Prevalence of fosfomycin resistance and gene mutations in clinical isolates of methicillin-resistant Staphylococcus aureus

Yi-Chien Lee, Pao-Yu Chen, Jann-Tay Wang, and Shan-Chwen Chang

Abstract

Background: Fosfomycin exhibits excellent in vitro activity against multidrug-resistant pathogens, including methicillin-resistant Staphylococcus aureus (MRSA). Increasing fosfomycin resistance among clinical MRSA isolates was reported previously, but little is known about the relative abundance of Fosfomycin resistance genes in MRSA isolates circulating in Taiwan.

Methods: All MRSA isolates, collected in 2002 and 2012 by the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program, were used in this study. Susceptibility to various antimicrobial agents, including fosfomycin, was determined by broth microdilution. Genetic determinants of fosfomycin resistance, including fosB carriage and murA, glpT and uhpT mutations, were investigated using PCR and sequencing of amplicons. Staphylococcal protein A (spa) typing was also performed to determine the genetic relatedness of MRSA isolates.

Results: A total of 969 MRSA strains, 495 in the year 2002 and 474 in the year 2012, were analyzed. The overall in vitro susceptibility was 8.2% to erythromycin, 18.0% to clindamycin, 29.0% to tetracycline, 44.6% to ciprofloxacin, 57.5% to trimethoprim/sulfamethoxazole, 86.9% to rifampicin, 92.9% to fosfomycin and 100% to linezolid and vancomycin. A significant increase in the fosfomycin resistance rate was observed from 3.4% in 2002 to 11.0% in 2012. Of 68 fosfomycin-resistant MRSA isolates, several genetic backgrounds probably contributing to fosfomycin resistance were identified. Twelve isolates harbored the fosB gene, and various mutations in murA, uhpT, and glpT genes were noted in 11, 59, and 66 isolates, respectively. The most prevalent gene mutations were found in the combination of uhpT and glpT genes (58 isolates). The vast majority of the fosfomycin-resistant MRSA isolates belonged to spa type t002.

Conclusions: An increased fosfomycin resistance rate of MRSA isolates was observed in our present study, mostly due to mutations in the glpT and uhpT genes. Clonal spread probably contributed to the increased fosfomycin resistance.

Keywords: Fosfomycin, Resistance, Gene mutations, Methicillin-resistant Staphylococcus aureus
Background
Fosfomycin, a phosphonic acid derivative produced by Streptomyces spp. and discovered in 1969 [1], displays broad-spectrum activity against both Gram-negative and Gram-positive pathogens. It is a bactericidal antimicrobial agent that interferes with the enzyme-catalyzed bacterial cell wall synthesis [2]. Numerous studies have demonstrated the excellent in vitro susceptibility of multidrug-resistant and extensively drug-resistant organisms (MDRO and XDRs) to fosfomycin, including vancomycin-resistant enterococci (VRE) (96%) [3], ESBL-producing Enterobacteriaceae (87.7%) [4], carbapenem-resistant Gram-negative bacteria (99%) [5], and methicillin-resistant Staphylococcus aureus (MRSA) (99.6%) [6]. Additionally, the synergistic effect of fosfomycin in combination with other relevant antibiotics against the above-mentioned MDR microorganisms, evaluated by time-kill experiments, checkerboard analysis and E-test methods [7–9], was promising. These studies indicated that fosfomycin could be a potential treatment option for the difficult-to-treat infections caused by drug-resistant organisms.

Among MDROs, MRSA is a major human pathogen which causes various dangerous infections, such as bacteremia, endocarditis, and abscess, in both community and hospital settings [10]. Fosfomycin alone or combined with other antimicrobial agents exhibited favorable in vitro activity against MRSA [6, 8, 11, 12], and more than 70% clinical cure was observed with fosfomycin administration for the treatment of MRSA infection [13, 14]. However, S. aureus with fosfomycin resistance developed and rose rapidly by 30–70% in China [15]. The mechanism of bacterial resistance to fosfomycin could involve either a chromosome-associated defective transport system or plasmid-mediated fosfomycin-inactivating enzymes. First, two key transporter systems, GlpT and UhpT, mediated fosfomycin-inactivating enzymes. First, two associated defective transport system or plasmid-fosfomycin could involve either a chromosome- or plasmid-encoded mechanism [15, 25]. The mechanism of bacterial resistance to fosfomycin was confirmed by analyzing the nucleotide sequence utilizing PCR products were sequenced, and the full nucleotide sequence of three genes (murA, uhpT, glpT) was determined by combing direct sequencing and primer walking with the individual PCR products. Primers, used in the present study, are shown in Table 1. The PCR and sequencing procedures were described in prior studies [15, 25].

Methods
Bacterial isolates
All MRSA strains, collected in 2002 and 2012 through the TSAR program from different hospitals in Taiwan, were used in this study. The principles of isolate collection by the TSAR program had been described clearly in a previous study [8]. Duplicate isolates were excluded, and a total of 969 MRSA isolates, 495 collected in 2002 and 474 in 2012, were analyzed. All these strains were identified as S. aureus by performing Gram staining, catalase-activity test, and a coagulase latex agglutination test (automated VITEK-2 system, Biorieux, France). Methicillin resistance was ascertained using agar disk diffusion (Kirby-Bauer), according to the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [24]. The study was approved by the Ethical Committee of the National Taiwan University Hospital (NTUH-IRB No. 201504056RINB).

Antimicrobial susceptibility
The antimicrobial susceptibility to clindamycin, ciprofloxacin, erythromycin, linezolid, rifampicin, trimethoprim/sulfamethoxazole, tetracycline, fosfomycin, and vancomycin was determined using a broth microdilution method according to the CLSI recommendations [24], and the results were interpreted using the criteria for S. aureus provided by the CLSI [24]. Staphylococcus aureus ATCC 29213 was used as the internal control for each run of the susceptibility test.

Genetic analysis
DNA of 68 fosfomycin-resistant MRSA isolates was harvested using a DNA Extraction System kit (Viogene, New Taipei City, Taiwan) according to the manufacturer’s instructions. The presence of fosB was detected by PCR using the previously described primers [25], and the full nucleotide sequence of three genes (murA, uhpT, glpT) was determined by combing direct sequencing and primer walking with the individual PCR products. Primers, used in the present study, are shown in Table 1. The PCR and sequencing procedures were described in prior studies [15, 25].

Molecular typing
Staphylococcal protein A (spa gene) typing was performed for 68 fosfomycin-resistant MRSA isolates. The highly variable X region in spa was amplified by PCR using the previously described primers [26]. The purified PCR products were sequenced, and the spa types were confirmed by analyzing the nucleotide sequence utilizing
BioNumerics Version 6.5 (Applied Maths NV, Sint-Martens-Latem, Belgium) [27].

Statistical analysis
Categorical variables were expressed as percentages, and Fisher’s exact test with two-sided comparison was utilized for the assessment of statistical significance.

Results
Susceptibilities to tested antimicrobial agents
The overall susceptibilities to various antibiotics was demonstrated in Table 2. Susceptibility rates to clindamycin, erythromycin, tetracycline, and trimethoprim/sulfamethoxazole increased from 2002 to 2012; however, the susceptibility rates to rifampicin and fosfomycin between 2002 and 2012 decreased statistically significantly, shown in Table 2. All tested MRSA isolates remained 100% susceptible to linezolid and vancomycin. Among the fosfomycin-resistant MRSA isolates, lower susceptibility rates to clindamycin, ciprofloxacin, erythromycin, and rifampicin were noted compared with those of the fosfomycin-susceptible group. In contrast, the susceptibility rates to trimethoprim/sulfamethoxazole and tetracycline within the fosfomycin-susceptible group was lower (Table 3).

Prevalence of fosfomycin resistance genes
Patients with fosfomycin-resistant MRSA colonization/infection have a median age of 79 years (interquartile range: 69–85), which is significantly elder than that of patients with fosfomycin-susceptible MRSA (median, 59; interquartile range: 33–76). Most of those fosfomycin-resistant MRSA isolates were collected from the hospitals located in central (37 isolates) or southern (20 isolates) Taiwan, but the majorities of fosfomycin-susceptible MRSA strains were isolated from central (34.9%) and northern Taiwan (28%). Of 68 fosfomycin-resistant MRSA isolates, 12 strains harbored the fosB gene with fosfomycin MICs ranging from 128 to > 2048 mg/L (Table 4). Classification of different mutations in the murA, uhpT, and glpT genes, including various nucleic acid deletions and amino acid substitutions, was defined as following: type ImurA, G257D; type IImurA, D278E; type IIImurA, deletion at position 717; type IVmurA, G322S; type VmurA, L162I; type VImurA, E271Q; type VIImurA, G240R; type IuhpT, A279V; type IIuhpT, A297V/E225D; type IIIuhpT, F267L/L281X; type IVuhpT, G161R; type IglpT, A434V; type IIglpT, W147X; type IIIglpT, F197I; type IVglpT, A434V/G399S; type VglpT, C57X; type VglpT, T313K. Nevertheless, a total of 11 isolates expressed a murA mutation, and seven different subtypes, including type I–VIImurA, were identified in these mutant genes. The most commonly encountered fosfomycin-resistant murA mutant was type I (4 isolates).

Sixty-six of 68 fosfomycin-resistant MRSA strains contained one of the six different mutations (type I–VIglpT) found in the glpT gene with the majority containing a type IglpT mutation (60 isolates). Each of these mutations caused amino acid substitutions within the GlpT protein. Furthermore, four different mutations (type I–IVuhpT) were recognized in the uhpT gene of the 59

| Table 1 | PCR primers of fosA, fosB, fosC, murA, glpT, and uhpT genes |
| --- | --- | --- | --- | --- |
| Primers | Genes | Primer sequences (5’ > 3’) | Product size | References |
| fosB-F | fosB | CAGAGATATTTCAGGAGCTGACA | 312 bp | [25] |
| fosB-R | fosB | CTCACTATCTACTTTAAGGATTCTG | 1600 bp<sup>a</sup> | NC_002745.2<sup>b</sup> |
| murA-F | murA | GCCCTTGAAAGAATGGTTCGT | 1600 bp<sup>a</sup> | NC_002745.2<sup>b</sup> |
| murA-R | murA | GTTACAACTGACGCGAACGLT | 1699 bp<sup>a</sup> | NC_002745.2<sup>b</sup> |
| glpT-F | glpT | TGAATTAACACAGACGGCAGAA | 1571 bp<sup>a</sup> | NC_002745.2<sup>b</sup> |
| glpT-R | glpT | TACATCCTTCTACGTCGACCAC | |
| uhpT-F | uhpT | TGTTTATGGTCAGTATTTTGGA | |
| uhpT-R | uhpT | TTTTCTACTTCTCAGCGCAC | |

<sup>a</sup> PCR product including surrounding sequences adjacent to target gene
<sup>b</sup> GenBank-EMBL-DDBL accession number

| Table 2 | Antibiotics susceptibilities grouped by study year |
| --- | --- | --- | --- | --- |
| Antibiotics<sup>a</sup> | Overall susceptibilities (%) (n = 968) | Susceptibilities by years (%) | | |
| | | 2002 (n = 495) | 2012 (n = 473) | P |
| Clindamycin | 174 (18.0) | 49 (9.9) | 125 (26.4) | <0.001 |
| Ciprofloxacin | 432 (44.6) | 224 (45.3) | 208 (44.0) | 0.699 |
| Erythromycin | 79 (8.2) | 17 (3.4) | 62 (13.1) | <0.001 |
| Linezolid | 968 (100) | 495 (100) | 473 (100) | — |
| Rifampicin | 841 (86.9) | 456 (92.1) | 385 (81.4) | <0.001 |
| SXT | 557 (57.5) | 236 (47.7) | 321 (67.9) | <0.001 |
| Tetracycline | 281 (29.0) | 70 (14.1) | 211 (44.6) | <0.001 |
| Fosfomycin | 900 (93.0) | 478 (96.6) | 422 (89.2) | <0.001 |
| Vancomycin | 968 (100) | 495 (100) | 473 (100) | — |

<sup>a</sup> Antibiotic abbreviation: SXT, trimethoprim/sulfamethoxazole
fosfomycin-resistant MRSA isolates with type I \textit{uhpT} as the most prevalent (55 isolates). Similarly, the four mutations resulted in amino acid substitutions within the UhpT protein. Likewise, 58 fosfomycin-resistant MRSA isolates displayed dual resistance mechanisms. The details of those fosfomycin-resistant MRSA isolates harboring different types of mutation genes were described in Table 4.

### Molecular typing

The 68 fosfomycin-resistant MRSA strains were classified into several \textit{spa} types, including t002 (52 isolates), t037 (5 isolates), and other \textit{spa} types. The 52 \textit{spa} t002 fosfomycin-resistant isolates had a greater proportion (51/52) of high fosfomycin MICs (1024 – > 2048) than in the other 16 (12/16) fosfomycin-resistant isolates ($p = 0.019$). Among them, 11 isolates harbored the \textit{fosB} gene;...
5 strains, the murA gene; 52 and 48 mutants, the glpT and uhpT genes, respectively.

Discussion

A unique mechanism of action of fosfomycin made cross-resistance to other antibiotic classes less common, which motivated physicians to reevaluate its ability to destroy drug-resistant pathogens, including MRSA [16]. In our study, elderly patients seemingly had the tendency of acquisition of fosfomycin-resistant MRSA infections, and the vast majority of those resistant strains were isolated from those hospitals located in central or southern Taiwan. The background mechanisms of this phenomenon need further investigation. Although the MRSA isolates exhibited high in vitro susceptibility to fosfomycin higher than 90%, a significant increase in fosfomycin resistance rate during past decades (from 3.4% in 2002 to 11.0% in 2012) was observed in Taiwan. Among the fosfomycin-resistant isolates, a higher resistance rate to clindamycin, ciprofloxacin, erythromycin, and rifampicin was noted; however, trimethoprim/sulfamethoxazole and tetracycline displayed more favorable susceptibility. Type I_uhpT and type I_glpT mutations predominantly susceptibility fosfomycin resistance in our MRSA isolates, and the vast majority of isolates belonged to spa type t002.

Little was known about the MRSA fosfomycin resistance mechanism in epidemiological research, and very few literature reports were previously published [15, 28, 29]. Of those studies, a large-scale surveillance conducted by Fu et al. [15] in China demonstrated a 13.4% (9/67) fosB-positive rate with two-thirds (6/9) belonging to ST5. A research study by Etienne et al. [28] revealed that 18 of 39 (46.2%) S. aureus isolates, containing the fosB gene, were highly resistant to fosfomycin, but only one MRSA isolate had the fosB gene (Zhejiang, China) [29]. In our present study, approximately one-fifth of the MRSA isolates with fosfomycin resistance carried the fosB gene with the dominant t002 spa typing (11/12), which belonged to ST5. Our finding was similar to that described in Fu’s report; it implicated the presence of clonal spread among the fosB-positive MRSA isolates, despite the previously reported triviality of fosB [30].

MurA, a target enzyme involved in the biosynthesis of bacterial peptidoglycan, could be inactivated by fosfomycin via its binding to the active site of the enzyme [16]. However, mutations of the murA gene resulted in amino acid substitutions, rendering susceptible clinical isolates resistant to fosfomycin [16]. Fu et al. [15] illustrated that a murA mutation played an unclear role in the fosfomycin resistance in their study, and a type I_murA mutant was the most common among all murA mutations. Our results were different in this regard. The difference in the source of clinical specimens in these two studies might indicate that the mechanisms of fosfomycin resistance are different in Taiwan and mainland China.

The vast majority of the MRSA isolates in the present study possessed at least one of glpT and/or uhpT mutations, implicating that the genes, encoding transporter mutants, contributed to fosfomycin resistance substantially. This result contrasted to the findings of the preceding study [15]. The prevailing subtype of mutations in the glpT and uhpT genes was also different from that reported by Fu et al. [15]. Forty-eight fosfomycin-resistant MRSA isolates with dual resistance mechanisms (glpT and uhpT mutations) belonged to spa type t002, again implying clonal spread of fosfomycin-resistant MRSA.

The most prevalent and the second most common spa types were t002 and t037 in our study, respectively, revealing that fosfomycin resistance in MRSA isolates were correlated to some spa types. A similar correlation was noticed in other countries, including Sweden, Korea, China, Iran, Africa, Canada, and Brazil [31]. An international, or even intercontinental spread of specific fosfomycin-resistant MRSA clones may be occurring.

In the present study, the susceptibility of the MRSA isolates to various antibiotics was similar to that reported in the previous studies from Taiwan [8, 27] but different from that in other countries [32–34]. Variation in drug susceptibility between geographic areas might be due to the presence of different prevalent MRSA clones and the difference in antibiotic selective pressure.

The major limitation of the present study is that it was conducted using the clinical MRSA isolates in Taiwan; thus, worldwide generalization of the results should be made carefully.

Conclusions

In conclusion, our study illustrated that the fosfomycin resistance rate of the MRSA isolates increased significantly in the past, and mutations in the glpT and/or uhpT genes were key for inducing fosfomycin resistance. These findings indicated to physicians that they should prescribe fosfomycin cautiously for treating MRSA infections empirically. Furthermore, t002 was the most frequently seen spa type among the fosfomycin-resistant MRSA isolates. This was comparable to that in other countries globally. Therefore, it is necessary to continuously monitor fosfomycin resistance and its mechanisms.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13756-020-00790-x.

Additional file 1.

Abbreviations

MRSA: methicillin resistant Staphylococcus aureus; TSAR: Taiwan Surveillance of Antimicrobial Resistance; PCR: polymerase chain reaction;
spas: Staphylococcal protein A; MDRO: multidrug-resistant organisms; XDR: extensively drug-resistant organisms; VRE: vancomycin-resistant enterococci; ESBL: extended spectrum beta-lactamasas; CLSI: Clinical and Laboratory Standards Institute

Acknowledgements
Not applicable.

Authors’ contributions
YCL and PYC wrote the article and revised it critically for important intellectual content. JTY collected the data and did the analysis and interpretation of data. SCC was responsible for the conception and design of the study. SCC had given the final approval of the version to be published. All authors had read and approved the final manuscript.

Funding
There were no external or internal sources of specific funding for this paper, and the data were generated as part of the department’s routine work.

Availability of data and materials
The datasets used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
The study was carried out in accordance with the principles stated in the Declaration of Helsinki, and approved by the Ethical Committee of National Taiwan University Hospital (NTUH-IRB No. 201504056RINB). The Review Board approved to waive informed consent due to the retrospective study design and the research posing no more than minimal risk.

Consent for publication
Not applicable.

Competing interests
The authors declared no conflict of interest.

Author details
1Department of Internal Medicine, Fu Jen Catholic University Hospital, Fu Jen Catholic University, New Taipei City, Taiwan. 2School of Medicine, College of Medicine, Fu Jen Catholic University, New Taipei City, Taiwan. 3Department of Internal Medicine, National Taiwan University Hospital, 7 Chung-Shan South Road, 100 Taipei, Taiwan. 4Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Tsa-Nan County, Taiwan.

Received: 8 February 2020 Accepted: 23 July 2020
Published online: 17 August 2020

References
1. Hendlin D, Stapley EO, Jackson M, Wallick H, Miller AK, Wolf FJ, et al. Phosphonycin, a new antibiotic produced by strains of streptomyces. Science. 1969;166(32–3.
2. Skarzynski T, Mistry A, Wonacott A, Hutchinson SE, Kelly VA, Duncan K. Structure of UDP-N-acetylglucosamine enolpyruvyl transferase, an enzyme essential for the synthesis of bacterial peptidoglycan, complexed with substrate UDP-N-acetylglucosamine and the drug fosfomycin. Structure. 1996;4:1465–74.
3. Pogue JM, Marchain D, Abreu-Lanfranco O, Sunkara B, Mynatt RP, Zhao JJ, et al. Fosfomycin activity versus carbapenem-resistant Enterobacteriaceae and vancomycin-resistant Enterococcus, Detroit, 2008-10. J Antimicrob Agents Chemother. 2013;66:262–7.
4. Cho YH, Jung SI, Chung HS, Yu HS, Hwang EC, Kim SO, et al. Antimicrobial susceptibilities of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniea in health care-associated urinary tract infection; focus on susceptibility to fosfomycin. Int Urol Nephrol. 2015;47: 1059–66.
5. Tuo F, Roca GL, Formighieri MS, Sfori S, Bertoldi MB, Palmeiro JK, et al. Clinical Fosfomycin susceptibility of isolates with blaKPC-2 from Brazil. Int J Infect. 2013;67:247–9.
6. Champion EA, Miller MB, Popowitch EB, Hobbs MM, Saiman L, Muhelebau MS, et al. Antimicrobial susceptibility and molecular typing of MRSA in cystic fibrosis. Pediatr Pulmonol. 2014;49:230–7.
7. Descoutouez JL, Jorgensen MR, Wegrin JE, Rose WE. Fosfomycin synergy in vitro with amoxicillin, daptoycin, and linezolid against vancomycin-resistant Enterococcus faecium from renal transplant patients with infected urinary stents. Antimicrob Agents Chemother. 2013;57:1518–20.
8. Lee YC, Chen PJ, Wang JT, Chang SC. A study on combination of daptomycin with selected antimicrobial agents: in vitro synergistic effect of MIC value of 1 mg/L against MRSA strains. BMC Pharmacol Toxicol. 2019;20:25.
9. Samonis G, Maraki S, Karageorgopoulos DE, Vouloumanou EK, Falagas ME. Synergy of fosfomycin with carbapenems, colistin, netilmicin, and tigecycline against multidrug-resistant Klebsiella pneumoniae, Escherichia coli, and Pseudomonas aeruginosa clinical isolates. Eur J Clin Microbiol Infect Dis. 2012;31:695–701.
10. Lowy FD. Staphylococcus aureus infections. N Engl J Med. 1998;339;520–32.
11. Rebiahi SA, Abdelouahid DE, Rahmoun M, Abdelali S, Azzouzi H. Emergence of vancomycin-resistant Staphylococcus aureus identified in the Tlemcen university hospital (north-West Algeria). Med Mal Infect. 2011;41:646–51.
12. Lingscheid T, Tobuscud S, Poeppl W, Mitteregger D, Burgmann H. In vitro activity of doripenem plus fosfomycin against drug-resistant clinical blood isolates. Pharmacology. 2013;91:214–8.
13. Dinh A, Salomon J, Bru JP, Bernard L. Fosfomycin: efficacy against infections caused by multidrug-resistant bacteria. Scand J Infect Dis. 2012;44:182–9.
14. Floret A, Chichmanian RM, Cua E, Pulcini C. Adverse events associated with intravenous fosfomycin. Int J Antimicrob Agents. 2011;37:82–3.
15. Fu Z, Ma Y, Chen C, Guo T, Hu F, Liu Y, et al. Prevalence of Fosfomycin resistance and mutations in murA, glpT, and upf1 in methicillin-resistant Staphylococcus aureus strains isolated from blood and cerebrospinal fluid samples. Front Microbiol. 2016;61:1544.
16. Aghamali M, Sedighi M, Zahedi Bakvaei A, Mohammadzadeh N, Abbasian S, Ghafouri Z, et al. Fosfomycin: mechanisms and the increasing prevalence of resistance. J Med Microbiol. 2019;68:11–25.
17. Takahata S, Ida T, Hirashiti T, Sakokabara S, Maebashi K, Terada S, et al. Molecular mechanisms of fosfomycin resistance in clinical isolates of Escherichia coli. Int J Antimicrob Agents. 2010;35:333–7.
18. Nilsson AI, Berg OG, Aspevall O, Kahlmeter G, Andersson DI. Biological costs and mechanisms of fosfomycin resistance in Escherichia coli. Antimicrob Agents Chemother. 2003;47:2850–8.
19. Horii T, Kimura T, Sato K, Shibayama K, Ohta M. Emergence of fosfomycin-resistant isolates of Shiga-like toxin-producing Escherichia coli O26. Antimicrob Agents Chemother. 1999;43:789–93.
20. Bennat BA, Laughlin LT, Armstrong RN. Fosfomycin resistance protein (FosA) is a manganese metalloglutathione transferase related to glyoxalase I and the extradiol dioxygenases. Biochemistry. 1997;36:3050–5.
21. Lee SY, Park YJ, Yu JK, Jung S, Kim Y, Jeong SH, et al. Prevalence of acquired fosfomycin resistance among extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae clinical isolates in Korea and β2S6-composite transposon surrounding fosA. J Antimicrob Chemother. 2012;67:2843–7.
22. Filigrove KL, Pahkomo VA, Schaab MR, Newcomer ME, Armstrong RN. Structure and mechanism of the genomically encoded fosfomycin resistance protein, FosX, from litteria monocytogenes. Biochemistry. 2007;46:8110–20.
23. Satry S, Doyi Y. Fosfomycin: resurgence of an old companion. J Infect Chemother. 2016;22:273–80.
24. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-eighth Informational Supplement M100-S20. Wayne: CLSI; 2018.
25. Chen C, Xu X, Qu T, Yu Y, Ying C, Liu Q, et al. Prevalence of the fosfomycin-resistance determinant, fosB3, in Enterococcus faecium clinical isolates from China. J Med Microbiol. 2014;63:1484–9.
26. Larson AR, Stegger M, Serum M. Spa typing directly from a meca+ spa and pvl multiplex PCR assay-a cost-effective improvement for methicillin-resistant Staphylococcus aureus surveillance. Clin Microbiol Infect. 2008;14:611–4.
27. Lee CT, Fang YP, Chang YF, Wu TH, Yang YY, Huang YC. Comparison of molecular epidemiology of bloodstream methicillin-resistant Staphylococcus aureus isolates between a new and an old hospital in Central Taiwan. Int J Infect Dis. 2019;79:162–8.
28. Etienne J, Gerbaud G, Fleurette J, Courvalin P. Characterization of staphylococcal plasmids hybridizing with the fosfomycin resistance gene fosB. FEMS Microbiol Lett. 1991;68:119–22.
29. Wu D, Chen Y, Sun L, Qu T, Wang H, Yu Y. Prevalence of Fosfomycin resistance in methicillin-resistant Staphylococcus aureus isolated from patients in a University Hospital in China from 2013 to 2015. Jpn J Infect Dis. 2018;71:312–4.

30. Falagas ME, Athanasaki F, Voulgaris GL, Triarides NA, Vardakas KZ. Resistance to fosfomycin: mechanisms, frequency and clinical consequences. Int J Antimicrob Agents. 2019;53:22–8.

31. Asadollahi P, Farahani NN, Mirzaal M, Khoramrooz SS, van Belkum A, Asadollahi K, et al. Distribution of the Most prevalent Spa types among clinical isolates of methicillin-resistant and -susceptible Staphylococcus aureus around the world: a review. Front Microbiol. 2018;9:163.

32. Liang Y, Tu C, Tan C, El-Sayed Ahmed MAE, Dai M, Xia Y, et al. Antimicrobial resistance, virulence genes profiling and molecular relatedness of methicillin-resistant Staphylococcus aureus strains isolated from hospitalized patients in Guangdong Province, China. Infect Drug Resist. 2019;12:447–59.

33. Stefanaki C, Jeronymaki A, Matoula T, Caroni C, Polythodoraki E, Chryssou SE, et al. Six-Year Retrospective Review of Hospital Data on Antimicrobial Resistance Profile of Staphylococcus aureus Isolated from Skin Infections from a Single Institution in Greece. Antibiotics (Basel). 2017;6:39.

34. Peng H, Liu D, Ma Y, Gao W. Comparison of community- and healthcare-associated methicillin-resistant Staphylococcus aureus isolates at a Chinese tertiary hospital, 2012-2017. Sci Rep. 2018;8:17916.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.