Prevalence of fosfomycin resistance and plasmid-mediated fosfomycin-modifying enzymes among carbapenem-resistant Enterobacteriaceae in Zhejiang, China

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Abstract

Two hundred and thirty-three nonduplicated clinical isolates of carbapenem-resistant Enterobacteriaceae were collected from four hospitals in Zhejiang, China. 45.1% (105/233) strains were resistant to fosfomycin, among which plasmid-mediated fosfomycin-modifying enzymes fosA, fosA2, fosA3 and fosA5 were positive, and the other fos genes were negative. 80% (12/15) Enterobacter cloacae isolates were positive for fosA. 100% (73/73) Klebsiella pneumoniae isolates were positive for fosA5. A conjugation experiment indicated that fosfomycin resistance could be transferred to an Escherichia coli recipient strain successfully. Fosfomycin still exhibits partial activity in carbapenem-resistant Enterobacteriaceae, especially carbapenem-resistant Escherichia coli. To our knowledge, plasmid-mediated fosfomycin-modifying enzymes account for the dominance in the carbapenem-resistant Enterobacteriaceae. Therefore, we need to pay attention to the plasmid-mediated fosfomycin-modifying enzymes fosA and fosA5 in Enterobacter cloacae and K. pneumoniae to prevent clonal dissemination in China.

Infections caused by carbapenem-resistant Enterobacteriaceae (CRE) are increasingly reported worldwide [1] and alternative options for the treatment of CRE are limited [2]. Fosfomycin was discovered in 1969 and has a broad spectrum of bactericidal activity against a wide range of Gram-positive and Gram-negative bacteria [3]. Fosfomycin inhibits the synthesis of peptidoglycan by binding irreversibly to UDP-N-acetylgalactosamine enolpyruvyl transferase [4], so fosfomycin is always effective in the treatment of multidrug-resistant Enterobacteriaceae [5].

Resistance to fosfomycin is mainly exerted through two mechanisms: (i) impermeability owing to chromosomal mutations affecting the hexose-6-phosphate or La-glycero-phosphate uptake systems; (ii) plasmid-encoded resistance by inactivating the antibiotic molecule, which have been identified as due to drug modification enzymes. The plasmids determinant of resistance to fosfomycin have been described as four main types: fosA, fosB, fosC, fosX genes and their subtypes: fosA, fosA2, fosA3, fosA4, fosA5 and fosC2 genes [6–9].

Till now, plasmid-mediated fosfomycin resistance in carbapenem-resistant Enterobacteriaceae had only been reported in the United Kingdom [10]. The aim of this study is to investigate the occurrence of plasmid-mediated fosfomycin resistance genes in carbapenem-resistant Enterobacteriaceae in Zhejiang, China.

Two hundred and thirty-three nonduplicated clinical isolates of CRE were collected from the Second Affiliated Hospital of Zhejiang University School of Medicine (n=64), Hangzhou Traditional Chinese Medicine Hospital (n=23), Zhejiang Provincial People’s Hospital (n=36) and the Second Hospital of Jiaxing (n=110) from January 2010 to December 2014. Each isolate was collected from the unique patient isolated for the first time. They were isolated from sputum, blood, urine, stool, bile, sanis, etc. The minimum inhibitory concentration (MIC) of fosfomycin was determined by agar dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2014) [11], and the MICs of other antibiotics were determined by broth microdilution method. Screening for the fos genes was carried out by PCR amplification using specific primers, including fosA, fosA2, fosA3, fosA4, fosA5, fosB, fosC, fosC2 and fosX [12–15] genes. One Enterobacter cloacae isolate, one Citrobacter freundii isolate and two Klebsiella pneumoniae isolates were selected randomly as donors for the fos gene conjugation experiment. Sodium azide-resistant Escherichia coli J53 was used as the recipient strain.
The *Escherichia coli* transconjugants were selected on Mueller-Hinton (MH) agar medium containing 100 mg l\(^{-1}\) sodium azide and 32 mg l\(^{-1}\) fosfomycin.

The 95% confidence interval (CI) of fosfomycin resistance rate in 233 CRE isolates was 38.6%–51.7%. The resistance rate of fosfomycin increased progressively from *Escherichia coli* (8.2%), *Serratia marcescens*(25.0%), *Enterobacter cloacae* (50.0%) to *K. pneumoniae* (61.9%) (Table 1). Seventy-three *K. pneumoniae* isolates were positive for fosA5, and 7 of them harbored fosA3 simultaneously. Gene fosA was the most prevalent in fosfomycin-resistant *Enterobacter cloacae* (n=13), one of which harbored fosA, fosA2, fosA3 and one of which harbored fosA, fosA3 simultaneously. One *C. freundii* isolate was positive for fosA5. Seventeen isolates containing S. marcescens (n=7), *Morganella morganii* (n=3), *C. freundii* (n=2), *Enterobacter cloacae* (n=1) and *Escherichia coli* (n=4) were negative for all fos genes. None of the fosB, fosC, fosC2 and fosX genes were detected (Table 1).

Fosfomycin resistance of the four selected isolates were all transferred to *Escherichia coli* J53 successfully by conjugation. Only the MICs of cefazolin, ampicillin-subactam and cefuroxime of *Enterobacter cloacae* were raised. The MICs of other antibiotics of all four selected isolates changed little except for fosfomycin. Four *Escherichia coli* transconjugants all produced fos gene (data not shown).

Infections caused by carbapenem-resistant Enterobacteriaceae were reported all over the world in the last few years [1, 16–19], and the rate of CRE among all Enterobacteriaceae is about 7% in these four hospitals. With an accompanying problem of few choices of other antibiotics, certainly as an old antibiotic, fosfomycin goes into the doctor’s view again.

Fosfomycin is usually prescribed for urinary tract infection caused by *Escherichia coli* [20, 21]. There are some reports about the susceptibility of Enterobacteriaceae to fosfomycin *in vitro* [22, 23], and the overall rate of fosfomycin resistance in all Enterobacteriaceae is about 25% in our study. But there are few investigations about the antimicrobial activity of fosfomycin against carbapenem-resistant Enterobacteriaceae. Here in China we find that the resistance rate of fosfomycin to carbapenem-resistant Enterobacteriaceae is 45.1% (38.6%–51.7%), and the resistance rate of fosfomycin against carbapenem-resistant *Escherichia coli* and *S. marcescens* is as low as 8.2% (2.3%–19.6%) and 25.0% (10.7%–44.9%). In consequence, the antimicrobial susceptibility results indicate that fosfomycin can be used as a supplementary drug for severe infection caused by carbapenem-resistant Enterobacteriaceae (especially *Escherichia coli* and *S. marcescens*).

The mechanism of fosfomycin resistance may be due to chromosomal mutations or plasmid-mediated fosfomycin-modifying enzymes. We found that 17 fosfomycin-resistant CRE isolates were negative for all fos genes, which may have mutations in the chromosome genes including *murA*, *glpT*, *uhpT*, *uhpA*, *ptsI* and *cyaA* [24]. Plasmid-mediated fosfomycin-modifying enzymes can transfer in the same species or in different species rapidly. The fosA5 gene was found in *Escherichia coli* first in 2014 [5], however 73 *K. pneumoniae* isolates all produce fosA5 gene in our study, which might indicated that fosA5 gene was transferred from *Escherichia coli* to *K. pneumoniae* through whole plasmid transmission or mobile genetic element transmission. Our study demonstrated that plasmid-mediated fosfomycin-modifying enzymes accounted for a majority of the fosfomycin resistance. Continuous monitoring will be necessary to prevent further dissemination of fosfomycin resistance genes, together with prudent use of fosfomycin in clinical settings.

### Table 1. Fosfomycin resistance in carbapenem-resistant Enterobacteriaceae

<table>
<thead>
<tr>
<th>Strain</th>
<th>No.</th>
<th>MIC (mg l(^{-1}))</th>
<th>Resistance (No.)</th>
<th>RR (%) (95% CI)</th>
<th>fos gene type (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC(_{50})</td>
<td>MIC(_{90})</td>
<td>range</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>49</td>
<td>1</td>
<td>32</td>
<td>&lt;0.0625–2048</td>
<td>4</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>118</td>
<td>256</td>
<td>&gt;2048</td>
<td>4–2048</td>
<td>73</td>
</tr>
<tr>
<td><em>S. marcescens</em></td>
<td>28</td>
<td>8</td>
<td>&gt;2048</td>
<td>1–2048</td>
<td>7</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>30</td>
<td>128</td>
<td>&gt;2048</td>
<td>1–2048</td>
<td>15</td>
</tr>
<tr>
<td><em>M. morganii</em></td>
<td>4</td>
<td>512</td>
<td>&gt;2048</td>
<td>1–2048</td>
<td>3</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>4</td>
<td>512</td>
<td>512</td>
<td>128–512</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>233</td>
<td>128</td>
<td>&gt;2048</td>
<td>&lt;0.0625–2048</td>
<td>105</td>
</tr>
</tbody>
</table>

*No RR (resistance rate) statistics because of few isolates.*

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### Conflicts of interest

The authors declare that there are no conflicts of interest.

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