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Transparency declarations
None to declare.

References

Identification of a novel fosXcc gene conferring fosfomycin resistance in Campylobacter

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Keywords: foodborne pathogens, multidrug resistance, food-producing animal, food safety

Sir,
Fosfomycin [(2R,3S)-3-methyloxiran-2-yl]phosphonic acid is a broad-spectrum antibiotic with bactericidal activity against both Gram-positive and Gram-negative bacteria. Fosfomycin inhibits bacterial cell wall synthesis by inactivating the enzyme UDP-N-acetylgalactosamine-3-enolpyruvyl transferase (MurA), which is essential for the catalysis of bacterial peptidoglycan biosynthesis. Thus, modification or overexpression of MurA can contribute to the development of fosfomycin resistance in bacteria. Other common mechanisms of resistance to fosfomycin include decreased drug uptake and inactivation of the antibiotic by addition of various groups. To date, several fosfomycin resistance genes have been functionally characterized in bacteria. FomA and FomB enzymes can inactivate fosfomycin by phosphorylation. FosA, FosB and FosX can inactivate fosfomycin by catalysing the addition of glutathione, l-cysteine and H2O, respectively, to its epoxide ring.

Campylobacter species are the leading foodborne pathogens worldwide, accounting for 400–500 million cases of diarrhoea each year. Over the past few decades, Campylobacter species have become increasingly resistant to several clinically important antimicrobial agents, compromising the effectiveness of clinical therapy. Various mechanisms of resistance to macrolides, fluoroquinolones, tetracyclines, aminoglycosides and β-lactams in Campylobacter have been extensively described. In addition, clinical trials have indicated that fosfomycin can effectively treat Campylobacter enteritis in humans. To date, no mechanism of fosfomycin resistance has been characterized in Campylobacter species. Here, we identified a novel fosX-like gene from Campylobacter coli (designated fosXcc), located in the multidrug resistance genomic island (MDRGI), which confers fosfomycin resistance to Campylobacter species.

C. coli DZB4 was isolated from swine faeces in China in 2012. Our recent study indicated that DZB4 contains a type II MDRGI consisting of 10 ORFs that is inserted between cadrF and CC01582 on the chromosome (GenBank accession number KC876749) and this MDRGI can mediate antibiotic resistance, at least to macrolides, aminoglycosides and tetracycline. Further analysis revealed that the fifth ORF, immediately upstream of erm(B), in type II MDRGI encodes a 136 amino acid protein that exhibits 26.9%, 34.2% and 63.9% identity to fosfomycin resistance determinants FosA, FosB and FosX found in Serratia marcescens (M85195), Staphylococcus haemolyticus (X89875) and Listeria monocytogenes (NP_465227.1), respectively. Protein structure prediction indicated that this putative protein has identical quaternary structure to FosX and contains the conserved active site residue E44, which plays a key role in catalysis in FosX but not in FosA or FosB.

Considering that the fosfomycin MIC for isolate DZB4 was >512 mg/L, which is much higher than that for reference strains
species. These results indicate that Campylobacter strains, which could be explained by the lack of the glucose-
not increase the bioactivity of fosfomycin in Campylobacter fosXbaI-F and fosXbaI-R. The MICs of fosfomycin were increased containing kanamycin and were confirmed by PCR using primers minants. The transformants were selected on Mueller–Hinton agar C1-89 and C114-1 (Table 1), without fosfomycin resistance deter-
strain NCTC 11168 as well as two Campylobacter isolates, C1-89 and C114-1 (Table 1), without fosfomycin resistance deter-
in this study, we successfully transferred fosXCC from donor strain C. coli DZB4 to the recipient strains C. jejuni NCTC 11168 and 81-176 using a natural transformation method. fosXCC was detected in the transformants 11168DZB4 and 81-176DZB4 by PCR using primers fosXbaI-F and fosXbaI-R. 11168DZB4 and 81-176DZB4 exhibited >16- and 32-fold increases in the MIC of fosfomycin compared with the WT DH5α strain (Table 1). This result confirmed that the fosfomycin determinant and its associated MDRGI are transferable between Campylobacter strains.

In conclusion, this is the first report of the novel fosfomycin resistance gene fosXCC in Campylobacter. Although fosfomycin is seldom used in livestock, fosXCC was identified in this swine C. coli isolate. This gene is associated with a chromosomal MDRGI that harbours multiple antibiotic resistance determinants, including to macrolides, aminoglycosides and tetracycline, suggesting that selection pressure for other antibiotics used for clinical treatments or as feed additives could cause the selection and persistence of fosXCC in Campylobacter. In view of the fact that fosfomycin has a broad spectrum of antimicrobial activity, it could be a drug of choice to treat Campylobacter enteritis; however, the presence of fosXCC in Campylobacter would severely limit the use of fosfomycin in the clinical setting. Thus, enhanced efforts are needed to mon-

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Description or relevant genotype</th>
<th>+G6P</th>
<th>−G6P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli DH5α</td>
<td>plasmid propagation E. coli strain</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>DH5α+pRRKfosXCC</td>
<td>DH5α containing pRRKfosXCC plasmid</td>
<td>256 (1512)c</td>
<td>1024 (12048)</td>
</tr>
<tr>
<td>DZB4</td>
<td>C. coli, isolated from swine faeces in 2012</td>
<td>&gt;512</td>
<td>&gt;512</td>
</tr>
<tr>
<td>NCTC 11168</td>
<td>WT C. jejuni</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>11168DZB4</td>
<td>NCTC 11168 derivative, containing MDRGI</td>
<td>&gt;512 (&gt;16);</td>
<td>&gt;512 (&gt;16);</td>
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<tr>
<td>11168+aphA3</td>
<td>NCTC 11168 derivative, rs::aphA3</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>11168+aphA3+fosXCC</td>
<td>NCTC 11168 derivative, rs::aphA3+fosXCC</td>
<td>&gt;512 (&gt;16);</td>
<td>&gt;512 (&gt;16);</td>
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<tr>
<td>81-176</td>
<td>WT C. jejuni</td>
<td>8</td>
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</tr>
<tr>
<td>81-176DZB4</td>
<td>81-176 derivative, containing MDRGI</td>
<td>256 (132)</td>
<td>256 (132)</td>
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<td>CI-89</td>
<td>C. jejuni chicken isolate</td>
<td>32</td>
<td>32</td>
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<tr>
<td>CI-89+aphA3+fosXCC</td>
<td>CI-89 derivative, rs::aphA3+fosXCC</td>
<td>&gt;512 (&gt;16);</td>
<td>&gt;512 (&gt;16);</td>
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<td>CI114-1</td>
<td>C. jejuni chicken isolate</td>
<td>32</td>
<td>32</td>
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<tr>
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<td>&gt;512 (&gt;16);</td>
<td>&gt;512 (&gt;16);</td>
</tr>
</tbody>
</table>

aPresence of glucose-6-phosphate.
bAbsence of glucose-6-phosphate.
cNumbers in parentheses indicate fold changes, increase from the susceptible strains (†).
MDR ST2179-CTX-M-15 Escherichia coli co-producing RmtD and AAC(6’)-Ib-cr in a horse with extraintestinal infection, Brazil

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Keywords: E. coli, equine, ESBLs, methylases, plasmid-mediated quinolone resistance, MLST, PMQR

Sir,

In recent years, ESBL genes have been increasingly reported in Enterobacteriaceae from various animal species. In this regard, recent reports published in this journal have documented the emergence of CTX-M-15-producing strains in horses from different European countries, even the pandemic Escherichia coli sequence types ST131 and ST68. In this report, we describe the emergence of an MDR ST2179-CTX-M-15 E. coli strain co-producing RmtD and AAC(6’)-Ib-cr in a foal with extraintestinal infection.

In March, 2012, a 3-month-old English thoroughbred foal was admitted to a private equine clinic with signs of diarrhoea, mild dehydration, moderate colic, anorexia and prostration. The owner reported watery diarrhoea for 3 days, anorexia and weakness for over a week. Physical examination showed moderate to severe dehydration, pale mucous membranes, hyperthermia, rectal temperature of 41°C, abdominal discomfort and distension, moderate colic, weakness and low body condition score. Treatment was initiated with intense fluids (lactated ringer solution), dexamethasone (0.2 mg/kg intravenously once a day) and 3 mg/kg gentamicin twice a day for 2 days. The clinical signs did not improve and the animal died 2 days after admission. Organ samples were aseptically collected and sent to the laboratory for microbiological investigation, where bacteriological cultures were determined by Etest or agar dilution methods. 4,5 The presence of ESBLs was screened by using Etest ESBL strips (bioMérieux) and CHROMagar® ESBL (France), and a range of...