Prevalence and molecular characterization of methicillin-resistant *Staphylococcus aureus* with mupirocin, fusidic acid and/or retapamulin resistance

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Abstract

Data on the prevalence of resistance to mupirocin (MUP), fusidic acid (FA) and retapamulin (RET) in methicillin-resistant *Staphylococcus aureus* (MRSA) from China are still limited. In this study we examined these three antibiotics resistance pheno and geno-typically in 1206 MRSA clinical isolates. Phenotypic MUP, FA and RET resistance was determined by MICs, and genotypically by PCR and DNA sequencing examining genes mupA / B , fusB - D , cfr and vgaA / Av , and mutations in ileS , fusA / E , rplC , and 23S RNA V domain. The genetic characteristics of resistance isolates were conducted by PFGE and MLST. Overall MRSA MUP, FA and RET resistance was low (5.1%, 1.0% and 0.3%, respectively). The mupA was the mechanism of high-level MUP resistance. All low-level MUP resistance isolates possessed an equivocal mutation N213D in IleS, and 2 of them additionally had the reported V588F mutation impacting the Rossman fold. FusA mutations, such as L461K, H457Q, H457Y and V90I, were the primary FA resistance mechanisms among high-level resistance isolates, most of which contained fusC ; however, all low-level resistance strains carried fusB . No resistance mechanisms detected were found among RET resistance isolates. Genetic analysis demonstrated clone spread for MUP resistance isolates. In conclusion, MUP, FA and RET exhibited highly activity against MRSA isolates. Acquired genes and chromosome-borne genes mutations were responsible for MUP and FA resistance, and further investigation is needed to uncover the RET resistance mechanisms. Moreover, the surveillance to MUP in MRSA should be strengthened to prevent resistance increase due to the expansion of clones.

Background

Methicillin-resistant Staphylococcus aureus (MRSA) is a major pathogen responsible for various hospital- and community-associated infectious on a worldwide scale [1]. Because of strong resistance, MRSA brings great challenges to clinical anti-infective therapy, leading to high risk of mortality and expensive medical expenses [1].

Skin and soft tissue infections (SSTIs) are one of the common diseases caused by S. aureus, including MRSA [2], which are able to be treated with topical antibiotics mupirocin (MUP) and fusidic acid (FA) [3]. However, long-term unreasonable use of these drugs leads to the emergence of resistance, which has become a significant public health problem [3]. Therefore, novel topical antimicrobial agent retapamulin (RET) is developed for the treatment of S. aureus SSTIs [3].

MUP resistance in staphylococcus is divided into two phenotypes: high-level (MuH, MIC ≥ 512 μg/ml) and low-level (MuL, MIC = 8–256 μg/ml) [3, 4]. The MuH is mediated by gene mupA or mupB, and the MuL is related to point mutations in the isoleucyl-tRNA synthetase gene (ileS) [3, 5]. For FA resistance (MIC ≥ 2 μg/ml) [3, 6], the molecular mechanisms are associated with mutations in fusA or fusE located in chromosome (conferring high-level resistance, FAH) and/or acquired fusB family genes, including fusB, fusC and fusD (mediating low-level resistance, FAL) [3, 7]. RET, belonging to pleuromutilin antibiotics, has been licensed in USA and Europe for the topical treatment of SSTIs caused by methicillin-sensitivity S. aureus and Streptococcus pyogenes [8]. RET resistance in S. aureus is often mediated by the point mutations of ribosomal protein L3 (encoded by rplC) or the 23S rRNA V domain, or efflux pumps VgaA/VgaAv (encoded by the vgaA or vgaAv gene), or methylation of the 23S rRNA subunit [methylated by methyltransferase encoded by chloramphenicol-florfenicol resistance (cfr) gene] [3].

Several previous studies reported the resistance of MUP and FA in Eastern China [9, 10–12]. However, in view that Eastern China is a region with a vast territory, the antibiotic resistance spectrum and the resistance mechanisms may change in diverse hospitals. Therefore, those data in the aforementioned documents on the resistance of both drugs in clinical isolates of S. aureus are limited. In addition, to the best of our knowledge, there is no information on RET resistance in China.

In this study, we determined the prevalence of MUP, FA and RET resistance among MRSA isolates from Shanghai and Zhejiang province in Eastern China, and analyzed the resistance mechanisms in the resistant isolates. Furthermore, to understand the genetic characterization of resistant isolates, PFGE and MLST genotyping was also carried out.

Methods

Bacterial isolates

A total of 1206 non-duplicate MRSA isolates from various clinical specimens were separated from 8 hospitals collection in Shanghai and Zhejiang province, Eastern China [Shanghai General Hospital (1037 isolates from July 2010 to June 2015), Ruijin Hospital (22 isolates during January 2011 to December 2011), Shanghai Sixth People's Hospital (36 isolates between Decermber 2010 and Decermber 2012), Shanghai People's Hospital of Putuo District (45 isolates from January 2013 to May 2014), Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (45 isolates from February 2014 to September 2014), Shanghai Armed Police Corps Hospital (9 isolates from January 2014 to June 2014), Zhejiang Xiaoshan Hospital (8 isolates from March 2012 to October 2012) and The Central Hospital of Lishui City, Zhejiang Province (28 isolates, July 2013 to September 2014)]. Most of these isolates were isolated from respiratory samples (80%). Intensive care units, respiratory medicine, geriatric medicine, thoracic surgery and nephrology wards were the units impacted by MRSA isolates. All isolates were identified using VITEK microbial identification system (bioMérieux, Marcy l' Etoile, France). The resistance to methicillin was detected with a 30 μg cefoxitin disk (Oxoid, Basingstoke, UK) [4]. MuH (MIC ≥ 512 μg/mL), MuL (MIC = 8 to 256 μg/mL), FAH (MIC ≥ 8 μg/mL), FAL (MIC = 2 to 4 μg/mL) and RET resistance (MIC ≥ 1 μg/mL was defined as resistance in this study) were screened from all the isolates collected by broth microdilution method [4, 6, 12, 13]. For three-antibiotic resistant isolates, the methicillin resistance was further confirmed by amplification of mecA and mecC genes [14]. MUP, FA and RET were purchased from Shanghai Boyle Chemical Co., Ltd., China. S. aureus ATCC 25923 and ATCC 29213 were used as quality control strains for antibiotic susceptibility testing.

DNA Extraction

The cultures of MRSA with MUP, FA and/or RET resistance were incubated with lysostaphin (1 mg/mL) (Sangon, Shanghai, China) at 37 °C for 0.5 h. Then, the DNA was extracted according to the instructions of the bacterial genomic DNA kit (Tiangen, Beijing, China), and utilized as a template for PCR assays.

Detection of mupirocin, fusidic acid and retapamulin resistance genes

PCR was used to detect determinants mediating MUP [mupA, mupB and ileS (amplifying three fragments of Smr, Mrm and Lmr, which possibly possess the mutations mediating mupirocin resistance)], FA (fusA, fusB, fusC, fusD and fusE) and RET (rplC, cfr, vgaA/Av and 23S RNA V) resistance [5, 7, 12, 15–19]. The primers for the amplification of genes were presented in Table 1. DNA sequencing of one randomly selected PCR product for mupA, mupB, fusB, fusC, fusD, cfr and vgaA/Av was used for the identification of target fragments. All the PCR products for ileS-Smr, ileS-Mrm, ileS-Lmr, fusA, fusE, rplC and 23S RNA V were sequenced to determine the possible mutations.

Table 1
Primers for detecting mupirocin, fusidic acid and retapamulin resistance genes in this study.

Gene	Primer name	Primer sequence (5′-3′)	Size	Reference
mupA	mupA-F	TATATTATGCGATGGAAGGTTGG	457 bp	18
	mupA-R	AATAAAATCAGCTGGAAAGTGTTG		
mupB	mupB-F	CTAGAAGTCGATTTTGGAGTAG	674 bp	9
	mupB-R	AGTGTCTAAAATGATAAGACGATC		
ileS (including the following 3 fragments)				
Smr	Smr-F	ATAAAGGTAAAAAGCCAGTTTATTGGT	200 bp	21
	Smr-R	TAATCGCAACATTTGATGGAATTGTC		
Mrm	Mrm-F	TCCCAGCAGATATGTATTTAGAAGGT	450 bp	
	Mrm-R	AACCACTTGGTCAGGTACAATCACA		
Lmr	Lmr-F	GTAAATCTTTAGGTAATGTGATTGTAC	690 bp	
	Lmr-R	TCTTCTTTAACATGTGGTGTATGAGA		
fusA	fusA-F	TTTACCCTGAGTGTGTTCT	2250 bp	11
	fusA-R	TACATTTAAGCTCACCTTGT		
fusB	fusB-F	TCATATAGATGACGATATTG	496 bp	22
	fusB-R	ACAATGAATGCTATCTCGAC		
fusC	fusC-F	GATATTGATATCTCGGACTT	128 bp	
	fusC-R	AGTTGACTTGATGAAGGTAT		
fusD	fusD-F	TGCTTATAATTCGGTCAACG	525 bp	
	fusD-R	TGGTTACATAATGTGCTATC		
fusE	fusE-F	CCTAGTGACGTAACAGTAAC	505 bp	
	fusE-R	CGGCGWACRTATTCACCTTG		
rplC	rplC-F	AACCTGATTTAGTTCCGTCTA	822 bp	12
	rplC-R	GTTGACGCTTTAATGGGCTTA		
cfr	cfr-F	GAGATAACAGATCAAGTTTTA	1050 bp	23
	cfr-R	CGAGTATATTCATTACCTCAT		
vgaA	vgaA-F	TCACATGATCGCGCTTTTTTAGAT	770	44
	vgaA-R	TCGCTCTCCACCACTTAAGACACT		
vgaAv	vgaAv-F	CTCTTTGTACGAGTATATGG	770 bp	24
	vgaAv-R	GTTTCTTAGTAGCTCGTTGAGC		
23S RNA V	23S RNA-F	TGGGCACTGTCTCAACGA	634 bp	25
	23S RNA-R	GGATAGGGACCGAACTGTCTC		
MLST typing				27
arcC	arcC-F	TTGATTCACCAGCGCGTATTGTC	456 bp	
	arcC-R	AGGTATCTGCTTCAATCAGCG		
aroE	aroE-F	ATCGGAAATCCTATTTCACATTC	456 bp	
	aroE-R	GGTGTTGTATTAATAACGATATC		
glpF	glpF-F	CTAGGAACTGCAATCTTAATCC	465 bp	
	glpF-R	TGGTAAAATCGCATGTCCAATTC		
gmk	gmk-F	ATCGTTTTATCGGGACCATC	429 bp	
	gmk-R	TCATTAACTACAACGTAATCGTA		
pta	pta-F	GTTAAAATCGTATTACCTGAAGG	474 bp	

Gene	Primer name	Primer sequence (5'-3')	Size	Reference
	pta-R	GACCCTTTTGTTGAAAAGCTTAA		
tpi	tpi-F	TCGTTCATTCTGAACGTCGTGAA	402 bp	
	tpi-R	TTTGCACCTTCTAACAATTGTAC		
yqiL	yqiL-F	CAGCATACAGGACACCTATTGGC	516 bp	
	yqiL-R	CGTTGAGGAATCGATACTGGAAC		

PFGE Typing

Pulsed field gel electrophoresis (PFGE) was performed for MUP, FA and/or RET resistant strains as described by previous investigation [20]. BioNumerics software 7.0 was used for the analysis of DNA fingerprint profiles. An 80% cutoff value for similarity was utilized.

MLST Typing

Multilocus sequence typing (MLST) was conducted by sequencing of internal fragments of 7 housekeeping genes arcC, aroE, glpF, gmk, pta, tpi and yqiL on MUP, FA and/or RET resistant MRSA using the primers (listed in Table 1) as depicted previously [21]. Sequence types (STs) were determined on the basis of data from the MLST database for S. aureus (http://saureus.mlst.net/).

Results

Prevalence of mupirocin, fusidic acid and retapamulin resistance

By screening of 1206 MRSA isolates using broth microdilution assay, we identified 49 MuH, 12 MuL, 6 FAH, 6 FAL, 2 RET resistant and 1 MuH-RET resistant isolates. The detailed MIC data of the MUP, FA and RET resistance isolates were listed in Fig. 1.

Mechanisms Of Mupirocin Resistance

Forty-nine MuH and 1 MuH-RET resistant isolates all contained the mupA gene, and no isolates were mupB positive (Table 2). The sequences of Smr, Mrm and Lmr DNA fragments were compared with the known ileS gene of S. aureus (Gene bank accession no. X74219). The findings showed that all 12 MuL isolates possessed N213D mutation in Smr fragment, and 2 MuL isolates had V588F mutation in Mrm fragment. No mutations were identified in Lmr fragment. In addition, no mupA or mupB gene was detected in MuL isolates.

Table 2

Resistance mechanisms of 76 mupirocin, fusidic acid and/or retapamulin-resistant clinical MRSA isolat

Strain	Resistance phenotype and mechanism														
	Mupirocin							Fusidic acid							
	Phenotype	mupA	mupB	IleS mutation				Phenotype	Fus A mutation	fusB	fusC	fusD	Fus E mutation		Phenc
				Smr	Mrm	Lmr									
PT300		MuL	—	—	N213D	V588F	NM		S						
PT343		MuH	+	—					S						
355		MuH	+	—					S						
546		MuL	—	—	N213D	NM	NM		S						
370		MuH	+	—					S						
532		MuL	—	—	N213D	NM	NM		S						
519		MuH	+	—					S						
472		MuL	—	—	N213D	NM	NM		S						
409		MuH	+	—					S						
671		MuH	+	—					S						
443		MuH	+	—					S						
530		MuH	+	—					S						
120		MuH	+	—					S						
322		MuH	+	—					S						
328		MuH	+	—					S						
448		MuH	+	—					S						
474		MuH	+	—					S						
526		MuL	—	—	N213D	NM	NM		S						
565		MuH	+	—					S						
600		MuH	+	—					S						
606		MuL	—	—	N213D	NM	NM		S						
623		MuH	+	—					S						
642		MuH	+	—					S						
673		MuL	—	—	N213D	NM	NM		S						
694		MuH	+	—					S						
697		MuH	+	—					S						
70		MuH	+	—					S						
701		MuH	+	—					S						
717		MuH	+	—					S						
PT317		MuH	+						S						
318		MuH	+	—					S						
106		MuH	+	—					S						
331		MuH	+	—					S						
364		MuH	+	—					S						
486		MuH	+	—					S						
553		MuH	+	—					S						

MRSA, methicillin-resistant S. aureus; +, Positive; —, Negetive; IleS, isoleucyl-tRNA synthetase; MuH, high-level mupirocin resistance; MuL, low-level mupirocin low-level fusidic acid resistance; NM, No mutation; R, resistance; I, intermediate; S, susceptibility.

Strain	Resistance phenotype and mechanism											
	Mupirocin						Fusidic acid					
777	MuL	—	—	N213D	NM	NM	S					
504	MuH	+	—				S					
394	MuH	+	—				S					
805	MuH	+	—				S					
453	MuH	+	—				S					
727	MuH	+	—				S					
764	MuH	+	—				S					
433	MuH	+	—				S					
541	MuH	+	—				S					
631	MuH	+	—				S					
696	S						FAL	NM	+	—	—	NM
635	MuL	—	—	N213D	NM	NM	S					
320	MuH	+	—				S					
348	MuH	+	—				S					
349	MuH	+	—				S					
110	S						FAH	L461K	—	+	—	NM
829	S						FAH	H457Q	—	+	—	NM
PT301	S						FAH	L461K	—	+	—	NM
PT308	S						FAH	L461K	—	+	—	NM
399	MuH	+	—				S					
422	MuH	+	—				S					
419	MuH	+	—				S					
390	MuH	+	—				S					
125	MuH	+	—				S					
755	S						FAL	NM	+	—	—	NM
783	S						FAL	NM	+	—	—	NM
361	S						FAL	NM	+	—	—	NM
611SG	S						FAL	NM	+	—	—	NM
58	S						S					
765	S						FAL	NM	+	+	—	NM
323	MuH	+	—				S					
386	MuH	+	—				S					
575	MuL	—	—	N213D	NM	NM	S					
LY26	S						FAH	E8K, V90I, L461K	—	—	—	NM
LY32	S						FAH	H457Y	—	—	—	NM
WU9	MuL	—	—	N213D	V588F	NM	S					
308	MuL	—	—	N213D	NM	NM	S					
513	MuH	+	—				S					
LS964	S						S					

MRSA, methicillin-resistant S. aureus; +, Positive; —, Negetive; IleS, isoleucyl-tRNA synthetase; MuH, high-level mupirocin resistance; MuL, low-level mupirocin
low-level fusidic acid resistance; NM, No mutation; R, resistance; I, intermediate; S, susceptibility.

Strain	Resistance phenotype and mechanism			
	Mupirocin			Fusidic acid
314	MuH	+	—	S

MRSA, methicillin-resistant S. aureus; +, Positive; —, Negetive; IleS, isoleucyl-tRNA synthetase; MuH, high-level mupirocin resistance; MuL, low-level mupirocin low-level fusidic acid resistance; NM, No mutation; R, resistance; I, intermediate; S, susceptibility.

Mechanisms Of Fusidic Acid Resistance

To uncover the mechanisms of FA resistance among 6 FAH isolates, the full-length of fusA and fusE genes was sequenced, and compared with that of S. aureus ATCC 25923. We identified that 2 isolates contained H457Q missense mutation, and 4 had mutation L461K in FusA. Among 4 strains with L461K mutation, 1 simultaneously possessed E8K and V90I mutations (Table 2). All FA resistance isolates were detected for fusB, fusC and fusD. For 6 FAH isolates, 4 carried fusC gene. Among 6 FAL isolates, all were fusB positive, and only one carried fusC gene (Table 2). No mutations were found in fusE, and no isolates were fusD positive.

Mechanism Of Retapamulin Resistance

For 3 isolates with resistance to RET, no resistance mechanisms detected in this study were observed.

PFGE

All isolates (76 isolates) with MUP, FA and/or RET resistance were divided into 16 patterns (type A to P) (Fig. 1). Among 49 MuH strains, 40 belonged to type B, 4 were type E, 5 belonged to type A, F, J, K and N, respectively. One MuH-RET resistant isolate was also type B. Among 12 MuL isolates, 9 were type B, and 3 belonged to type A, M and N, respectively. Type C was the most frequent type in FAH strains (3/6; 50%). Type G was the most common pattern in FAL strains (4/6; 66.7%). Two RET resistant MRSA belonged to type O and H, respectively.

MLST

Fifteen different STs were identified among 76 isolates studied (Fig. 1). ST764 (24/76, 31.6%) was the most frequent pattern, followed by ST630 (11/76, 14.5%), ST239 (9/76, 11.8%) and ST5 (7/76, 9.2%) and 11 additional STs, namely ST4631 and ST1821 (4/76 each, 5.3%), ST9, ST3262 and ST4184 (3/76 each, 3.9%), ST1 and ST20 (2/76 each, 2.6%), ST965, ST398, ST4359 and ST97 (1/76 each, 1.3%).

Discussion

Mupirocin is effective for the prevention and treatment of MRSA infections. However, the resistance (including MuL) is able to lead to MRSA treatment and eradication failure [22, 23]. The prevalence rate of MUP resistance is various in MRSA clinical isolates worldwide, from 0.5–10.1% for MuH and 2.4–8.6% for MuL in America, from 0–75% for MuH and 0%% to 46.7% for MuL in Asia, and from 0.8–98% for MuH and 0–31.2% for MuL in Europe [22]. In our present study, the isolation rates of MuH and MuL were low, namely 4.1% (50/1206) and 1.0% (12/1206), respectively. Recent large studies displayed that the prevalence of MuH is mediated by plasmid-borne mupA gene [22], this is the same as our results. Although mupB, also a plasmid-borne gene, is correlated with MuH [5], this mechanism is rarely examined in staphylococci, including the isolates investigated in the present study. The point mutations in the ileS gene, resulting in amino changes in MUP-binding site (also named Rossman fold), are the main mechanisms determining MuL [22]. V588F and V631F are well identified the most frequent mutations in IleS responsible for MuL [22]. In this study, only two MuL isolates (PT300 and wu9) contained the V588F mutation, and no MuL isolates harbored the V631F mutation. Notably, all MuL isolates harbored N213D mutation, which located in a hotspot amino acid sequence between 200 to 350 described by Lee et al [24]. The N213D mutation had been previously reported, and are considered to have no impact on the sensitivity of MUP [25]. Although the mupA gene located on chromosome is also associated with MuL [3], we did not detect the gene in our MuL isolates. In addition, no other mutations in IleS were found. Lee et al. [24] reported that a mutation of S634F could confer phenotype of susceptibility or MuL in diverse isolates. In view of this phenomenon, the contribution of N213D mutation to MuL should be evaluated further.

Fusidic acid is a steroidal antimicrobial agent, and suppresses the production of bacterial proteins by stopping the dissociation of elongation factor G (EF-G) from ribosome [6, 26]. In clinic, the main applications of topical FA are the treatment of SSTIs and decolonization of S. aureus including MRSA, which is similar to those for MUP [3]. The prevalence of FA resistance reported by recent large studies varies in MRSA isolates from USA (0%-0.3%), Australian (4.1%-5.1%), Denmark (17.8%), Greece (57.0%) and other European countries (9.9%) [3]. In China, the resistance levels in MRSA are also different in different areas, for example 3.0%-5.3% in MRSA from Beijing, Shanghai, Shenyang and Shenzhen cities [10, 27], and 27.1% in MRSA from Wenzhou city [9]. Compared with the aforementioned data from China, our results showed a very low resistance rate (1.0%, 12/1026).

In S. aureus, the mutations in fusA (encoding EF-G) or fusE (coding for ribosome protein L6, RplF) lead to a decreased affinity of FA for the EF-G ribosome complex [3, 28]. There are over thirty point mutations in FusA sequence to be described, however, only a few were experimentally verified playing a role in FA resistance [3, 29, 30]. The mutations V90I, H457Y, H457Q and L461K observed in this study have been previously identified causing FAH in S. aureus [29, 31], and the L461K is the most prevalent mechanism among clinical FAL S. aureus strains [3]. In our results, the L461K also existed in most (80%, 4/6) FAL isolates. One substitution with E8K located in domain I (amino acids 1 to 280) of EF-G was identified for the first time, and occurred with the mutations of V90I and L461K in a FAH isolate. Whether the novel mutation is associated with FA resistance is not clear and needs further clarification.

Protection of EF-G by FusB family molecules is another mechanism conferring the resistance (low-level) of FA [3]. FusB family proteins (including FusB, FusC and FusD) are able to restore the translation of protein by binding to EF-G when FA exists [3]. Previous studies showed that fusB was the most prevalent in Netherlands and mainland China [9, 10, 32], and fusC main existed in isolates from Taiwan, Australia, USA and European collections [16, 33, 34]. In our isolates with FA resistance, the fusB existed in all FAL isolates (6/6), and the fusC was most prevalent in FAH isolates (66.7%, 4/6). The fusD gene was identified in Staphylococcus saprophyticus, and relates to the "intrinsic resistance of FA" among this species [26]. To date, this determinant is rarely detected in S. aureus strains.

Retapamulin, a semisynthetic drug, represses the synthesis of bacterial proteins by interacting with domain V of 50S ribosomal subunit [3]. This drug has a potency to act as an alternative to MUP to eradicate the S. aureus colonization, except used for the treatment of SSTIs of S. aureus [8, 35]. For RET resistance, very little published data are available among clinical S. aureus strains worldwide. The resistance rates of 664 UK S. aureus (74% of them were MRSA), 155 USA MRSA, 403 USA MRSA and 400 USA S. aureus from several different studies were 0.15%, 2.6%, 0.25%, and 9.5%, respectively [8, 35–37]. In this study, the prevalence of RET resistance was very low (0.24%, 3/1226). In UK and USA, the RET resistance among S. aureus or MRSA with MUP resistance was < 1–2.6% [8, 37]. In the present study, only one MRSA isolate was observed to be simultaneous resistance to RET and MUP. In our 3 RET resistance isolates, no resistance mechanisms studied were examined. The genetic basis for resistance to RET in these isolates remains unclear, and other potential mechanisms may need to be further explored.

ST239 and ST5 are two predominant sequence types in China. However, in this study our strains were mainly belonged to ST764 (31.6%), which was more than the total percentage of ST239 and ST5 (16/76, 21.1%). ST764 MRSA, first reported in Japan, is a single-locus of ST5 nosocomial MRSA clone with or without the arginine catabolic mobile element (ACME, a feature of CA-MRSA) [38, 39]. In recent years, several studies have reported the S. aureus clone with ST764 in China [11, 14]. Notably, multiple MUP resistance MRSA clones with different genetic patterns, such as PFGE B-ST764, PFGE B-ST1821, PFGE B-ST239, PFGE B-ST5 and PFGE B-ST630, mainly occurred in the same hospital (Shanghai General Hospital) (Fig. 1), which indicates that the dissemination of different clones is responsible for the resistance of MUP in this hospital. However, for FA resistance isolates, the genetic background (PFGE-ST) exhibited more heterogeneity. This may be due to the situation that these isolates were from different hospitals (Fig. 1).

Conclusion

MRSA isolates in this study exhibited a low prevalence of resistance to MUP, FA and RET, especially to the latter two, and cross-resistance to these three antibiotics was rare. The mupA gene was the mechanism mediated MuH. The contribution of the mutation N213D in IleS found in our MuL isolates to low-level resistance of mupirocin is still confusing. FusA mutations, FusB and FusC were the frequent genetic mechanisms mediated FA resistance. Phylogenetic detection showed the transmission of a main clonal isolate (PFGE B-ST764) of MUP resistant MRSA in one hospital. Because of the concern of resistance development and clonal dissemination in healthcare settings, continuous surveillance for the resistance of these topic antibiotics in S. aureus is essential in China.

Abbreviations

MUP:mupirocin; FA:fusidic acid; RET:retapamulin; PFGE:pulsed-field gel electrophoresis; MLST:multilocus sequence typing; MuH:high-level mupirocin resistance; MuL:low-level mupirocin resistance; FAH:high-level fusidic acid resistance; FAL:low-level fusidic acid resistance; IleS:isoleucyl-tRNA synthetase; MIC:minimum inhibitory concentration; PCR:polymerase chain reaction; MRSA:methicillin-resistant Staphylococcus aureus; PFGE, Pulsed field gel electrophoresis; MLST, Multilocus sequence typing; MIC, minimum inhibitory concentration; SGH, Shanghai General Hospital; SSH, Shanghai Sixth People's Hospital; PT, Shanghai People's Hospital of Putuo District; SAH, Shanghai Armed Police Corps Hospital; LSH, the Central Hospital of Lishui City, Zhejiang Province; SGHA, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine

Declarations

Acknowledgments

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Authors' contributions

QZL designed and conceived the investigation; WJC and CYH carried out the experiments; WJC, CYH, HY, RT, ZLC, SW and CLZ analyzed the experiment data; WJC and QZL wrote and revised this manuscript. All authors had read and agreed with the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

None declared.

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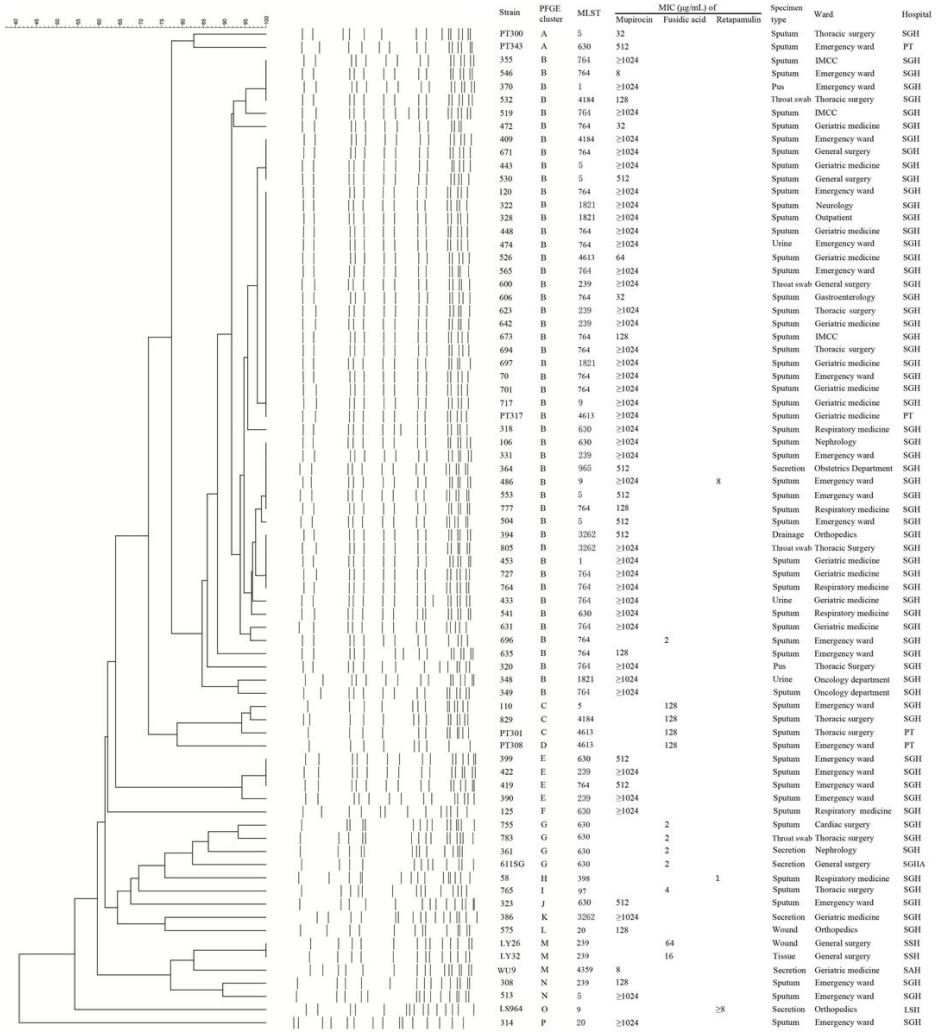
Figures



Strain	PFGE cluster	MLST	MIC (μg/mL) of			Specimen type	Ward	Hospital
			Mupirocin	Fusidic acid	Retapamulin			
PT300	A	5	32			Sputum	Thoracic surgery	SGH
PT343	A	630	512			Sputum	Emergency ward	PT
355	B	764	≥1024			Sputum	IMCC	SGH
546	B	764	8			Sputum	Emergency ward	SGH
370	B	1	≥1024			Pus	Emergency ward	SGH
532	B	4184	128			Throat swab	Thoracic surgery	SGH
519	B	764	≥1024			Sputum	IMCC	SGH
472	B	764	32			Sputum	Geriatric medicine	SGH
409	B	4184	≥1024			Sputum	Emergency ward	SGH
671	B	764	≥1024			Sputum	General surgery	SGH
443	B	5	≥1024			Sputum	Geriatric medicine	SGH
530	B	5	512			Sputum	General surgery	SGH
120	B	764	≥1024			Sputum	Emergency ward	SGH
322	B	1821	≥1024			Sputum	Neurology	SGH
328	B	1821	≥1024			Sputum	Outpatient	SGH
448	B	764	≥1024			Sputum	Geriatric medicine	SGH
474	B	764	≥1024			Urine	Emergency ward	SGH
526	B	4613	64			Sputum	Geriatric medicine	SGH
565	B	764	≥1024			Sputum	Emergency ward	SGH
600	B	239	≥1024			Throat swab	General surgery	SGH
606	B	764	32			Sputum	Gastroenterology	SGH
623	B	239	≥1024			Sputum	Thoracic surgery	SGH
642	B	239	≥1024			Sputum	Geriatric medicine	SGH
673	B	764	128			Sputum	IMCC	SGH
694	B	764	≥1024			Sputum	Thoracic surgery	SGH
697	B	1821	≥1024			Sputum	Geriatric medicine	SGH
70	B	764	≥1024			Sputum	Emergency ward	SGH
701	B	764	≥1024			Sputum	Geriatric medicine	SGH
717	B	9	≥1024			Sputum	Geriatric medicine	SGH
PT317	B	4613	≥1024			Sputum	Geriatric medicine	PT
318	B	630	≥1024			Sputum	Respiratory medicine	SGH
106	B	630	≥1024			Sputum	Nephrology	SGH
331	B	239	≥1024			Sputum	Emergency ward	SGH
364	B	965	512			Secretion	Obstetrics Department	SGH
486	B	9	≥1024		8	Sputum	Emergency ward	SGH
553	B	5	512			Sputum	Emergency ward	SGH
777	B	764	128			Sputum	Respiratory medicine	SGH
504	B	5	512			Sputum	Emergency ward	SGH
394	B	3262	512			Drainage	Orthopedics	SGH
805	B	3262	≥1024			Throat swab	Thoracic Surgery	SGH
453	B	1	≥1024			Sputum	Geriatric medicine	SGH
727	B	764	≥1024			Sputum	Geriatric medicine	SGH
764	B	764	≥1024			Sputum	Respiratory medicine	SGH
433	B	764	≥1024			Urine	Geriatric medicine	SGH
541	B	630	≥1024			Sputum	Respiratory medicine	SGH
631	B	764	≥1024			Sputum	Geriatric medicine	SGH
696	B	764		2		Sputum	Emergency ward	SGH
635	B	764	128			Sputum	Emergency ward	SGH
320	B	764	≥1024			Pus	Thoracic Surgery	SGH
348	B	1821	≥1024			Urine	Oncology department	SGH
349	B	764	≥1024			Sputum	Oncology department	SGH
110	C	5		128		Sputum	Emergency ward	SGH
829	C	4184		128		Sputum	Thoracic surgery	SGH
PT301	C	4613		128		Sputum	Thoracic surgery	PT
PT308	D	4613		128		Sputum	Emergency ward	PT
399	E	630	512			Sputum	Emergency ward	SGH
422	E	239	≥1024			Sputum	Emergency ward	SGH
419	E	764	512			Sputum	Emergency ward	SGH
390	E	239	≥1024			Sputum	Emergency ward	SGH
125	F	630	≥1024			Sputum	Respiratory medicine	SGH
755	G	630		2		Sputum	Cardiac surgery	SGH
783	G	630		2		Throat swab	Thoracic surgery	SGH
361	G	630		2		Secretion	Nephrology	SGH
611SG	G	630		2		Secretion	General surgery	SGHA
58	H	398			1	Sputum	Respiratory medicine	SGH
765	I	97		4		Sputum	Thoracic surgery	SGH
323	J	630	512			Sputum	Emergency ward	SGH
386	K	3262	≥1024			Secretion	Geriatric medicine	SGH
575	L	20	128			Wound	Orthopedics	SGH
LY26	M	239		64		Wound	General surgery	SSH
LY32	M	239		16		Tissue	General surgery	SSH
W19	M	4359	8			Secretion	Geriatric medicine	SAH
308	N	239	128			Sputum	Emergency ward	SGH
513	N	5	≥1024			Sputum	Emergency ward	SGH
LS964	O	9			≥8	Secretion	Orthopedics	LSH
314	P	20	≥1024			Sputum	Emergency ward	SGH

Figure 1

Characteristics of 76 clinical MRSA isolates with mupirocin, fusidic acid and/or retapamulin resistance.