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Predominance of ST5-II-t311 clone among healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates recovered from Zhejiang, China

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ABSTRACT

Objectives: To determine the molecular characteristics and antimicrobial susceptibility of healthcare-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) in Zhejiang Province.
Methods: A total of 391 HA-MRSA isolates were collected from 12 hospitals in five cities of Zhejiang Province, between January 2012 and May 2013. Susceptibility to vancomycin, teicoplanin, linezolid, tigecycline, and daptomycin was determined. Resistant isolates were screened for resistance mutations. Ten isolates from each hospital were then chosen at random for molecular typing.
Results: The isolates showed good susceptibility to all five anti-MRSA agents; only five sporadic non-susceptible isolates were detected. CC5/ST5-MRSA-II-t311 (39/120, 32.5%) was found to be the predominant HA-MRSA clone and was spread between the different hospitals in Hangzhou. CC5/ST5-MRSA-II-t002 was the most prevalent clone in Ningbo, while CC239/ST239-MRSA was epidemic only in certain hospitals in Wenzhou and Shaoxing. Fifteen ST59 isolates (15/120, 12.5%) were identified among the HA-MRSA isolates.
Conclusions: CC5/ST5-MRSA-II-t311 has become the predominant HA-MRSA clone in Hangzhou, Zhejiang Province. ST59 MRSA has spread into hospitals. The isolates showed good susceptibility to all five anti-MRSA agents.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has caused worldwide concern because of its high incidence and undesirable outcomes (Hassoun et al., 2017). The Active Bacterial Core Surveillance of the United States reported an overall national adjusted incidence rate of invasive MRSA infection of approximately 22.72 per 100 000 population per year and mortality rate of 2.88 per 100 000 population per year (Centers for Disease Control and Prevention, 2014). A high prevalence of MRSA has also been seen in China. Recent data from the CHINET Surveillance system indicated that MRSA accounted for 38.4% of all clinical staphylococcal isolates in China in 2016 (Fupin Hu et al., 2017). To control the spread of MRSA, it is essential to know the genotypic characteristics of MRSA clones in certain geographic regions. Previous studies have demonstrated ST239-III-t030 and ST239-III-t037 to be the most prevalent clones of MRSA in China (Xiao et al., 2013). However, the National Surveillance system cannot completely represent the status in each province.

The global MRSA epidemic calls for an intervention, but the options for the treatment of MRSA infection are limited. Vancomycin has been considered the gold standard treatment for MRSA infection. However, its role has been questioned and debated by researchers over the past 5 to 10 years, because of heteroresistance and reduced vancomycin susceptibility (Koh et al., 2016; Moravvej et al., 2013). Linezolid and teicoplanin have been used increasingly worldwide over the last decade. Daptomycin and tigecycline are two new antibiotics that came into use in

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China over the last 5 years. Although still rare, the number of clinical MRSA isolates resistant to these antimicrobial agents is growing (Molina and Huang, 2016).

The aim of this study was to identify the healthcare-associated MRSA (HA-MRSA) clonal types currently circulating in Zhejiang Province and update the information on MRSA clonal evolution. Another objective was to evaluate the in vitro activity of different anti-MRSA agents, compare the antimicrobial susceptibility among the different clonal types, and investigate the possible resistance mechanisms.

Materials and methods

Bacterial isolation and collection, MRSA identification, and DNA extraction

From January 2012 to May 2013, a total of 391 non-duplicate HA-MRSA isolates were collected from 12 tertiary-care teaching hospitals in five cities of Zhejiang Province with a population of 50 million. Seven hospitals were located in Hangzhou, two hospitals were located in Wenzhou, and the remaining three hospitals were located in Ningbo, Shaoxing, and Jiaxing. HA-MRSA was defined as described previously (Li et al., 2013). The isolates were recovered from various clinical sources, including blood, drainage, pus, central venous catheter, and sputum. Isolates of *S. aureus* were identified with a Vitek microbial identification system (Vitek 2; bioMérieux, France), and phenotypic methicillin resistance was confirmed using the cefoxitin disk diffusion method and *mecA* gene detection by PCR (Hu et al., 2013). Only one isolate per patient per infection episode was included in the surveillance sample. Ten MRSA isolates from each hospital, i.e., a total 120 isolates, were selected at random for further molecular typing.

Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of five antimicrobial agents, including vancomycin, teicoplanin, linezolid, tigecycline, and daptomycin, were determined. Broth microdilution (BMD) susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) standards (CLSI, 2017). *S. aureus* ATCC 29213 was used as a quality control. Susceptibility interpretive criteria were based on the CLSI (CLSI, 2017), except for daptomycin and tigecycline, for which European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) breakpoints were used.

Nucleotide sequencing and mutation detection

For non-susceptible isolates, resistance mediating mutations were investigated (Heidary et al., 2018; Howden et al., 2010). For isolates resistant to teicoplanin and daptomycin, the *vraSR*, *yvqF*, *vraFG*, *graSR*, *walKR*, *mprF*, *trfAB*, *tcaRAB*, *sigB*, *rsbUVW*, *cls2*, *clpP*, *rpoC*, and *rpoB* genes or operons were screened. For isolates resistant to tigecycline, the *mepA*, *mepR*, and *rpsJ* genes were screened. For linezolid-resistant isolates, *rplC*, *rplD*, and *rplV* were screened. The full-length and bidirectional sequences were aligned and compared to the reference genomes N315 (GenBank accession number **BA000018.3**) and TW20 (GenBank accession number **FN433596.1**) according to the sequence types (STs) of the resistant strains. For linezolid-resistant isolates, the *cfr* and *optrA* genes and mutations in 23S rRNA were screened as described previously (Toh et al., 2007). The primers are shown in the Supplementary material, Table S1.

Multilocus sequence typing (MLST)

MLST was performed according to the methods described by Enright et al. (Enright et al., 2000). eBURST software was used to assign the STs to clonal complexes (CCs) (http://eburst.mlst.net/v3/enter_data/single/). The evolutionary relationship between the isolates was assessed using the Minimal Spanning Tree (MST) algorithm implemented in Bionumerics v.6.06 (Applied Maths, St-Martens-Latem, Belgium).

Pulsed field gel electrophoresis (PFGE)

PFGE was performed according to the method described by Bannerman et al. with some modifications (Bannerman et al., 1995). The PFGE profiles were analyzed using Bionumerics software v.6.06 (Applied Maths, St-Martens-Latem, Belgium). Similarity coefficients were calculated and a dendrogram was constructed using the Dice coefficient with a tolerance of 1.5% and optimization of 1% and the unweighted pair group method with arithmetic averages (UPGMA) (Yan et al., 2012). Isolates with ≥80% similarity were considered closely related, while those with ≥90% similarity were considered nearly identical (Molina et al., 2008).

Staphylococcal cassette chromosome mec *(SCC*mec*) typing*

The SCC*mec* types were determined using a multiplex PCR strategy developed by Zhang et al. (Zhang et al., 2005). Non-typeable (NT) types were defined as isolates showing unexpected fragments. International clones of SCC*mec* types I to V were used as quality controls.

*Staphylococcal protein A (*spa*) gene typing*

spa typing was performed as described previously (Harmsen et al., 2003). The amplified products were sequenced and analyzed based on the *spa* database website (http://spatyper.fortinbras.us/).

Table 1
Minimum inhibitory concentration distributions of 391 healthcare-associated methicillin-resistant *Staphylococcus aureus* isolates and the profiles of the main sequence types.

Antimicrobial agent	MIC (μg/ml)				Breakpoint[a] (μg/ml)			MIC_{50}/MIC_{90} of the main STs (μg/ml)			Characterization of non-susceptible isolates (*n*, ST, MIC)
	50	90	Range	S%/R%	S	I	R	ST5 (*n* = 55)	ST59 (*n* = 15)	ST239 (*n* = 28)	
Vancomycin	1	1	0.25–2	100/0	≤2	4–8	≥16	1/1	1/1	1/1	
Teicoplanin	1	2	0.25–16	99.7/0	≤8	16	≥32	1/2	1/1	1/2	1[b], ST5, MIC 16 μg/ml
Linezolid	2	4	1–16	99.7/0.3	≤4	–	≥8	2/4	2/4	2/2	1, ST5, MIC 16 μg/ml
Daptomycin	0.5	1	0.12–2	99.5/0.5	≤1	–	>1	0.5/1	0.5/1	0.5/1	2 (1[b]), ST5, MIC 2 μg/ml
Tigecycline	0.12	0.5	0.06–1	99.5/0.5	≤0.5	–	>0.5	0.125/0.5	0.125/0.25	0.125/0.5	2, ST239, MIC 1 μg/ml

MIC, minimum inhibitory concentration; ST, sequence type; S, susceptible; I, intermediate; R, resistant.
[a] Criteria were based on the Clinical and Laboratory Standards Institute guidelines (CLSI, 2017), except for daptomycin and tigecycline, for which the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2017) breakpoints were used.
[b] SA1089 was resistant to daptomycin and intermediate to teicoplanin.