

RESEARCH ARTICLE

Open Access



# Dissemination of fusidic acid resistance among *Staphylococcus aureus* clinical isolates

Fangyou Yu<sup>1\*</sup>, Yunling Liu<sup>2</sup>, Chaohui Lu<sup>2</sup>, Jinnan LV<sup>1</sup>, Xiuqin Qi<sup>1</sup>, Yu Ding<sup>1</sup>, Dan Li<sup>1</sup>, Xiaoying Huang<sup>2</sup>, Longhua Hu<sup>3</sup> and Liangxing Wang<sup>2\*</sup>

## Abstract

**Background:** A significant trend towards increased fusidic acid (FA) resistance among *Staphylococcus aureus* with increased duration of use is of concern. The aim of the present study is to investigate the dissemination of fusidic acid resistance among *Staphylococcus aureus* clinical isolates.

**Methods:** The susceptibility of *S. aureus* isolates to antimicrobial agents was determined by disc-diffusion method. The minimal inhibitory concentrations (MICs) of fusidic acid and vancomycin for fusidic acid-resistant isolates were determined by agar dilution method. FA resistance determinants were determined by PCR and DNA sequencing. SCCmec typing, spa typing and multi-locus sequence typing were used for the determination of molecular characteristics for *S. aureus* isolates.

**Results:** A total of 392 non-duplicate *S. aureus* isolates including 181 methicillin-resistant *S. aureus* (MRSA) isolates, which were isolated from the clinical specimens of patients at a Chinese tertiary hospital from January, 2012 to September, 2013, were collected for investigating FA resistance. Among 392 *S. aureus* clinical isolates tested, 56 (14.3 %) with FA MIC values ranging from 2 µg/ml to ≥128 µg/ml were resistant to FA. The proportions of FA resistance among MRSA and MSSA isolates were 27.1 % (49/181) and 3.3 % (7/211). There was a trend of rapidly increased FA resistance among *S. aureus* and MRSA isolates from 5.2 % and 8.9 % in 2012 to 24.9 % and 45.1 % in 2013. Acquired FA resistance gene, *fusB*, was present in 73.2 % (41/56) of FA-resistant *S. aureus* isolates. *fusC* and *fusA* mutation were not found in any of tested isolates. A total of 9 sequence types (STs) and 12 spa types were identified among the 56 FA-resistant *S. aureus* isolates. ST5 accounting for 66.1 % (37/56) was the most prevalent ST. The majority (92.9 %, 52/56) of the isolates tested belonged to clonal complex 5 (CC5). t2460 was the most prevalent spa type, accounting for 67.9 % (38/56). ST5-MRSA-II-t2460 was predominant clone, accounting for 75.5 % (37/49) of FA-resistant MRSA isolates and 66.1 % (37/56) of FA-resistant *S. aureus* isolates. Five of 7 FA-resistant MSSA isolates belonged to ST630-MSSA.

**Conclusion:** Increased FA resistance among *S. aureus* isolates was found in China. *fusB* was predominant FA resistance determinant. The spread of CC5 clone, especially novel ST5-MRSA-II-t2460 clone with high-level resistance to FA, was responsible for the increase of FA resistance.

**Keywords:** *Staphylococcus aureus*, Fusidic acid, Resistance, Resistance determinants, Molecular characteristic

## Background

*Staphylococcus aureus*, especially methicillin-resistant *S. aureus* (MRSA), is an important and often encountered human pathogen responsible for many infectious diseases from mild superficial skin infection to life-threatening diseases, including skin and soft tissue infections (SSTIs),

foreign-body infections, pneumonia, septic arthritis, endovascular infections, osteomyelitis, sepsis, and bloodstream infections (BSI) in both hospitals and community settings [1]. Since the first European isolate of MRSA was detected in 1960s, MRSA with resistance to all available penicillins and other β-lactam antimicrobial agents has become a leading cause of nosocomial infections in hospitals and other healthcare facilities. In addition to β-lactam, MRSA isolates are often resistant to erythromycin, clindamycin, aminoglycosides and quinolones. Since the mid-1990s, however, MRSA isolates are found to be responsible for

\* Correspondence: wzxyfy@163.com; wangliangxin2014@163.com

<sup>1</sup>Department of Laboratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

<sup>2</sup>Department of Respiratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China

Full list of author information is available at the end of the article

severe infections in individuals lacking risk factors for exposure to the health care system [1, 2]. MRSA infections has become a global concern [1, 3]. As vancomycin has relatively clean safety profile and durability against the development of resistance, it has been considered to be the optimal option for the treatment of invasive MRSA infections [4]. However, the emergence of vancomycin- hetero-resistant *S. aureus* (hVISA), vancomycin-intermediate *S. aureus* (VISA) and vancomycin-resistant *S. aureus* (VRSA) limits the use of vancomycin for the treatment of serious MRSA infections [5–7]. In addition, the drawbacks of vancomycin, including poor tissue penetration, serious nephrotoxicity and neutropenia and unpredictable synergy with other antimicrobial agents, also limit the selection of the alternatives for the treatment of MRSA infections. Fusidic acid (FA), is an effective agent for the treatment of SSTIs, acute osteomyelitis, chronic osteomyelitis, vertebral infection, septic arthritis, and prosthetic and other device related infections caused by MRSA and methicillin-susceptible *S. aureus* (MSSA) [8–10]. FA dose of 250–500 mg every 8–12 h was recommended for the treatment of MRSA infections [8]. However, recent study reported that front-loaded dosing of  $\geq 1200$  mg every 12 h (q12h) has better activity against MRSA infections than non-front-loaded dosing (600 mg q12 h) [11]. Although FA has not been approved for use in the USA by the US FDA, this drug has been widely used in Europe, Canada, Australia and some Asian countries for decades [8]. FA is a valuable alternative to vancomycin for the treatment of MRSA infections. In the 1990s, the prevalence of resistance to FA in *S. aureus* remained low. But there is a concern that there is a significant trend towards increased FA resistance among *S. aureus* with increased duration of use. In a surveillance report on FA resistance in 13 European countries, the prevalence of resistance to FA among *S. aureus* isolates was 10.7 %, with the highest resistance rates of 62.4 % among the isolates from Greece [12]. Although many Asian countries have reported FA resistance among *S. aureus* isolates, these data in China are limited. The aim of the present study is to investigate the dissemination of fusidic acid resistance among *S. aureus* clinical isolates from a tertiary hospital between 2012 and 2013 in Wenzhou, east China.

## Methods

### Collection of *S. aureus* clinical isolates

From Jan. 2012 to Sep. 2013, a total of 392 non-duplicate *S. aureus* isolates (single isolate per patient) from the various specimens of patients at the first Affiliated Hospital of Wenzhou Medical University locating in Wenzhou, east China, were selected for investigating the prevalence of FA resistance. The numbers of *S. aureus* isolates included in 2012 and 2013 were 211 and 181. Isolates were identified as *S. aureus* by using Gram's stain, positive catalase

and coagulase test results, and Vitek microbiology analysis (BioMérieux, Marcy l'Etoile, France). *S. aureus* ATCC25923 was used as a control strain for the identification of bacteria. Because the present study focused on bacteria, the Ethics Committee of the first Affiliated Hospital of Wenzhou Medical University exempted this investigation from review. Informed consents were required from patients for sample collection.

### Antimicrobial susceptibility testing

*S. aureus* susceptibilities to penicillin (10  $\mu$ g), erythromycin (15  $\mu$ g), clindamycin (2  $\mu$ g), rifampicin (5  $\mu$ g), tetracycline (30  $\mu$ g), linezolid (30  $\mu$ g), quinupristin/dalfopristin (15  $\mu$ g), trimethoprim/sulfamethoxazole (1.25/23.75  $\mu$ g), gentamicin (10  $\mu$ g), ciprofloxacin (5  $\mu$ g), chloramphenicol (30  $\mu$ g), fusidic acid (5  $\mu$ g) and nitrofurantoin (300  $\mu$ g) were determined using the disk diffusion test recommended by the Clinical and Laboratory Standards Institute (CLSI) [13]. Every assay was replicated for three times. The mean value was applied for the assay. All disks were obtained from Oxoid Ltd. Vancomycin MICs for *S. aureus* isolates were determined by the agar dilution method in accordance with the CLSI guidelines [14]. Interpretive standards of antimicrobial susceptibility testing for *S. aureus* isolates tested was in accordance with the guidelines provided by CLSI [13]. Initial screening of FA resistance was performed by disk diffusion method with 5  $\mu$ g FA containing disks. The isolates with an inhibition zone  $\leq 17$  mm in diameter were further determined for FA MIC values by an agar dilution method. The interpretive criterion of FA susceptibility is in accordance with the European Committee for Antimicrobial Susceptibility Testing (EUCAST)/ criteria (susceptible, MIC  $< 2$   $\mu$ g/ml; resistant, MIC  $\geq 2$   $\mu$ g/ml). *S. aureus* ATCC 25923 and *Escherichia coli* ATCC25922 were used as reference strains for antimicrobial susceptibility testing.

### DNA extraction

*S. aureus* isolates with FA resistance were cultured on blood agar overnight at 35 °C. Then, three to four bacterial colonies were suspended in 150  $\mu$ l sterile distilled water with lysostaphin (1 mg/mL) (Sangon, Shanghai, China) and incubated at 37 °C for an hour. Finally, DNA was extracted following the instructions of the Genomic DNA Extraction kit (Sangon, Shanghai, China), stored at  $-20$  °C and prepared for PCR assays.

### MRSA identification

A simplex PCR was used for the detection of *mecA* using MRSA N315 as positive control strain. The isolates positive for *mecA* were identified as MRSA. And cefoxitin disk diffusion test was also used for the detection of MRSA in accordance with the guideline provided by the Clinical and Laboratory Standards Institute (CLSI) [13].

### Detection of FA resistance determinants by PCR

Detection of *fusA* mutations and acquired FA resistance determinants including *fusB* and *fusC* was detected by PCR assays with primers and reaction conditions described previously [15]. DNA sequencing was used for the identification of genotypes of acquired FA resistance determinants. The *fusA* mutations were determined by sequencing entire *fusA* gene.

### SCCmec typing

SCCmec typing of MRSA isolates was performed using a battery of multiplex PCRs as described previously [16]. The MRSA isolates with unanticipated fragments or lacking fragments by multiplex PCRs were defined as non-typeable (NT). MRSA NCTC 10442 (SCCmec I), MRSA N315 (SCCmec II), MRSA 85/2082 (SCCmec III), MRSA JCSC 4744 (SCCmec IV) and MRSA WZ153 (SCCmec V) were used as control strains for SCCmec typing.

### spa typing

The *spa* variable repeat region from each *S. aureus* isolate was amplified using simplex PCR oligonucleotide primers as previously described [17, 18]. Following their purification and sequencing, *spa* types were assigned using the *spa* database website (<http://spaserver.ridom.de>).

### Multilocus sequence typing (MLST)

MLST typing of *S. aureus* isolates was performed using amplification of internal fragments of the seven house-keeping genes (*arcc*, *aroe*, *glpf*, *gmk*, *pta*, *tpi* and *yqil*) of *S. aureus* as described previously [19]. Following purification and sequencing of these genes, the sequences were compared with the existing sequences available on the MLST website for *S. aureus* (<http://saureus.mlst.net>), and STs were assigned according to the allelic profiles.

## Results and discussion

### Prevalence of FA resistance among *S. aureus* isolates

Among 392 *S. aureus* isolates tested including 181 MRSA isolates, 56 (14.3 %) with FA MIC values ranging from 2 to  $\geq 128$   $\mu\text{g/ml}$ , including 49 (87.5 %, 49/56) MRSA and 7 (12.5 %, 7/56) MSSA, were resistant to FA. The proportions of FA resistance among MRSA and MSSA isolates were 27.1 % (49/181) and 3.3 % (7/211). The sources for the isolation of the 56 FA-resistant isolates included sputum (42, 75 %), pus (10, 17.9 %), catheter (1, 1.8 %), blood (1, 1.8 %) and exudates (2, 3.6 %). Compared with previous reports from China with FA resistance rates of 3.5 % (4/116) from Shanghai and 2.2 % (4/186) from Chinese children with SSTIs [20, 21], the prevalence of FA resistance among *S. aureus* clinical isolates in the present study was very high. The resistance rates to FA among *S. aureus* isolates are different from

country to country. The resistance rates of *S. aureus* isolates to FA were very low in the USA (0.3 %) and relatively high in Canada (7.0 %) and Australia (7.0 %) [22]. In an updated surveillance report on FA resistance in 13 European countries, Israel, Italy, Poland, Spain and Sweden had low rates (1.4–3.1 %), while Greece (62.4 %) and Ireland (19.9 %) had the highest resistance rates [12]. In most Asian countries, FA resistance rates are relatively low (<10 %) [8]. FA resistance was observed more frequently among MSSA isolates (0.6 %) than among MRSA isolates from the United States [22]. On the contrary, the prevalence of FA resistance among MRSA isolates was significantly higher than that among MSSA isolates in the present study. There is a significant trend towards increased FA resistance among *S. aureus* since FA has been used widely. In Kuwait, the rate of FA resistance increased rapidly from 22 % in 1994 to 92 % in 2004 [23]. There was a trend of rapidly increased FA resistance among *S. aureus* and MRSA isolates from 5.2 % and 8.9 % in 2012 to 24.9 % and 45.1 % in 2013 in this investigation. The MIC values of FA for 56 FA-resistant isolates were as follows:  $\geq 128$   $\mu\text{g/ml}$ , 43; 16  $\mu\text{g/ml}$ , 2; 8  $\mu\text{g/ml}$ , 1; 4  $\mu\text{g/ml}$ , 7; and 2  $\mu\text{g/ml}$ , 3. In 2012, only 5 *S. aureus* isolates (45.5 %, 5/11) with FA MIC value of  $\geq 128$   $\mu\text{g/ml}$  were identified. Surprisingly, the FA MIC values for 38 (84.4 %) of 45 FA-resistant isolates were  $\geq 128$   $\mu\text{g/ml}$  in 2013. The resistance rates of FA-resistant *S. aureus*, MRSA and MSSA isolates to antimicrobials tested were showed in Table 1. All 56 FA-resistant isolates were susceptible to vancomycin, quinupristin-dalfopristin, linezolid, and

**Table 1** Antimicrobial resistance profiles of FA-resistant *S. aureus*, MRSA and MSSA isolates

	<i>S. aureus</i> (n = 56)	MRSA (n = 49)	MSSA (n = 7)
	R <sup>a</sup> (%)	R(%)	R(%)
Tetracycline	75	81	28.6
Gentamicin	28.6	30.6	14.3
Penicillin	100	100	100
Clindamycin	89.3	91.8	71.4
Erythromycin	92.9	95.9	71.4
Ciprofloxacin	82.1	87.8	42.9
Linezolid	0	0	0
Rifampicin	10.7	10.2	14.3
Trimethoprim/ sulfametho-xazole	3.6	4.1	0
Nitrofurantoin	0	0	0
Chloramphenicol	5.4	6.1	0
Quinupristin/ dalfopristin	0	0	0
Vancomycin	0	0	0

a: R, resistance

nitrofurantoin. The antimicrobials with the resistance rates of more than 50 % among FA-resistant *S. aureus* isolates were penicillin (100 %), erythromycin (92.9 %), clindamycin (89.3 %), ciprofloxacin (82.1 %), and tetracycline (75.0 %), respectively. The resistance rates of the 56 isolates to gentamycin, rifampin, trimethoprim/sulfamethoxazole and chloramphenicol were relatively low. In contrast to the report from Taiwan among which the resistance rate to rimethoprim/sulfamethoxazole among FA-resistant MRSA isolates was high to 97 % [24], only 4.9 % (4/81) of FA-resistant MRSA isolates were resistant to this antimicrobial.

### Prevalence of FA resistance determinants

Two major FA resistance mechanisms have been reported in *S. aureus*, including the alteration of the drug target site, which is due to mutations in *fusA* encoding elongation factor G (EF-G) or *rplF* encoding ribosome protein L6), and the protection of the drug target site by FusB family proteins including FusB, FusC, and FusD [8]. In staphylococci, high-level FA resistance is usually associated with mutations in *fusA* encoding EF-G, while low-level resistance is generally caused by the horizontally transferable genes including *fusB*, *fusC* and *fusD* [25]. The spontaneous mutations in *fusA* lead to modification of the drug target and reduced susceptibility [26]. The *fusB*-type resistance involves the recruitment of an exogenous resistance determinant whose product binds EF-G and protects it from FA [27]. In this study, the results from PCR and DNA sequencing demonstrated that acquired resistance gene, *fusB* was present in 73.2 % (41/56) of FA-resistant isolates tested. However, *fusC* and *fusA* mutations were not found in any of tested isolates. The previous

investigation from China found that all 4 clinical isolates with FA resistance were positive for *fusB* and negative for *fusC* and *fusA* mutations [20]. These data indicated that *fusB* is the predominant determinant responsible for FA resistance among *S. aureus* in China. In Netherlands, *fusB* was also found to be predominant FA resistance determinant which was detected in about 90 % of FA-resistant *S. aureus* isolates [28]. On the contrary, a report from northern Taiwan found that 84 % of FA-resistant MRSA isolates had *fusA* mutations and another report from a Taiwanese hospital found that *fusC* was the most common FA resistance determinant [15, 24]. *S. aureus* isolates from Australia with FA MIC values ranging from 2 to 32 µg/ml were predominantly *fusC* positive [22]. In U.S. and European collections, *fusC* was also more prevalent than *fusB* in FA-resistant *S. aureus* isolates [12, 22]. In contrast to previous report from Taiwan among which the distribution of FA resistance determinants including *fusA* mutations, *fusB* and *fusC* was quite different between MRSA and MSSA groups and no MRSA isolates carried *fusB* [15].

### Molecular characteristics of FA-resistant *S. aureus* isolates

The molecular characteristics of FA-resistant *S. aureus* isolates were listed in Table 2. A total of 9 STs were identified among the 56 FA-resistant *S. aureus* isolates, among which ST5 accounting for 66.1 % (37/56) was the most common ST, followed by ST630 (12.5 %, 7/56). Only 3 MRSA isolates belonged to ST239 which is the predominant ST in China. The STs for two isolates were not identified. The antimicrobial resistance profiles of ST5 and ST239 FA-resistant MRSA isolates were showed in Table 3. The clonal complex 5 (CC5) was the

**Table 2** Molecular characteristic of FA-resistant *S. aureus* isolates

CC <sup>a</sup> (No.)	ST <sup>b</sup> (No.)	<i>spa</i> types (No.)	MRSA (No.)	MSSA (No.)	SCC <sub>mec</sub> (No.)	<i>fusB</i> (No.)	<i>fusC</i> (No.)	FA MICs (No.)	
5(52)	ST5(37)	t2460(37)	37	0	II(36)III(1)	32	0	≥128(36);4(1)	
		ST630(7)	t4549(3)	2	1	III(1)V(1)	1	0	2(1);4(1);16(1)
			t377(2)	0	2		1	0	4(1);16(1)
			t257(1)	0	1		0	0	4(1)
			t2196(1)	0	1		1	0	2(1)
	ST239(3)	t030(3)	2	1	III(2)	2	0	≥128(2); 4(1)	
	ST25(2)	t078(2)	2	0	II(2)	1	0	≥128(2)	
	ST15(1)	t2460	1	0	II	0	0	4(1)	
	ST72(1)	t664	1	0	III	0	0	≥128(1)	
	ST2831(1)	t030	1	0	III	1	0	2(1)	
7(1)	ST7(1)	t091(1)	0	1		0	0	≥128(1)	
59(1)	ST59(1)	t437	1	0	V	1	0	8(1)	
Singleton(2)	NT(2)	t11497(1)	1	0	II	0	0	4(1)	
		t5137(1)	1	0	III	1	0	≥128(1)	

a: CC, clone complex; b: ST, sequence type



**Table 3** Antimicrobial resistance profiles of FA-resistant ST5 and ST239 MRSA isolates

	ST5 MRSA isolates (n = 37)	ST239 MRSA isolates (n = 3)
	R <sup>a</sup> (%)	R(%)
Tetracycline	94.6	33.3
Gentamicin	70.3	100
Penicillin	100	100
Clindamycin	100	100
Erythromycin	100	100
Ciprofloxacin	97.3	100
Linezolid	0	0
Rifampicin	5.4	100
Trimethoprim/ sulfamethoxazole	3.7	0
Nitrofurantoin	0	0
Chloramphenicol	5.4	0
Quinupristin/ dalfopristin	0	0
Vancomycin	0	0

a: R, resistance

predominant CC, accounting for the majority (92.9 %, 52/56) of the isolates tested. Twelve *spa* types were identified among the 56 FA-resistant *S. aureus* isolates, among which *t2460* (67.9 %, 38/56) was the most prevalent *spa* type. The *spa* types for 5 isolates were not identified. *t2460* was identified among 43.9 % of MRSA isolates from 3 intensive care units at a Korean tertiary hospital in 2007–2008 [29]. Among the 49 MRSA isolates, 40, 7 and 2 harbored SCC *mec* types II, III and V, respectively.

In the present study, ST5-MRSA- II-*t2460* was predominant clone, accounting for 75.5 % (37/49) of FA-resistant MRSA isolates and 66.1 % (37/56) of FA-resistant *S. aureus* isolates. ST5-MRSA- II is usually associated with *t002* [30]. ST5-MRSA- II-*t002* was one of two major clones in China [30]. However, ST5-MRSA- II was linked to *t2460* in this study. This fairly unusual clone was only found among 6 MRSA isolates from Shanghai in China [30]. FA MIC values for 36 of 37 ST5-MRSA- II-*t2460* isolates were  $\geq 128$   $\mu\text{g/ml}$ . ST5-MRSA- II-*t2460* clone first emerged in 2012. However, this newly emerging clone with FA resistance increased dramatically in 2013, accounting for 77.8 % (35/45) of FA-resistant isolates. The origin of 35 isolates belonged to ST5-MRSA- II-*t2460* in 2013 was confined to only 4 wards including neurosurgery ward (28 isolates), orthopaedic ward (three isolates), intensive care unit (three isolates) and hematology ward (one isolate), indicating that outbreak of infections caused by this newly clone has occurred in our hospital, mainly in neurosurgery

ward. Clonal spread is responsible for the increase in FA resistance in UK, France and other European countries [31, 32]. CC80-MRSA-IV clone carrying *fusB* has made a major contribution to the prevalence of FA resistance among MRSA isolates in Europe [25]. The highest prevalence of FA resistance in 2013 in this investigation was associated with the spread of ST5-MRSA- II-*t2460* clone with high-level FA resistance. Among 7 FA-resistant MSSA isolates, 5 belonged to ST630-MSSA.

## Conclusions

In conclusion, increased FA resistance among *S. aureus* isolates was found in China. *fusB* was predominant FA resistance determinant. The spread of CC5 clone, especially novel ST5-MRSA- II-*t2460* clone with high-level resistance to FA, was responsible for the increase of FA resistance.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

YLL, CHL, JNL, XQQ, YD and DL performed the laboratory measurements. FYY and LXW made substantial contributions to conception and design. FYY and LXW revised the manuscript critically for important intellectual content. XYH and LHH participated in experimental design and data analysis. FYY drafted the manuscript. All authors read and approved the final manuscript.

## Acknowledgement

We are grateful to Pro. T. Ito for the kind gift of MRSA NCTC 10442 (SCC*mec* I), MRSA N315 (SCC*mec* II), MRSA85 / 2082 (SCC*mec* III), and MRSA JCSC4744 (SCC*mec* IV). This study was supported by grants from Natural Science fund of China (81271906H2002 and 81472011H2002) and the Program for Zhejiang Provincial Leading Team for S&T innovation (2011R50018-11).

## Author details

<sup>1</sup>Department of Laboratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China. <sup>2</sup>Department of Respiratory Medicine, the First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China. <sup>3</sup>Department of Laboratory Medicine, the Second Affiliated Hospital of Nanchang University, Nanchang 336000, China.

Received: 14 February 2015 Accepted: 7 October 2015

Published online: 13 October 2015

## References

- David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev*. 2010;23(3):616–87.
- Said-Salim B, Mathema B, Kreiswirth BN. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. *Infect Control Hosp Epidemiol*. 2003;24(6):451–5.
- Cohen PR. Community-acquired methicillin-resistant *Staphylococcus aureus* skin infections: implications for patients and practitioners. *Am J Clin Dermatol*. 2007;8(5):259–70.
- Daschner FD, Kropec A. Glycopeptides in the treatment of staphylococcal infections. *Eur J Clin Microbiol Infect Dis*. 1995;14 Suppl 1:S12–17.
- Centers for Disease Control and Prevention (CDC). Vancomycin-resistant *Staphylococcus aureus*—Pennsylvania, 2002. *MMWR Morb Mortal Wkly Rep*. 2002;51(40):902.
- Hiramatsu K. The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. *Am J Med*. 1998;104(5A):75–105.
- Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, et al. Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. *Lancet*. 1997;350(9092):1670–3.

8. Wang JL, Tang HJ, Hsieh PH, Chiu FY, Chen YH, Chang MC, et al. Fusidic acid for the treatment of bone and joint infections caused by methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents*. 2012;40(2):103–7.
9. Schofer H, Simonsen L. Fusidic acid in dermatology: an updated review. *Eur J Dermatol*. 2010;20(1):6–15.
10. Whitby M. Fusidic acid in the treatment of methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents*. 1999;12 Suppl 2:S67–71.
11. Tsuji BT, Okusanya OO, Bulitta JB, Forrest A, Bhavnani SM, Fernandez PB, et al. Application of pharmacokinetic-pharmacodynamic modeling and the justification of a novel fusidic acid dosing regimen: raising Lazarus from the dead. *Clin Infect Dis*. 2011;52 Suppl 7:S513–519.
12. Castanheira M, Watters AA, Mendes RE, Farrell DJ, Jones RN. Occurrence and molecular characterization of fusidic acid resistance mechanisms among *Staphylococcus* spp. from European countries (2008). *J Antimicrob Chemother*. 2010;65(7):1353–8.
13. CLSI. Performance standards for antimicrobial susceptibility testing, 18th informational supplement (M100-S22). Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2012.
14. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard-eighth edition. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2009.
15. Chen HJ, Hung WC, Tseng SP, Tsai JC, Hsueh PR, Teng LJ. Fusidic acid resistance determinants in *Staphylococcus aureus* clinical isolates. *Antimicrob Agents Chemother*. 2010;54(12):4985–91.
16. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN, Etienne J, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome mec type assignment: rapid identification system for mec, ccr, and major differences in junkyard regions. *Antimicrob Agents Chemother*. 2007;51(1):264–74.
17. Koreen L, Ramaswamy SV, Graviss EA, Naidich S, Musser JM, Kreiswirth BN. spa typing method for discriminating among *Staphylococcus aureus* isolates: implications for use of a single marker to detect genetic micro- and macrovariation. *J Clin Microbiol*. 2004;42(2):792–9.
18. Harmsen D, Claus H, Witte W, Rothganger J, Turnwald D, Vogel U. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442–8.
19. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol*. 2000;38(3):1008–15.
20. Huang J, Ye M, Ding H, Guo Q, Ding B, Wang M. Prevalence of fusB in *Staphylococcus aureus* clinical isolates. *J Med Microbiol*. 2013;62 (Pt 8):1199–203.
21. Liu Y, Geng W, Yang Y, Wang C, Zheng Y, Shang Y, et al. Susceptibility to and resistance determinants of fusidic acid in *Staphylococcus aureus* isolated from Chinese children with skin and soft tissue infections. *FEMS Immunol Med Microbiol*. 2012;64(2):212–8.
22. Castanheira M, Watters AA, Bell JM, Turnidge JD, Jones RN. Fusidic acid resistance rates and prevalence of resistance mechanisms among *Staphylococcus* spp. isolated in North America and Australia, 2007–2008. *Antimicrob Agents Chemother*. 2010;54(9):3614–7.
23. Udo EE, Al-Sweih N, Mokaddas E, Johnny M, Dhar R, Gomaa HH, et al. Antibacterial resistance and their genetic location in MRSA isolated in Kuwait hospitals, 1994–2004. *BMC Infect Dis*. 2006;6:168.
24. Chen CM, Huang M, Chen HF, Ke SC, Li CR, Wang JH, et al. Fusidic acid resistance among clinical isolates of methicillin-resistant *Staphylococcus aureus* in a Taiwanese hospital. *BMC Microbiol*. 2011;11:98.
25. McLaws FB, Larsen AR, Skov RL, Chopra I, O'Neill AJ. Distribution of fusidic acid resistance determinants in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2011;55(3):1173–6.
26. Nagaev I, Bjorkman J, Andersson DI, Hughes D. Biological cost and compensatory evolution in fusidic acid-resistant *Staphylococcus aureus*. *Mol Microbiol*. 2001;40(2):433–9.
27. O'Neill AJ, Chopra I. Molecular basis of fusB-mediated resistance to fusidic acid in *Staphylococcus aureus*. *Mol Microbiol*. 2006;59(2):664–76.
28. Rijnders MI, Wolffs PF, Hopstaken RM, den Heyer M, Bruggeman CA, Stobberingh EE. Spread of the epidemic European fusidic acid-resistant impetigo clone (EEFIC) in general practice patients in the south of The Netherlands. *J Antimicrob Chemother*. 2012;67(5):1176–80.
29. Kim T, Yi J, Hong KH, Park JS, Kim EC. Distribution of virulence genes in spa types of methicillin-resistant *Staphylococcus aureus* isolated from patients in intensive care units. *Korean J Lab Med*. 2011;31(1):30–6.
30. Cheng H, Yuan W, Zeng F, Hu Q, Shang W, Tang D, et al. Molecular and phenotypic evidence for the spread of three major methicillin-resistant *Staphylococcus aureus* clones associated with two characteristic antimicrobial resistance profiles in China. *J Antimicrob Chemother*. 2013;68(11):2453–7.
31. O'Neill AJ, Larsen AR, Skov R, Henriksen AS, Chopra I. Characterization of the epidemic European fusidic acid-resistant impetigo clone of *Staphylococcus aureus*. *J Clin Microbiol*. 2007;45(5):1505–10.
32. Laurent F, Tristan A, Croze M, Bes M, Meugnier H, Lina G, et al. Presence of the epidemic European fusidic acid-resistant impetigo clone (EEFIC) of *Staphylococcus aureus* in France. *J Antimicrob Chemother*. 2009;63(2):420–1. author reply 421.

**Submit your next manuscript to BioMed Central and take full advantage of:**

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at  
[www.biomedcentral.com/submit](http://www.biomedcentral.com/submit)

