Hong Kong during 1996–2008, were used to investigate the prevalence and molecular epidemiology of plasmid-mediated fosfomycin (*fos*) resistance genes. Eighteen of the 1878 clinical *E. coli* isolates were fosfomycin resistant, of which six were *fosA*³ positive and two were positive for another *fosA* variant (designated *fosKP96*). No isolates had the *fosC2* gene. The clones of the eight isolates were diverse: sequence type (ST) 95 (n=2), ST118 (n=1), ST131 (n=1), ST617 (n=1), ST648 (n=1), ST1488 (n=1) and ST2847 (n=1). In the isolates, *fosA*³ and *bla*<sub>CTX-M</sub> genes were co-harboured on conjugative plasmids with *F<sub>2</sub>A*-carrying plasmids (F–:A<sup>+</sup>) (n=2), N (n=1), F–:A<sup>+</sup>:B<sup>+</sup> and N (n=1) and untypable (n=2) replicons. Both *fosKP96*-carrying plasmids belonged to replicon N. RFLP analysis showed that the two *F<sub>2</sub>A<sup>-</sup>*-carrying plasmids carrying *fosA*³ and *bla*<sub>CTX-M</sub>-³ genes shared the same pattern. Complete sequencing of one of the two *F<sub>2</sub>A<sup>-</sup>*-carrying plasmids, pFOS-HK151325 (69 768 bp) demonstrated it to be >99 % identical to the previously sequenced plasmid pHK23a originating from a pig *E. coli* isolate in the same region. This study demonstrated the dissemination of *fosA*³ genes in diverse *E. coli* clones on multiple *bla*<sub>CTX-M</sub>-³ carrying plasmid types, of which *F<sub>2</sub>A<sup>-</sup>*-carrying plasmids closely related to pHK23a were shared by isolates from human and animal sources.

INTRODUCTION

The increasing prevalence of multidrug-resistant *Enterobacteriaceae* has rekindled interest in the old antibiotic, fosfomycin. As the agent is usually active against fluoroquinolone-resistant and extended-spectrum β-lactamases (ESBL)-producing *Escherichia coli*, it is now one of the first-line antibiotics recommended for treatment of urinary tract infections (UTIs) (Gupta et al., 2011). Historically, impaired drug uptake is the main mechanism for the acquisition of fosfomycin resistance in *E. coli*. This often occurs as a result of mutation in genes encoding the hexose phosphate transport and the glycerol-3-phosphate permase pathways (Takahata et al., 2010). Nonetheless, recent studies have demonstrated the emergence of the plasmid-mediated fosfomycin resistance gene *fosA*³ in *E. coli* and *Klebsiella pneumoniae* isolates, especially among those producing the CTX-M type ESBL (Lee et al., 2012; Wachino et al., 2010). Isolates carrying *fosA*³ have been found in livestock animals, pets and wild rodents from China (Ho et al., 2013a, b; Hou et al., 2012) and in human isolates from Korea (Lee et al., 2012). In Japan, another plasmid-mediated fosfomycin resistance gene, *fosC*², has also been described among CTX-M-producing *E. coli* isolates (Wachino et al., 2010). In China, the multiresistance plasmid pHKP96 from *K. pneumoniae* was reported to carry another fosfomycin resistance gene variant and *bla*<sub>CTX-M-24</sub> (Shen et al., 2008). The *fosA* gene in pHKP96 (designated *fosKP96*) was approximately 74 % identical in nucleotide sequence to the early *fosA* gene described in *Serratia marcescens* plasmids (Mendoza et al., 1980). So far, information on the distribution of these plasmid-mediated determinants in clinical isolates is limited. Therefore, this study was conducted to investigate the prevalence and molecular epidemiology of plasmid-mediated fosfomycin...
resistance genes in human *E. coli* clinical isolates, collected over different time periods.

**METHODS**

**Bacterial strains and susceptibility testing.** A total of 1711 urinary isolates and 167 blood-culture isolates, collected from multiple study sites in Hong Kong between 1996 and 2008, were investigated retrospectively (Table 1). The sources and patient demographic for the isolate collections have been reported previously (Ho et al., 2007, 2010, 2012b). Susceptibility of the isolates to fosfomycin was determined by the disc diffusion method and interpreted according to Clinical and Laboratory Standards Institute criteria (inhibition zone diameters: $\geq 16$ mm, sensitive; intermediate, 13–15 mm; and $\leq 12$, resistant; CLSI, 2012).

**Genotyping.** The major *E. coli* phylogenetic group was determined by PCR analysis (Doumith et al., 2012). Multilocus sequence typing (MLST) was carried out by the University College Cork scheme (Ho et al., 2012b).

**Detection of antibiotic resistance genes.** PCR and sequencing were used to investigate antibiotic resistance genes. Primers used for investigation of the plasmid-mediated fosfomycin resistance genes (*fosA*, *fosA3*, *fosC2* and *fosKP96*) are listed in Table 2. Primers for the *blaCTX-M* (consensus, CTX-M-1, -2, -8, -9 and -25 groups) and 16S rRNA methylase (*rmrA-E*) genes have been described previously (Lo et al., 2010, 2013; Zhou et al., 2010).

**Characterization of the genetic environment of *fosA3*.** The sequences flanking the ORF of *fosA3* were determined by PCR mapping. The primers for PCR mapping were selected to cover insertion sequences (IS26, IScep1 and IS10) described previously to occur in the regions flanking *fosA3* (Ho et al., 2013b).

**Plasmid studies.** Transferability of the fosfomycin resistance determinant in all the fosfomycin-resistant (*Fos\(^5\)*) isolates was investigated by filter mating using *E. coli* J53 (azide-resistant) as the recipient. Transconjugants were selected on MacConkey agar containing 150 $\mu$g sodium azide ml\(^{-1}\), 4 $\mu$g fosfomycin ml\(^{-1}\) (Sigma) and 25 $\mu$g glucose-6-phosphate ml\(^{-1}\) (Sigma) (Ho et al., 2013b). The transfer frequencies were expressed as the number of transconjugants per donor cell. Plasmids were sized by the S1 nuclease PFGE method (Ho et al., 2011b).

Identification of the plasmid replicons was confirmed by sequencing the PCR products. The F plasmids were further categorized by hybridization using specific PCR products as probes (Ho et al., 2011a). The two *fosA3*-carrying F2A–B– plasmids in the transconjugants were analysed further by RFLP (Ho et al., 2012a). Purified plasmids were digested with EcoRI (Takara) and hybridized with the *E. coli* pUC18 plasmid (pHKU1) carrying each as the only plasmid. pFOS-HK151325 and pHKU1 were sequenced by 454 GS-FLX system (Roche) and Ion Torrent system (Life Technologies), respectively. As either system is equally suitable for the sequencing purpose, the choice was entirely operational. The reads obtained for pPfos-HK151325 were assembled by the *GS de novo Assembler* (version 2.6) into ten contigs and the mean coverage was $\sim$200-fold. The reads for pHKU1 were assembled by using TMAP 3.2.2 into four contigs and the mean coverage was $\sim$200-fold. The gaps in both plasmids were closed by additional PCRs and Sanger sequencing. The final plasmid assembly sequences were verified by separate digestion of the plasmids with EcoRI and comparison with the *in silico* restriction analysis results. The plasmids were annotated using the RAST Server and each predicted ORF was further blasted against the National Center for Biotechnology Information (NCBI) non-redundant protein database using BLASTP.

**Plasmid sequencing.** Two plasmids including one *fosA3*-carrying F2A–B– plasmid (pPfos-HK151325 originating from strain 151325) and one *fosKP96*-carrying IncN plasmid (pHKU1 originating from strain 72710) were chosen for complete sequencing. Plasmid DNA was prepared as described previously (Ho et al., 2011b) from an *E. coli* J53 transconjugant (pFOS-HK151325) and a DH5α transformant (pHKU1) carrying each as the only plasmid. pFOS-HK151325 and pHKU1 were sequenced by 454 GS-FLX system (Roche) and Ion Torrent system (Life Technologies), respectively. As either system is equally suitable for the sequencing purpose, the choice was entirely operational. The reads obtained for pPfos-HK151325 were assembled by the *GS de novo Assembler* (version 2.6) into ten contigs and the mean coverage was $\sim$200-fold. The reads for pHKU1 were assembled by using TMAP 3.2.2 into four contigs and the mean coverage was $\sim$200-fold. The gaps in both plasmids were closed by additional PCRs and Sanger sequencing. The final plasmid assembly sequences were verified by separate digestion of the plasmids with EcoRI and comparison with the *in silico* restriction analysis results. The plasmids were annotated using the RAST Server and each predicted ORF was further blasted against the National Center for Biotechnology Information (NCBI) non-redundant protein database using BLASTP.

**Table 1.** Prevalence of fosfomycin resistance and *fosA3* in *Escherichia coli* according to collection periods and specimen sources

<table>
<thead>
<tr>
<th>Isolate/year</th>
<th>No. isolates</th>
<th>No. (%) ESBL-producing</th>
<th>No. (%) <em>Fos(^5)</em> isolates</th>
<th><em>fos</em> genes found (no. isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine isolates</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004–2005†</td>
<td>1440</td>
<td>125 (8.7)</td>
<td>8 (0.6)</td>
<td><em>fosA3</em> (2), <em>fosKP96</em> (1)</td>
</tr>
<tr>
<td>2006–2008‡</td>
<td>271</td>
<td>14 (5.2)</td>
<td>5 (1.8)</td>
<td><em>fosA3</em> (1)</td>
</tr>
<tr>
<td>Blood isolates§</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1996–1999</td>
<td>50</td>
<td>50 (100)</td>
<td>0 (0)</td>
<td>–</td>
</tr>
<tr>
<td>2007–2008</td>
<td>117</td>
<td>117 (100)</td>
<td>5 (4.3)</td>
<td><em>fosA3</em> (3), <em>fosKP96</em> (1)</td>
</tr>
</tbody>
</table>

†The isolates were obtained from adult females with UTIs including 1214 outpatients and 226 inpatients. The isolates were collected from six private hospital laboratories and seven stand-alone community laboratories as described previously (Ho et al., 2007). The collection overlapped with 1067 isolates reported previously (Ho et al., 2007).

‡The isolates were obtained from female outpatients with uncomplicated cystitis at 54 study sites. The patient details have been described previously (Ho et al., 2010).

§The isolates were obtained from inpatients with bacteraemia in a university teaching hospital (Ho et al., 2012b). The prevalence of ESBL-producing *E. coli* among all blood-culture *E. coli* isolates in 1996–1999 and 2007–2008 was 6.6 % (74/1127) and 26.8 % (253/945), respectively. In 1996–1999, 67.6 % (50/74) of the ESBL-producing isolates remained viable and all were included. In 2007–2008, 46.2 % (117/253) of the ESBL-producing isolates from 253 patients (one per patient) were randomly selected for inclusion.
FosR isolates.
Among the urine isolates, 13 isolates were FosR, of which 4 isolates were ESBL positive. Three of the four FosR/ESBL-positive isolates had resistance could be demonstrated in 8 of the 18 FosR isolates.
Overall, 18 (1.0 %) isolates were FosR, of which 6 were positive for the fosA3 gene. All blood isolates were ESBL positive and 9 isolates were ESBL producers, of which five were FosR. Among the urine isolates, 13 isolates were FosR, of which 4 isolates were ESBL positive and 9 isolates were ESBL producers, of which five were FosR. Three of the four FosR/ESBL-positive isolates had resistance could be demonstrated in 8 of the 18 FosR isolates.

## RESULTS

### Prevalence of fosfomycin resistance and plasmid-mediated determinants

Overall, 18 (1.0 %) isolates were FosR, of which 6 were positive for fosA3 and 2 were positive for fosKP96 (Table 1). Among the urine isolates, 13 isolates were FosR, of which 4 isolates were ESBL positive and 9 isolates were ESBL negative. Three of the four FosR/ESBL-positive isolates were positive for the fosA3 gene. One FosR/ESBL-negative isolate was positive for the fosKP96 gene. All blood isolates were ESBL producers, of which five were FosR. Three of the FosR isolates were fosA3 positive and one was fosKP96 positive. No fosA or fosC2 gene was detected among the FosR isolates.

### Characteristics of fos-carrying E. coli isolates and plasmids

In the conjugation experiments, transferable fosfomycin resistance could be demonstrated in 8 of the 18 FosR isolates. The frequency of transfer ranged from 10^{-1} to 10^{-5} per donor cell. All PCR fos-positive isolates could transfer the resistance trait to the recipient J53 strain. No transconjugants for the remaining ten FosR isolates without fos genes could be obtained, despite repeated attempts. The characteristics of the eight fos-carrying E. coli isolates and plasmids are summarized in Table 3. Phylogenetic group analysis revealed that three isolates belonged to group B2, two to group A and three to group D. MLST revealed that the clones of the fos-carrying isolates were diverse. Two isolates were ST95, while the remaining ones were singletons (ST118, ST131, ST617, ST648, ST1488 and ST2847). All eight parent isolates were resistant to three or more non-β-lactam drugs. All seven ESBL-positive isolates were positive for bla_{CTX-M}; three had bla_{CTX-M-3}, two had bla_{CTX-M-65} and two had bla_{CTX-M-14}. In the transconjugants, there was co-transfer of ESBL resistance and bla_{CTX-M} (six isolates). Four transconjugants had co-transfer of resistance to other non-β-lactam drugs. The plasmids carrying fosA3 were 60–200 kb. The two plasmids carrying fosKP96 were 50 kb. The replicon types of the fosA3 plasmids were IncFI1 (F2:A:B; n=2), IncN (n=1), multireplicon IncN and Inc FIB (F--:A:B1; n=1) and untypable (n=2). In all the transconjugants, hybridization experiments confirmed that the fosA3 and bla_{CTX-M} genes were harboured on the same plasmids. The regions surrounding fosA3 were explored by PCR mapping and sequencing. The results showed that fosA3 was flanked by IS26 at both ends as a transposon-like structure. The intergenic regions between fosA3 and the downstream IS26 included sequences of two different lengths having homology to regulatory genes (orf1, orf2 and orf3) in the chromosome of K. pneumoniae 342 (GenBank accession no. CP000964). Both fosKP96-carrying plasmids belonged to replicon N.

### Analysis of pFOS-HK151325

To investigate the relationship between the fosA3-carrying F2:A:B-- plasmids detected in human and animal isolates, one of the two F2:A:B-- plasmids in the transconjugants was studied further by sequencing. The assembled plasmid pFOS-HK151325 had 69 768 bp, a mean G+C content of 51.7 mol% and 103 putative ORFs (GenBank accession no. JX627737). pFOS-HK151325 had a plasmid scaffold typical for the IncFII plasmids in general (Fig. S1, available in JMM online), and was highly similar to the fosA3-carrying plasmid, pHK23a (GenBank accession no. JQ432559) originating from a slaughter pig in December 2008 in Hong Kong (>99.9 % query coverage, >99.9 % identity by blastn analysis at NCBI).

#### Table 2. Oligonucleotide primers for the detection of plasmid-mediated fos resistance genes

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Target</th>
<th>Nucleotide sequence (5’→3’)</th>
<th>Position (nt)</th>
<th>GenBank accession no.</th>
<th>Amplicon size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>fosA-F131</td>
<td>fosA</td>
<td>ATCGTTGCTGGTGCTCTGTGAGG</td>
<td>2031–2051</td>
<td>FJ829469</td>
<td>271</td>
<td>Hou et al. (2012)</td>
</tr>
<tr>
<td>fosA-R401</td>
<td>fosA</td>
<td>ATGCCCGCATAGGGCTTCTTCTG</td>
<td>C2301–2282</td>
<td>AB522970</td>
<td>234</td>
<td>Ho et al. (2013b)</td>
</tr>
<tr>
<td>fosA3-F</td>
<td>fosA3</td>
<td>CACCAATGTTATCCAGGACGTT</td>
<td>54–73</td>
<td>AB522969</td>
<td>217</td>
<td>Ho et al. (2012)</td>
</tr>
<tr>
<td>fosA3-R</td>
<td>fosA3</td>
<td>CGGGTATCTTTCCATACCTCAGG</td>
<td>C287–266</td>
<td>AB522969</td>
<td>217</td>
<td>Ho et al. (2012)</td>
</tr>
<tr>
<td>fosC2-F125</td>
<td>fosC2</td>
<td>TGGAGGCATCCTGATGTTG</td>
<td>125–143</td>
<td>AB522969</td>
<td>217</td>
<td>Ho et al. (2012)</td>
</tr>
<tr>
<td>fosC2-R341</td>
<td>fosC2</td>
<td>AGGCTACCGCTATGGATTTC</td>
<td>c341–323</td>
<td>AB522969</td>
<td>217</td>
<td>Ho et al. (2012)</td>
</tr>
<tr>
<td>fosA96_FW</td>
<td>fosKP96</td>
<td>TATTAGGGAAGGCGATTTGCC</td>
<td>212–234</td>
<td>EU195449</td>
<td>188</td>
<td>This study</td>
</tr>
<tr>
<td>fosA96-var–RV</td>
<td>fosKP96</td>
<td>CCCCCTTATACGGTCTCG</td>
<td>c399–380</td>
<td>EU195449</td>
<td>188</td>
<td>This study</td>
</tr>
</tbody>
</table>

(Aziz et al., 2008; Ho et al., 2011a). The comparison of overall plasmid sequence was conducted by WebACT and Geneious Pro (version 5.0.1; Biomatters Limited) (Ho et al., 2013a).
Table 3. Characteristics of *E. coli* strains and plasmids carrying plasmid-mediated fosfomycin resistance determinants

<table>
<thead>
<tr>
<th>Strain</th>
<th>Origin</th>
<th>Isolation date</th>
<th>Sex/Age (y)</th>
<th>Phylogenetic group</th>
<th>MLST</th>
<th>Size (kb)*</th>
<th>Conjugation frequency</th>
<th>Replicon*</th>
<th>Resistance phenotype†</th>
<th>Resistance gene content†‡§</th>
</tr>
</thead>
<tbody>
<tr>
<td>039647</td>
<td>Blood</td>
<td>Mar 07</td>
<td>M/69</td>
<td>A</td>
<td>ST1488</td>
<td>70</td>
<td>$10^{-1}$</td>
<td>F2:A→:B→</td>
<td>ESBL, Cip, Gen, Nit, Sxt, Tet</td>
<td>fosA3, bla&lt;sub&gt;CTX-M-3&lt;/sub&gt;</td>
</tr>
<tr>
<td>151325</td>
<td>Blood</td>
<td>Oct 07</td>
<td>F/60</td>
<td>B2</td>
<td>ST95</td>
<td>70</td>
<td>$10^{-3}$</td>
<td>F2:A→:B→</td>
<td>ESBL, Cip, Gen, Sxt, Tet</td>
<td>fosA3, bla&lt;sub&gt;CTX-M-3&lt;/sub&gt;</td>
</tr>
<tr>
<td>10240</td>
<td>Urine</td>
<td>May 06</td>
<td>F/52</td>
<td>B2</td>
<td>ST131</td>
<td>60</td>
<td>$10^{-1}$</td>
<td>N</td>
<td>ESBL, Cip, Gen, Sxt</td>
<td>fosA3, bla&lt;sub&gt;CTX-M-14&lt;/sub&gt;</td>
</tr>
<tr>
<td>4904890</td>
<td>Urine</td>
<td>Oct 04</td>
<td>F/80</td>
<td>D</td>
<td>ST2847</td>
<td>200</td>
<td>$10^{-5}$</td>
<td>F→A→:B1, N</td>
<td>ESBL, Cip, Gen, Sxt, Nit</td>
<td>fosA3, bla&lt;sub&gt;CTX-M-65&lt;/sub&gt;</td>
</tr>
<tr>
<td>18363</td>
<td>Urine</td>
<td>Dec 05</td>
<td>F/80</td>
<td>B2</td>
<td>ST95</td>
<td>70</td>
<td>$10^{-5}$</td>
<td>Untypable</td>
<td>ESBL, Cip, Gen, Sxt</td>
<td>fosA3, bla&lt;sub&gt;CTX-M-3&lt;/sub&gt;</td>
</tr>
<tr>
<td>064838</td>
<td>Blood</td>
<td>May 07</td>
<td>M/77</td>
<td>A</td>
<td>ST617</td>
<td>70</td>
<td>$10^{-1}$</td>
<td>Untypable</td>
<td>ESBL, Amk, Cip, Gen, Nit</td>
<td>fosA3, bla&lt;sub&gt;CTX-M-65&lt;/sub&gt;, rmtB</td>
</tr>
<tr>
<td>76726</td>
<td>Urine</td>
<td>Aug 04</td>
<td>F/57</td>
<td>D</td>
<td>ST118</td>
<td>50</td>
<td>$10^{-1}$</td>
<td>N</td>
<td>Amk, Cip, Gen, Nit, Sxt, Tet</td>
<td>fosKP96</td>
</tr>
<tr>
<td>72710</td>
<td>Blood</td>
<td>May 07</td>
<td>M/52</td>
<td>D</td>
<td>ST648</td>
<td>50</td>
<td>$10^{-4}$</td>
<td>N</td>
<td>ESBL, Cip, Tet, Sxt</td>
<td>fosKP96</td>
</tr>
</tbody>
</table>

*Size of fosA3 or fosKP96-carrying plasmid. Underlining indicates where the plasmid location of the replicon and the fosA3 and bla<sub>CTX-M</sub> genes has been confirmed by hybridization.
†All parent strains and transconjugants were resistant to fosfomycin. Resistance phenotype indicates additional resistance phenotypes involving ESBL, amikacin (Amk), ciprofloxacin (Cip), chloramphenicol (Chl), gentamicin (Gen), nitrofurantoin (Nit), tetracycline (Tet), and trimethoprim/sulfamethoxazole (Sxt). Those that were co-transferred to the transconjugants are underlined.
‡As determined by PCR assays in the transconjugants.
§The regions flanking fosA3 in the six strains were as follows: type A, IS26-fosA3-orf1-orf2-Δorf3-IS26 (n=5) and type B, IS26-fosA3-orf1-Δorf2-IS26 (n=1). All except strain 064838 had type A pattern.
Analysis of pHKU1

To investigate the relationship between the fosKP96 plasmid identified in this study and the previously reported pKP96 (GenBank accession no. EU195449), one of the two fosKP96-carrying IncN plasmids was sequenced. The assembled plasmid pHKU1 had 54373 bp, a mean G+C content of 50.2 mol% and 74 putative ORFs (GenBank accession no. KC960485). pHKU1 had a plasmid scaffold typical for the IncN plasmids, including a replicon (repA) and genes encoding UV protection (mucA–mucB), plasmid stability (stabABC operon), antirestriction functions (ardA–ardB and ardK–ardR), protection from type 1 restriction system (cgc system) and conjugative apparatus (two transfer gene clusters, locus tral and locus tr2). In pHKU1, the variable region was inserted adjacent to the resolvase gene, usp1, and could be broadly divided into a class I integron region and a fosKP96-containing region. The integron consisted of aac(6’)-Ib-cr, blaOXA-1, catB3, arr-3, qacEdelata1 and sul1 and an array sequence is similar to In37 (GenBank accession no. AY259086). The remaining part of the variable region was mosaic and consisted of fosKP96 flanked by two copies of IS10, a restriction system (EcoRI, EcoRIlm), two other insertion elements (IS6100 and a truncated insB) and three hypothetical orf genes. Overall, the structure of pHKU1 was highly similar to pKP96 but diminished by two resistance genes (qnrA1 and blaCTX-M-24). The IncN plasmid from strain 76726 gave similar RFLP patterns to pHKU1 when digested by EcoRI, indicating that the two plasmids were highly related.

DISCUSSION

This is the first study to investigate the prevalence of plasmid-mediated fos genes among a large collection of urinary and blood E. coli isolates. In our collection, the prevalence of fosfomycin resistance was low (0–4.3 %) and only 18 isolates were FosR. Therefore, the proportions of fosfomycin gene-positive isolates in the entire collection was low (<1%, 8/1878). Nonetheless, it showed that a substantial proportion of the fosfomycin resistance (44%, 8/18) was attributed to the plasmid-mediated fosfomycin genes, especially the fosA3 variant, which have been reported to be emerging among animal isolates in China (Ho et al., 2013b; Hou et al., 2012). By comparison, we previously showed that 6.0% (101/1693) of E. coli isolates carried by livestock animals, dogs, cats and wild rodents in 2008–2010 were fosfomycin resistant, of which 96% (97/101) were fosA3 positive (Ho et al., 2013b).

The six patients with infection by the fosA3-carrying E. coli isolates had no history of exposure to fosfomycin. Previous studies have shown that fosA3 and blaCTX-M are often co-carried on the same plasmid in the animal isolates (Ho et al., 2013b). This study showed that a similar situation occurred among fosA3-positive human isolates. Moreover, fosA3-carrying plasmids may contain determinants encoding resistance to other unrelated antibiotics. Therefore, the presence of fosA3 in the isolates could be a result of selection pressure by other antibiotics such as cephalosporins and aminoglycosides, which are widely used for therapy of human infections. Notably, the multi-replicon plasmid encodes transferable resistance to five antibiotic classes – cephalosporins (blaCTX-M), fosfomycin (fosA3), tetracycline, chloramphenicol and nitrofurantoin. Further investigation is required to find out the additional resistance mechanisms involved.

Our findings showed an increase in the detection rate of fosR, ESBL-producing E. coli. Molecular analysis demonstrated that the association between ESBL production and fosfomycin resistance was related to the co-carriage of fos and blaCTX-M genes by the same plasmids. Taking into account the limited options for oral treatment of ESBL-producing E. coli infections, it may be prudent to reserve fosfomycin for management of culture-confirmed, multi-drug-resistant infections rather than using it as first-line agent for empirical therapy of common UTIs (Gupta et al., 2011). In our region, nitrofurantoin instead of fosfomycin is recommended as a first-line agent for empirical therapy of uncomplicated cystitis (Ho et al., 2010).

An important finding of our work is that it confirmed the occurrence of highly similar IncFII (F2:A−:B−) plasmids carrying fosA3 and blaCTX-M-3 in isolates from two humans and a pig. In animal E. coli isolates, fosA3 has been reported to be commonly carried by IncFII plasmids including subtypes F2:A−:B−, F16:A1:B−, F24:A−:B− and F33:A−:B− (Ho et al., 2013b; Hou et al., 2012). In China, F2:A−:B− plasmids carrying fosA3 and blaCTX-M-3 have been found among isolates from pigs and pets, indicating the wide distribution of this plasmid subtype (Ho et al., 2013b; Hou et al., 2012). The F33:A−:B− variant, which predominate among isolates from pets and carries the fosA3, blaCTX-M-65 and mrtB genes (Hou et al., 2012), was not found in our human isolates. As the IncFII plasmids occur widely among E. coli populations (Ho et al., 2011a, 2012a, b), antibiotic selection pressure arising from antibiotic use in human and animal hosts might accelerate the spread of the fosA3 gene to other parts of the world.

Plasmid types other than IncF that have been reported previously to carry fosA3 include IncN, IncB/O and IncI (Ho et al., 2013b; Hou et al., 2012). As in previous studies (Ho et al., 2013b; Lee et al., 2012), we found that the fosA3 gene was flanked by IS26 elements in different plasmid types. This finding further highlights the importance of IS26 elements in the horizontal dissemination of fosA3 genes. The strains in the present study belonged to diverse clones. However, two strains with different types of fosA3-carrying plasmids were of ST95 and one strain was of the widespread ST131 clone.

This study showed that pKP96-like multiresistance plasmids were carried by two isolates recovered in 2004 and 2007. The original pKP96 was recovered from a K. pneumoniae isolate originating from the sputum sample of a patient in Zhejing, China, in 2002. While this fos gene variant seems to be rare, the identification of pKP96-like
over an extended period of time in different parts of China indicated that the plasmid is maintained in this region. As the primers described previously for identification of fos could not amplify this variant (Hou et al., 2012), the prevalence of this resistance mechanism has probably been underestimated.

In conclusion, this study showed that the prevalence of plasmid-mediated fos genes in clinical E. coli isolates remains low. However, highly similar IncFII-type plasmids carrying fosA3 are shared by multidrug-resistant E. coli isolates originating from human and animal sources. Acquisition of the fosA3 gene by successful E. coli clones and efficient resistance plasmid vectors may undermine the usefulness of fosfomycin in human medicine. Fosfomycin should be used carefully and the prevalence of fosA3 and other fos genes monitored closely.

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