Clonal Diversity of Escherichia coli Isolates Carrying Plasmid-Mediated Fosfomycin Resistance Gene fosA3 from Livestock and Other Animals

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In Escherichia coli, fosfomycin resistance is uncommon and is mainly caused by mutations in the chromosomally encoded drug transporters (1). Nonetheless, recent studies have demonstrated the emergence of fosA3, the plasmid-mediated fosfomycin resistance gene, among CTX-M-producing and multidrug-resistant E. coli strains originating from animals and patients in China, Japan, and South Korea (2–6). Although the genetic sequences surrounding the fosA3 gene and the plasmids carrying them have been extensively investigated, there is only limited information about the clonal diversity of the fosA3-positive E. coli populations (2–6).

Here, we investigated the clonal structure of fosA3-positive isolates originating from different animals (5). The antimicrobial susceptibility and characteristics of the regions surrounding the fosA3 have previously been published (5). In brief, 101 of 1,693 E. coli isolates recovered from 2,106 animals (210 beef cattle, 214 pigs, 460 broiler chickens, 389 stray cats, 368 stray dogs, and 456 wild rodents) during 2008 to 2010 in Hong Kong were fosfomycin resistant; among those isolates, 97 were fosA3 positive (5). Among the 97 fosA3-positive isolates, 95.9% were blaCTX-M positive and 66.0% had coresistance to 2/4 non-β-lactam drugs (amikacin, chloramphenicol, ciprofloxacin, cotrimoxazole, gentamicin, nalidixic acid, netilmicin, nitrofurantoin, and tetracycline) (5). Multilocus sequence typing (MLST) was carried out by the University College Cork scheme (http://mlst.warwick.ac.uk/mlst/), and results were analyzed by eBURST v3. The major E. coli phylogenotypes were determined by multiplex PCRs (6).

The phylogroup distribution for the 97 fosA3-positive isolates was as follows: A (38.1%, 37/97), B1 (52.6%, 51/97), B2 (2.1%, 2/97), and D (7.2%, 7/97). MLST analysis revealed 52 different sequence types (STs) under six clonal complexes (CCs) and 15 singletons, including overrepresentation of CC58/phylogroup B1 (38/97, 39.2%) and CC10/phylogroup A (30/97, 30.9%) (Table 1). The proportions of fosA3-positive isolates distributed into the two CCs by animal sources were 62.5% (5/8) for dogs, 66.7% (2/3) for cats, 60% (3/5) for rodents, 74.3% (26/35) for chickens, 61.5% (16/26) for pigs, and 80% (16/20) for cattle.

Our findings showed that a large proportion of the plasmid-mediated fosA3 E. coli isolates were included in two CC lineages. However, it is not clear if the two CCs represent the major E. coli population in the normal flora of the animals or are merely prevalent among the chosen subset. Studies conducted elsewhere showed that both CC10 and CC58 isolates are commonly found as part of the normal flora in animals and humans (7, 8). Sato et al. recently described five fosA3-positive, CTX-M-producing E. coli isolates originating from fecal flora of healthy individuals; among those isolates, three had STs (ST155, ST224, and ST3054) belonging to CC58 (9).

As our previous studies revealed, fosA3 and blaCTX-M were of-

### Table 1: Clonal structure for 97 fosA3-positive E. coli isolates from animals

<table>
<thead>
<tr>
<th>E. coli clone</th>
<th>n</th>
<th>MLST result(s)</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Dogs</td>
</tr>
<tr>
<td>CC58</td>
<td>38</td>
<td>12 different STs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>CC10</td>
<td>30</td>
<td>20 different STs&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
</tr>
<tr>
<td>CC117</td>
<td>4</td>
<td>ST117 (n = 4)</td>
<td>1</td>
</tr>
<tr>
<td>CC156</td>
<td>4</td>
<td>ST156 (n = 4)</td>
<td>1</td>
</tr>
<tr>
<td>CC88</td>
<td>3</td>
<td>ST23 (n = 2), ST2851 (n = 1)</td>
<td>1</td>
</tr>
<tr>
<td>CC847</td>
<td>2</td>
<td>ST2599 (n = 2)</td>
<td>1</td>
</tr>
<tr>
<td>Others</td>
<td>16</td>
<td>15 different STs&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td></td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> CC, clonal complex; MLST, multilocus sequence typing; ST, sequence type.
<sup>b</sup> Data include 15 new STs (ST3058, ST3894, ST3896, ST3897, ST3899, ST3900, ST3901, ST4149, ST3936, ST3937, ST3938, ST3939, ST3940, ST3941, and ST3942) assigned for the first time in this study.
<sup>c</sup> ST17 (n = 1), ST20 (n = 1), ST58 (n = 3), ST155 (n = 3), ST162 (n = 9), ST224 (n = 3), ST297 (n = 9), ST602 (n = 2), ST1081 (n = 1), ST1196 (n = 2), ST3714 (n = 3), ST3939 (n = 1).
<sup>d</sup> ST10 (n = 5), ST48 (n = 2), ST93 (n = 1), ST165 (n = 1), ST189 (n = 1), ST209 (n = 1), ST215 (n = 1), ST226 (n = 2), ST746 (n = 2), ST1638 (n = 2), ST2913 (n = 1), ST3489 (n = 1), ST3896 (n = 1), ST3897 (n = 1), ST3899 (n = 1), ST3936 (n = 1), ST3937 (n = 1), ST3941 (n = 1), ST3942 (n = 2).
<sup>e</sup> The following STs had one isolate each (with the exception of ST1589, which was shared by two cattle isolates): ST75, ST101, ST131, ST192, ST351, ST322, ST795, ST3209, ST3058, ST3894, ST3900, ST3901, ST3935, ST3938, and ST3940.
ten cohabored on the same plasmids which sometimes carry additional resistance determinants (4, 5). In China, the fosA3 genes were often carried by IncF (F2:A-:B-, F16:A1:B1, F24:A-:B-, and F33:A-:B-) plasmids, but they have also been found on other plasmid groups (IncN, IncB/O, IncI, and untypeable) (4–6, 10). Besides plasmids, the finding highlights the potential for the spread of this resistance mechanism by clonal expansion.

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REFERENCES