Short Communication

Prevalence of Fosfomycin Resistance in Methicillin-Resistant Staphylococcus aureus Isolated from Patients in a University Hospital in China from 2013 to 2015

Dandan Wu1,2†, Yan Chen1,3†, Lu Sun1, Tingting Qu1, Haiping Wang1, and Yunsong Yu1,5*  
1Department of Infectious Diseases, 1Department of Hospital Epidemiology and Infection Control, Sir Run Run Shaw Hospital, 2Department of Infectious Diseases, Second Affiliated Hospital, School of Medicine, and 3State Key Laboratory for Diagnosis and Treatment of Infectious Disease, First Affiliated Hospital, College of Medicine, Zhejiang University, Zhejiang; and 5Key Laboratory for Microorganism Technology and Bioinformatics Research of Zhejiang Province, Zhejiang, China

SUMMARY: In this study, we investigated the fosfomycin susceptibility rates among different methicillin-resistant Staphylococcus aureus (MRSA) clones. A total of 293 MRSA isolates obtained from Sir Run Run Shaw hospital during 2013–2015 were tested for fosfomycin susceptibility. The overall fosfomycin resistance rate among these MRSA isolates was 53.2%. Although 91.9% of the ST5 MRSA isolates (MIC50 > 1,024 mg/L) were resistant to fosfomycin, no fosfomycin-resistant isolate was found among the 69 ST59 MRSA isolates (MIC50/90, 0.5/4 mg/L). The fosfomycin resistance rate among the MRSA isolates recovered from skin and soft tissue infections was 19.1%, which was lower than the rates detected among MRSA isolates from other types of invasive infections. The fosfomycin resistance rate in community-onset MRSA was 30.2%, which was lower than that detected in healthcare-associated MRSA of 70.7%. One MRSA isolate had the fosB gene, whereas most (127/156) of the fosfomycin-resistant MRSA isolates had deletions in glpT genes. These findings highlight the importance of monitoring the fosfomycin susceptibility in MRSA isolates for epidemiological purposes.

Methicillin-resistant Staphylococcus aureus (MRSA), which is resistant to almost all available beta-lactam antimicrobial drugs (except for 5th-generation cephalosporins), has been spreading worldwide since 1961 (1). According to clinical and epidemiological features, MRSA isolates are usually classified into healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) (2). MRSA is the leading cause of surgical site infections, and is also associated with severe invasive diseases, including skin and soft tissue infections (SSTIs), bloodstream infections, and pneumonia in both communities and hospitals. Owing to the lack of novel antimicrobial agents, fosfomycin, an old antibiotic agent discovered in 1969, has gained renewed attention because of its activity against both gram-positive and gram-negative multidrug-resistant bacteria, including MRSA (3).

Fosfomycin is a bactericidal agent that inhibits the enzyme-catalyzed reaction of cell-wall synthesis in both gram-positive and gram-negative pathogens. Several mechanisms of resistance to fosfomycin have been identified to date, including mutations in the murA, chromosomal glpT, and uhpT genes, and several fosfomycin-modifying enzymes (FosA, FosB, FosC, FosX) (3). According to its available formulation for oral treatment and its lower cost compared to other anti-staphylococcal agents, fosfomycin has great application prospects for MRSA treatment.

In many countries, MRSA infections are becoming increasingly common in both hospitals and communities. However, the antimicrobial susceptibility of fosfomycin is not routinely reported for MRSA in China. Therefore, in the present study, we evaluated the fosfomycin susceptibility activity against different lineages of MRSA isolated from patients at a tertiary hospital in China.

We performed fosfomycin susceptibility testing for 293 non-duplicated MRSA isolates obtained from patients at Sir Run Run Shaw hospital (SRRSH), a tertiary hospital affiliated to Zhejiang University in Hangzhou, China, from January 2013 to December 2015. Multilocus sequence typing was performed for the MRSA isolates using standard methods (http://saureus.mlst.net/) in our previous study (unpublished data). The minimal inhibitory concentration (MIC) of fosfomycin against the MRSA isolates was determined by the agar dilution method in medium supplemented with glucose-6-phosphate (25 mg/L) based on the Clinical and Laboratory Standards Institute recommendations (4). The results were interpreted according to 2017 European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (http://www.eucast.org/clinical-breakpoints/). S. aureus ATCC 29213 was used as a quality control strain. Presence of the fosA, fosB, and

Received January 16, 2018. Accepted March 30, 2018.
DOI: 10.7883/yoken.JJID.2018.013
*Corresponding author: Mailing address: Department of Infectious Diseases, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang 310016, China. Tel: +86-571-8600-6142, Fax: +86-571-8600-6142, E-mail: yysys119@zju.edu.cn
†These authors contributed equally to this work.
fosC genes and the murA, glpT, and uhpT genes were screened using primers and PCR conditions described previously (5,6). MRSA infection that occurred $> 48$ h following hospitalization was defined as a healthcare-onset MRSA (HO-MRSA) infection; otherwise, it was defined as community-onset MRSA (CO-MRSA).

The MICs of fosfomycin ranged from < 0.25 mg/L to $> 1,024$ mg/L in these MRSA isolates, and the total fosfomycin resistance rate among the MRSA isolates was 53.2% (156/293) according to the EUCAST criteria (MIC $> 32$ mg/L) (Table 1). The fosfomycin resistance rate decreased significantly from 69.1% (85/123) in 2013 to 35.4% (28/79) in 2015 ($P < 0.0001$; Fig. 1A). Activity of fosfomycin against the MRSA isolates varied among different sequence type (ST) lineages (Table 1). For example, 91.9% (136/148) of the ST5 MRSA isolates (MIC50, $> 1,024$ mg/L) were resistant to fosfomycin, whereas only 66.7% (12/18) of ST239 MRSA isolates (MIC50/90, 64/512 mg/L) showed fosfomycin resistance, which represented a statistically significant difference ($P = 0.0057$). However, no fosfomycin-resistant isolate was found among the 69 ST59 MRSA isolates (MIC50/90, 0.5/4 mg/L) ($P = 0.0108$).

The fosfomycin resistance rate among the MRSA isolates recovered from SSTI cultures was 19.1% (18/94), which was significantly lower than the resistance rates of MRSA isolates causing bloodstream infections (52.9%, 9/17; $P = 0.0054$), respiratory infections (77.7%, 101/130; $P < 0.0001$), and urinary tract infections (58.8%, 10/17; $P = 0.0014$) (Fig. 1B). The fosfomycin resistance rate in all CO-MRSA isolates was 30.2% (38/126), which was significantly lower ($P = 0.0001$) than that of HO-MRSA isolates at 70.7% (118/167) (Fig. 1C).

Among the 293 MRSA isolates, only one ST88 MRSA isolate with an MIC of 128 mg/L harbored the fosB7 gene (GenBank accession number CP019566), which had been described previously (7), and no isolate was positive for fosA or fosC. Among the 156 fosfomycin-resistant MRSA isolates, 126 harbored 1-bp deletions in the glpT gene resulting a frame shift. One ST5 MRSA isolate had an 8-bp deletion in the glpT gene. Several isolates had missense mutations that led to amino acid residue replacement such as E194K and A235G in the murA gene. The GenBank accession number for the sequences of glpT and murA are KY618542 and KY618549, respectively.

Fosfomycin is considered to be active against both gram-negative and gram-positive pathogens, including Enterococcus spp., S. aureus, and Staphylococcus epidermidis (3). According to a review published in 2009 that evaluated 22 studies, fosfomycin exhibited considerable activity against MRSA with a cumulative susceptibility rate of 86.7% (4,240/4,892 isolates) (8). Consistently, our results showed that nearly half of the MRSA isolates from SRRSH were resistant to fosfomycin.

### Table 1. Distribution of fosfomycin resistance in methicillin-resistant Staphylococcus aureus isolates from SRRSH, 2013–2015

<table>
<thead>
<tr>
<th>ST</th>
<th>Isolate No.</th>
<th>Resistance No. (%)</th>
<th>MIC (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. (%)</td>
<td>Range</td>
</tr>
<tr>
<td>ST5</td>
<td>148</td>
<td>136 (91.9)</td>
<td>2–1024</td>
</tr>
<tr>
<td>ST59</td>
<td>69</td>
<td>0 (0.0)</td>
<td>&lt;0.25–8</td>
</tr>
<tr>
<td>ST239</td>
<td>18</td>
<td>12 (66.7)</td>
<td>4–1024</td>
</tr>
<tr>
<td>ST630</td>
<td>10</td>
<td>0 (0.0)</td>
<td>2–8</td>
</tr>
<tr>
<td>ST88</td>
<td>8</td>
<td>1 (12.5)</td>
<td>0.5–64</td>
</tr>
<tr>
<td>ST965</td>
<td>5</td>
<td>0 (0.0)</td>
<td>0.5–8</td>
</tr>
<tr>
<td>ST3338</td>
<td>4</td>
<td>0 (0.0)</td>
<td>0.25–1</td>
</tr>
<tr>
<td>ST398</td>
<td>3</td>
<td>0 (0.0)</td>
<td>0.5–1</td>
</tr>
<tr>
<td>ST25</td>
<td>3</td>
<td>0 (0.0)</td>
<td>8</td>
</tr>
<tr>
<td>ST1</td>
<td>2</td>
<td>0 (0.0)</td>
<td>1–32</td>
</tr>
<tr>
<td>ST22</td>
<td>2</td>
<td>0 (0.0)</td>
<td>&lt;0.25–1</td>
</tr>
<tr>
<td>ST221</td>
<td>1</td>
<td>2 (100.0)</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>ST403</td>
<td>1</td>
<td>1 (100.0)</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>ST772</td>
<td>1</td>
<td>0 (0.0)</td>
<td>2</td>
</tr>
<tr>
<td>ST889</td>
<td>1</td>
<td>1 (100.0)</td>
<td>64</td>
</tr>
<tr>
<td>ST3194</td>
<td>1</td>
<td>1 (100.0)</td>
<td>&gt;1024</td>
</tr>
<tr>
<td>New</td>
<td>15</td>
<td>13 (86.7)</td>
<td>0.5–1024</td>
</tr>
</tbody>
</table>

*ST, sequence type.*

*New, novel sequence type.*

Fig. 1. Prevalence of fosfomycin resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from Sir Run Run Shaw hospital (SRRSH), 2013–2015. (A) Percentages of fosfomycin-resistant isolates among MRSA isolates from 2013 to 2015. (B) Percentages of fosfomycin-resistant isolates among MRSA isolates from different types of infections. (C) Percentages of fosfomycin-resistant isolates among healthcare-onset MRSA (HO-MRSA) and community-onset MRSA (CO-MRSA). Categorical variables were analyzed by $\chi^2$ test or Fisher exact test for small samples. **$P < 0.005$; ***$P < 0.0001$. 

---

313
However, the fosfomycin resistance rate has been decreasing in our hospital in the last 3 years.

Fu et al. (6) reported that approximately 69.8% (67/96) of the MRSA isolates from blood and cerebrospinal fluid samples at a teaching hospital in Shanghai, China between 2004 and 2014 were resistant to fosfomycin (MIC ≥ 64.0 mg/L). The overall resistance rate was higher than that obtained in the present study. However, it should be noted that the majority of the isolates included in the previous study belonged to lineages ST5 and ST239. Our data showed the fosfomycin resistance varied significantly among different MRSA clonal lineages. ST5 and ST239 MRSA, which are the predominant HA-MRSA clones in China, are usually resistant to fosfomycin (9). However, fosfomycin showed excellent activity against ST59 MRSA, which is a predominant CA-MRSA clone reported in Asian countries (2). ST59 MRSA are the most common causes of SSTIs and community-acquired infections, which may explain the relatively low resistance rates to fosfomycin of the isolates obtained from SSTI cultures and CO-MRSA in our study.

FosB is a plasmid-encoded enzyme that catalyzes the reaction between cysteine and fosfomycin in Staphylococcus spp. In the present study, only one MRSA isolate was found to carry the fosB7 gene, and the positive rate of fosB was lower than the rate of 13.4% (9/67) previously reported in Shanghai, China (10). Most of the ST5 and ST239 MRSA isolates with fosfomycin MICs above 1,024 mg/L had mutations in glpT, and several mutations were detected in murA. However, further studies should be conducted to confirm the relationship between these mutations and fosfomycin resistance.

Given the lack of new antibiotics against multidrug-resistant bacteria, physicians have now started to use older antibiotics. Under this circumstance, fosfomycin has gained attention recently, since it shows potential synergistic activity with other agents against multidrug-resistant or extensively drug resistant bacteria, including MRSA (3,11,12). Several studies have indicated that fosfomycin achieves high concentrations in the skin and soft tissues (13,14). Since increased numbers of CA-MRSA infections have been reported in China, mainly SSTIs caused by ST59 MRSA, fosfomycin might become an important alternative treatment for SSTIs (15).

In conclusion, we demonstrated that fosfomycin displays significantly different activities against MRSA isolates from different clonal lineages. Therefore, it is important to monitor the fosfomycin susceptibility in MRSA strains, not only for evaluating the spread of resistance determinants but also for tracing the epidemiological change of MRSA infections.

Acknowledgments  This work was supported by National Natural Science Foundation of China (No.81501788 and No.81371859) and Zhejiang Provincial Natural Science Foundation of China (No. Q16H190005 and No. LQ18H190002).

Conflict of interest  None to declare.

REFERENCES