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Short Communication

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

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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 *fosB7* 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

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fosC genes and the *murA*, *glpT*, and *uhpT* genes was 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

ST[1)]	Isolate No.	Resistance No. (%)	MIC (mg/L)		
			Range	MIC50	MIC90
ST5	148	136 (91.9)	2～> 1,024	> 1,024	> 1,024
ST59	69	0 (0.0)	< 0.25～8	0.5	4
ST239	18	12 (66.7)	4～> 1,024	64	512
ST630	10	0 (0.0)	2～8	2	8
ST88	8	1 (12.5)	0.5～64	1	128
ST965	5	0 (0.0)	0.5～8	1	8
ST338	4	0 (0.0)	0.25～1	0.5	1
ST398	3	0 (0.0)	0.5～1	1	1
ST25	3	0 (0.0)	8	8	8
ST1	2	0 (0.0)	1～32	1	32
ST22	2	0 (0.0)	< 0.25～1	1	1
ST221	2	2 (100.0)	> 1,024	> 1,024	> 1,024
ST403	1	1 (100.0)	> 1,024	> 1,024	> 1,024
ST772	1	0 (0.0)	2	2	2
ST889	1	1 (100.0)	64	64	64
ST3194	1	1 (100.0)	> 1,024	> 1,024	> 1,024
New[2)]	15	13 (86.7)	0.5～> 1,024	> 1,024	> 1,024

[1)]: ST, sequence type.
[2)]: New, novel sequence type.

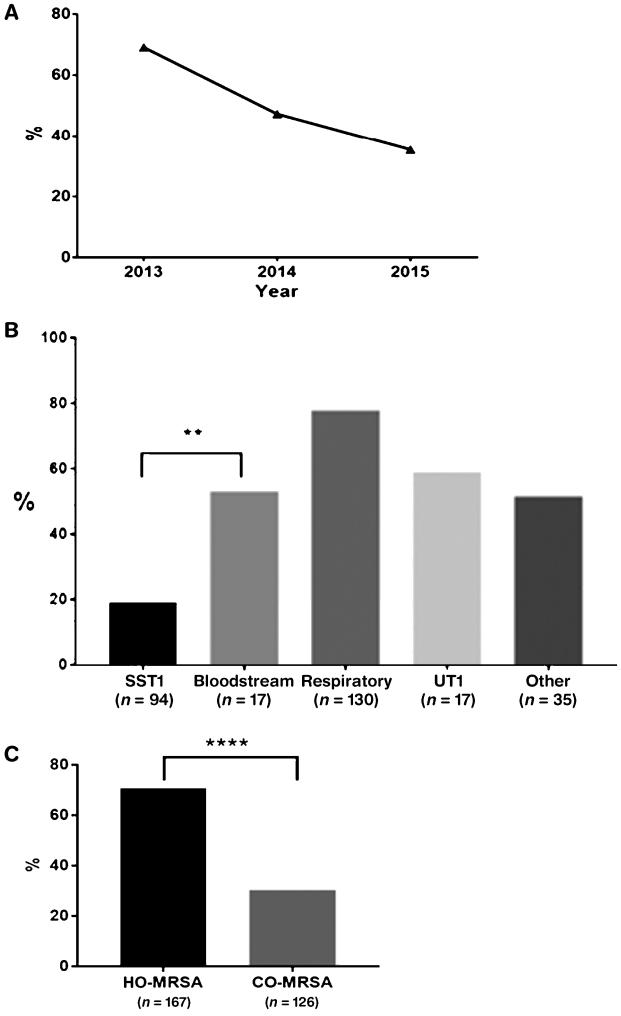

Fig. 1. Prevalence of fosfomycin resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from Sir Run Run Shaw hospital (SRRSH), 2013–2015. (A) Percentages of fosfomycin-resistant isolates among MRSA isolates from 2013 to 2015. (B) Percentages of fosfomycin-resistant isolates among 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. $^{**}P < 0.005$; $^{****}P < 0.0001$.

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 $\geq$ 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 linages. 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.

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Conflict of interest None to declare.

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