

Full Length Research Paper

Staphylococcal cassette chromosome mec (SCCmec) analysis and antimicrobial susceptibility profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in a teaching hospital, Shantou, China

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Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has now become a major public health concern. The aim of this study is to determine the antibiotic resistance pattern and prevalence of different staphylococcal cassette chromosome mec (SCCmec) types among the MRSA isolates from a teaching hospital in Shantou, China. The minimum inhibitory concentrations (MICs) of seventeen antimicrobial agents against MRSA isolates were determined using the micro broth dilution method. SCCmec types were identified by multiplex polymerase chain reaction (PCR) strategy. The results show that all MRSA isolates were resistant to ampicillin, oxacillin, gentamycin, erythromycin and ciprofloxacin. Fewer than 6% of isolates were resistant to doxycycline, but more than 90% were resistant to cefazolin, cefuroxime, cefotaxime, cefepime, sparfloxacin and tetracycline. There was no minocycline, chloramphenicol or vancomycin resistant. *S. aureus* was found in this study. SCCmec type III and IIIA were predominant in our study. Our data highlighted that multidrug-resistant strains of MRSA caused severe problems in Shantou, China. However, some of the old agents, such as minocycline, doxycycline and chloramphenicol are highly effective against MRSA isolates.

Key words: Methicillin-resistant *Staphylococcus aureus* (MRSA), staphylococcal cassette chromosome mec (SCCmec), antimicrobial agent resistance.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for a large percentage of clinical infections and is considered as a serious problem owing to the multidrug-resistant properties (Fishbain et al., 2003; Kuehnert et al., 2005). MRSA expresses a specific penicillin binding protein called PBP2a or PBP2' from mecA gene, which has a decreased binding affinity to β -lactams causing the resistance to most of β -lactams (Berger-Bachi and Rohrer, 2002). SCCmec is the chro-

mosomally integrated resistance element which carries the mecA gene. At most, seven types of SCCmec have been described differentiated according to their combinations of mec complex (Berglund et al., 2008; Deurenberg and Stobberingh, 2008; Higuchi et al., 2008; Oliveira and Lencastre, 2002; Oliveira et al., 2006). In China, MRSA has now emerged as an important pathogen as in other countries (Zhang et al., 2006). Therefore, it becomes necessary to know the prevalence of MRSA and their current antimicrobial profile for the selection of appropriate treatment. This study was designed to determine the antibiotic resistance patterns and prevalence of different SCCmec types among the MRSA isolates from a teaching hospital in Shantou (south of China).

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MATERIALS AND METHODS

Bacterial strains

115 *S. aureus* isolates were collected consecutively from patients attending the First Affiliated Hospital of Shantou University, a 1200 bed teaching hospital from January 2005 to January 2006. Only one isolate per patient was included. The isolated strains were defined as *S. aureus* using the VITEK32 Automated Microbiology System combined with standard biochemical methods. Cefoxitin susceptibility was tested to screen the MRSA isolates using disc diffusion method according to Clinical Laboratory and Standards Institute (CLSI) guidelines (2009). The standard strain of *S. aureus* (ATCC 25923) which is a MSSA (methicillin-sensitive *Staphylococcus aureus*) was used for a control strain.

Reagents and culture media

The antimicrobial agents used in this study all came from the Sigma Chemical Company. We used cation adjusted Muller Hinton broth (CAMHB) and Muller-Hinton agar manufactured by Oxoid Ltd. (Basingstoke, Hampshire, England).

Detection of *mecA* gene

The isolates resistant to cefoxitin were further tested by *mecA* gene detection. DNA templates were prepared as follows: 2.0 ml of overnight bacterial broth culture was centrifuged at 10,000 rpm for 2 min. Pellet was resuspended in 180 μ l of 20 mM Tris-HCl, pH 8.4 (containing 20 mg/ml lysozyme and 0.1 mg/ml lysostaphin) and then incubated at 37°C for 40 min. DNA extraction was performed by using the bacterial genomic DNA extraction Kit (Tangen Biotech, China). To confirm the identity of MRSA isolates, the polymerase chain reaction (PCR) assay was performed in a DNA thermal cycler, by using the PCR primers for the *mecA* gene (449F [5'AAA CTA CGG TAA CAT TGA TCG CAA C-3' and 761R] 5'CTT GTA CCC AAT TTT GAT CCA TTT G-3') (Zhao et al., 2001).

Determination of minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations (MICs) of ampicillin, oxacillin, cephazoline, cefuroxime, cefotaxime, cefepime, imipenem, tetracycline, doxycycline, minocycline, sparfloxacin, ciprofloxacin, erythromycin, gentamicin, rifampicin, chloramphenicol and vancomycin were determined by the micro broth dilution method recommended by clinical laboratory standard institute (CLSI, 2009).

Identification of SCCmec typing

Multiplex PCR for SCCmec types were performed as described by (Oliveira and Lencastre, 2002). The following MRSA reference strains were used as controls: Harmany 21 (SCCmec I), Harmany 24 (SCCmec II), Harmany 20 (SCCmec III), Harmany 22 (SCCmec IV) and HS662 (SCCmec V).

RESULTS

The screening of MRSA isolates

All the cefoxitin resistant *S. aureus* isolates contained *mecA* gene and were therefore confirmed as MRSA strains. 80% (n = 92) of *S. aureus* isolates were MRSA

among the 115 collected *S. aureus* isolates. Most of the MRSA were cultured from respiratory tract (n = 69). Others were isolated from blood (n = 9), body fluids (n = 8) and wounds (n = 6).

Antibiotic susceptibility

The antimicrobial susceptibility profiles of MRSA isolates are summarized in Table 1. All MRSA isolates were resistant to ampicillin, oxacillin, gentamycin, erythromycin and ciprofloxacin. Fewer than 6% of MRSA isolates were resistant to doxycycline, but more than 90% were resistant to cefazolin, cefuroxime, cefotaxime, cefepime, sparfloxacin and tetracycline. No minocycline, chloramphenicol or vancomycin resistant *S. aureus* was found in this study.

SCCmec typing

Ninety isolates (97.8%) were identified as SCCmec type III (56 isolates) or IIIA (44 isolates). One strain was identified as a variant of SCCmec II (contains loci C and D) which has the same multiplex pattern as that of SCCmec II₆ reported by (Kim et al., 2007). A new variant of SCCmec III (contains loci C and F but loses the specific locus E of SCCmec III) was also detected in our study which was not reported previously (Figure 1). The expected results for SCCmec of all control strains type were also shown in Figure 1. There were no significant differences in resistance profiles between SCCmec types III and IIIA. Strains of SCCmec II₆ and the new variant of SCCmec III also have the similar resistance pattern to that of SCCmec types III and IIIA, and both of them are susceptible to rifampicin and chloramphenicol.

DISCUSSION

MRSA infection has now become a major public health concern and its prevalence is increasing globally (Zhang et al., 2006). It is usually resistance to multiple antibiotics and making infections difficult to treat that account for an increasing proportion of staphylococcal infections among hospitalized patients. Therefore, continuous bacterial susceptibility data update is necessary to control the MRSA infection in hospital. The prevalence of Hospital-Acquired MRSA (HA-MRSA) in Mainland China in 2005 was 50.3% and in Shanghai, China, the prevalence reached 80.3% (Wang et al., 2008). In this study, the rate of methicillin resistance among *S. aureus* isolates in Shantou was 80%, which is similar to that in Shanghai. The high prevalence of MRSA in our teaching hospital indicated that more intensified control policy is needed to reduce the MRSA infections. Antibiotic susceptibility test revealed that β -lactams, fluoroquinolones, gentamycin, erythromycin and tetracycline are all poorly active against MRSA.

Table 1. Antimicrobial susceptibility profiles of MRSA isolates in Shantou.

	MRSA strains			Range	MIC (mg/L)		Resistant rate (%)
	S	I	R		MIC50	MIC90	
Ampicillin	0	0	92	32 - 256	128	128	100.00
Oxacillin	0	0	92	128 - 512	256	256	100.00
Cefazolin	7	1	84	2 - 256	128	256	91.30
Cefuroxime	4	2	86	0.03 - 256	128	256	93.48
Cefotaxime	2	4	86	1 - 256	128	256	93.48
Cefepime	3	4	85	2 - 256	128	128	92.39
Imipemen	6	5	81	0.03 - 128	64	128	88.04
Tetracycline	1	1	90	2 - 256	128	128	97.83
Doxycycline	61	26	5	0.5 - 32	4	16	5.43
Minocycline	92	0	0	0.125 - 4	2	4	0.00
Gentamycin	0	0	92	128 - 512	256	256	100.00
Erythromycin	0	0	92	256 - 512	512	512	100.00
Chloramphenicol	87	5	0	0.5 - 16	2	8	0.00
Rifampin	52	1	39	0.03 - 64	0.5	32	42.39
Ciprofloroxacin	0	0	92	2 - 128	128	128	100.00
Sparfloxacin	0	2	90	1 - 16	16	16	97.83
Vancomycin	92	0	0	0.5 - 2	1	2	0.00

MRSA, methicillin-resistant *s. aureus*; MIC, minimum inhibitory concentration; S, sensitive; I, intermediate; R, resistance.

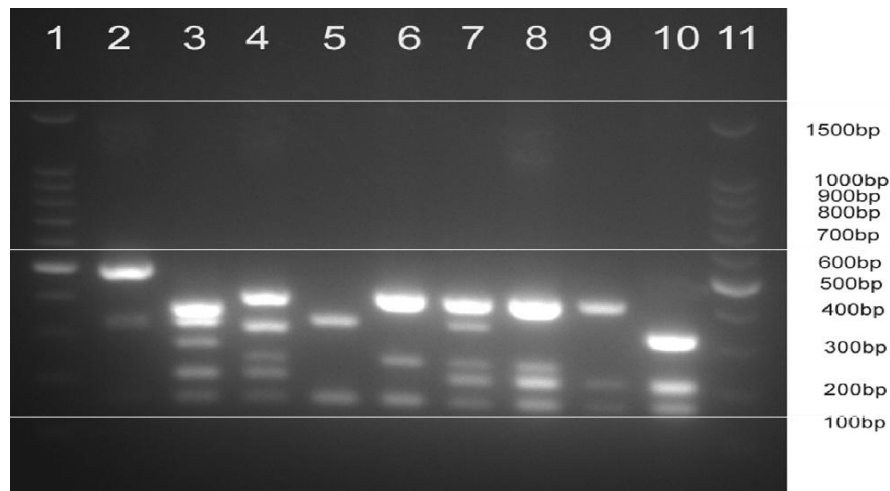


Figure 1. Multiplex PCR SCCmec typing of MRSA isolates. Lanes 1 and 11,; DNA 100-bp ladder; Lane 2, Harmany 21 (SCCmec I); Lane 3, Harmany 24 (SCCmec II); Lane 4, Harmany 20 (SCCmec III); Lane 5, Harmany 22 (SCCmec IV); Lane 6, HS662 (SCCmec V); Lane 7 and 8 tested MRSA isolates (identified as SCCmec types IIIA, and III, respectively); lane 9 tested MRSA isolate (a new variant of SCCmec III); Lane 10 tested MRSA (SCCmec II₆).

However, some old agents, such as chloram-phenicol, minocycline and doxycycline were still highly active against MRSA in this study, which indicated that these old agents may be used in the treatment of MRSA infections. Our results are also consistent with those of others who found that MRSA is resistant to most

antibiotics, but are almost always susceptible to chloram-phenicol, long-acting tetracyclines and vancomycin (Baddour et al., 2006; Cha et al., 2005; Cunha, 2006; Kim et al., 2004).

SCCmec types are usually related with the evolutionary origin of MRSA. SCCmec types I, II and III are most often

found in hospital-acquired MRSA, whereas SCCmec types IV and V are mainly associated with community-acquired MRSA (CA-MRSA) (Chmelnitsky et al., 2008). The SCCmec types I, as well as SCCmec type IV and SCCmec V, usually carry no additional drug resistance genes other than *mecA* (Robinson and Enright, 2003). SCCmec types II and III isolates carry additional genes that provide resistance to heavy metals and drugs other than β -lactams (Robinson and Enright, 2003).

Although, the multiplex PCR strategy used in this study was not able to identify SCCmec VI and SCCmec VII (Higuchi et al., 2008; Oliveira et al., 2006). It was still a useful tool for the rapid identification of the structural type of the *mec* elements in MRSA isolates (Oliveira and Lencastre, 2002). SCCmec analysis showed that 97.8% of MRSA isolates were identified as SCCmec type III and IIIA suggesting that most of the MRSA isolates in this study may have originated from HA-MRSA. These results were also consistent with previous reports that SCCmec III and IIIA types were predominant in most Asian countries (Fatholahzadeh et al., 2008; Ko et al., 2005). A variant of SCCmec II was found in an isolate which has the same multiplex pattern as the SCCmec II₆ described by Kim et al. (2007).

This was the first report of SCCmec II₆ strain isolated from China. We also found another variant of SCCmec III containing the loci C and F with an absence of locus E. We assume that this may be a new variant of SCCmec III. It was previously reported that SCCmec-IV and V strains were predominately isolated from skin and soft tissue and abscess (Kilic et al., 2008). No SCCmec IV or SCCmec V strains were found in the present study, probably due to the small numbers of isolates from wound. Other molecular techniques such as pulsed field gel electrophoresis, *spa* gene sequencing and multilocus sequence typing are required to fully investigate the SCCmec types and the epidemiology of MRSA within the hospital.

Conclusion

Our data highlights that multidrug-resistant strains of MRSA have caused severe problems in Shantou, China, which may be related to the inappropriate use of antibiotics. SCCmec type III and IIIA were predominant among the MRSA isolates in Shantou. Some of the old agents such as minocycline, doxycycline and chloramphenicol are still highly effective against MRSA isolates.

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