Prevalence of Enterotoxin Genes and spa Genotypes of Methicillin-resistant Staphylococcus aureus from a Tertiary Care Hospital in China

YANMENG LI, RUIKE ZHAO, XIANFENG ZHANG, QINGZHEN HAN, XUEFENG QIAN, GUOHAO GU, JINFANG SHI, JIE XU

ABSTRACT

Objectives: Methicillin-resistant Staphylococcus aureus (MRSA) is a major nosocomial pathogen that causes a variety of infections. MRSA has evolved resistance to multiple antibiotics. Genetic background and virulence differ in different geographic regions. The present study was aimed to investigate the prevalence of enterotoxin genes and spa genotypes of hospital-associated methicillin-resistant Staphylococcus aureus (HA-MRSA) isolated from a tertiary care hospital of Jiangsu province, China.

Materials and Methods: HA-MRSA isolates from August 2013 to April 2014 at a tertiary care hospital of China were collected. We investigated antimicrobial pattern, spa types, SCCmec types and the presence of 14 virulence genes.

Results: Eighty HA-MRSA isolates were collected. Results from SCCmec typing revealed that 73.8% were type II; 13.8% were type III; 12.5% were type V. There were 19 different spa types. Spa type t2460 was the most common (35.0%), followed by t002 (11.3%). CC5 was the predominant MLST CCs type (50%). The most frequent toxin genes were sea, seb, sed, sel, sen and seo (100.0%). None of the investigated isolates carried the sec or tst.

Conclusion: Genotypic and virulence evaluation of the isolated HA-MRSA revealed that the isolates with CCS and SCCmec II were the predominant type and highly homologous. The virulence profiles mainly existed in the genes of sea, seb, sed, sel, sen, seo and ser. The prevalence of t2460 was an outbreak and the predominant spa type.

Keywords: sea, seb, sed, sel, sen, seo, ser, t2460

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen of public health importance. Since the first European isolate of MRSA was detected in 1961, MRSA isolates has become a leading cause of hospital-acquired or healthcare-associated infections throughout the world [1-3]. In China, the mean prevalence rate of HA-MRSA isolates had reached 47.9 % by 2012 [4]. MRSA strains have acquired and integrated into their genome a 21-67 kb mobile genetic element, termed the staphylococcal cassette chromosome mec (SCCmec). SCCmec elements are highly diverse in their structural organization and genetic content and have been classified into types and subtypes. Strains with SCCmec types I, II and III are most commonly found in isolates from hospital-acquired infections, while community-acquired strains predominantly carry SCCmec types IV or V [5,6]. SCCmec type IV is also characteristic of some HA-MRSA clones. Spa typing based on the polymorphic staphylococcal protein A (spa) coding region is a common genotyping tool for MRSA [7]. Genotyping with spa has been showed discriminatory power similar to multi-locus sequence typing (MLST) [8].

Enterotoxins, toxic shock syndrome toxin 1 (TSST-1), exfoliative toxin (ET), haemolysins and coagulase are among various virulence factors produced by S. aureus. The enterotoxins, and TSST-1, belong to a family of superantigens. Eighteen Staphylococcal enterotoxins (SEs) have been recognized as: SEA, SEB, SEC, SEK, SEA, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, SEQ, SER and SEU. They are the main source of food poisoning and cause intensive intestinal peristalsis [9]. The present study aimed to identify the types of spa, SCCmec and the virulence genes among HA-MRSA isolates collected from a tertiary care hospital. Their association was examined to enhance our current knowledge of the pathogenicity and evolution of HA-MRSA.

MATERIALS AND METHODS

Selection of the strains

Eighty HA-MRSA strains were isolated from unrelated patients in the First Affiliated Hospital of Soochow University from September 2013 to June 2014. This hospital has 1800 beds and serves a population of 1,000,000 inhabitants in both urban and rural areas. These strains were obtained from sputum (71), wound swabs (9), secretions (3), Pharyngeal swabs (3), urine samples (3), body fluid (2), liquor puris (2), bone marrow (1), catheter (1) and others (1). The presence of methicillin resistance was evaluated using a cefoxitin disc (30µg; Oxoid). The presence of the resistance gene mecA was tested for PCR according to a protocol previously described [10].

Susceptibility testing

Antimicrobial susceptibility test for isolates of S. aureus was performed against cotrimoxazole (SXT, 25µg), vancomycin (VAN, 30µg), teicoplanin (TEC, 30µg) and linezolid (LZD, 30µg) (Oxoid, UK), by the disc diffusion method. The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI- 2011) [11].

DNA isolation

All isolates were cultured on blood agar and incubated overnight at 37°C. Genomic DNA was isolated from all strains with Wizard Genomic DNA purification kit (Promega, China), according to the manufacturer’s instructions and used as template for PCR.

Spa typing of strains

All HA-MRSA were characterized by comparative DNA analysis of the variable number of tandem repeats region of the S. aureus
protein A (spa) gene similar to a previously described method [12], using primers spa-1095F and spa-1517R. Calculation of the type ability, diversity, and concordance of the spa typing method with the results of alternative typing methods was implemented in Ridom SpaServer software (http://spa.ridom.de/index.shtml).

**SCCmec typing of strains**

MRSA strains were further characterized by simplex PCR of the SCCmec gene, as described elsewhere [13].

**Detection of virulence genes**

The genes encoding staphylococcal enterotoxins (sea, seb, sec, sed, selR, sen, seo, sep, seq, ser, seu), tst-1, pvl and cna were performed by single PCR as previously reported [14]. The primers used in this study are listed in [Table/Fig-1].

**RESULTS**

**Antimicrobial Susceptibility**

Overall, the resistance rates for the HA-MRSA strains were 100.0% (80/80) for cefoxitin (FOX), 100% (80/80) for penicillin (P), 93.8% (75/80) for ciprofloxacin (CIP), 62.5% (50/80) for clindamycin (DA), 13.8% (11/80) for sulfamethoxazole (SXT) [Table/Fig-2]. No resistance to vancomycin, teicoplanin, and linezolid was found. Almost all of the isolates except four which were included in this study, were found to be resistant to three or more groups of antibiotics which were tested and five different resistant patterns were observed amongst them [Table/Fig-3]. Most strains were resistant to cefoxitin, penicillin and ciprofloxacin.

**SCCmec typing and spa typing**

The distribution of spa types, spa types, virulence gene profile in isolates is shown in [Table/Fig-4]. Among the 80 HA-MRSA strains, SCCmec II, SCCmec III and SCCmec V were identified in 73.8% (59/80), 13.8% (11/80) and 12.5% (10/80) of strains, respectively.

There were 19 different spa types (t2460, t1002, t632, t030, t437, t211, t4549, t299, t163, t2310, t164, t377, t264, t279, t459 and t034) [Table/Fig-4]. Spa type t2460 were the most prevalent one (35.0%, 28/80), followed by spa type t002 (11.3%, 9/80). The prevalence of t2460 was thought to be an outbreak. It was previously reported that t002, t311, and t2460 were linked to MLST CC5, and t030, t211 and t037 were associated with CC8 [18,19]. CC5 is one of the major MLST CCs type (50%, 40/80) in Souh.

**Virulence factors genes analysis**

The presence of 14 virulence genes was in all 80 HA-MRSA isolates. The most frequent toxin genes were sea, seb, sec, sed, sel, sen, seo, sep, seq, ser, seu. The presence of 14 virulence genes was in all 80 HA-MRSA isolates. The most frequent toxin genes were sea, seb, sec, sed, sel, sen, seo, sep, seq, ser, seu. The prevalence of t2460 were the most frequent one (35.0%, 28/80), followed by spa type t002 (11.3%, 9/80). The prevalence of t2460 was thought to be an outbreak. It was previously reported that t002, t311, and t2460 were linked to MLST CC5, and t030, t211 and t037 were associated with CC8 [18,19]. CC5 is one of the major MLST CCs type (50%, 40/80) in Souh.

**Antibiotics**

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Resistant (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOX</td>
<td>80(100%)</td>
</tr>
<tr>
<td>P</td>
<td>80(100%)</td>
</tr>
<tr>
<td>CIP</td>
<td>75(93.8%)</td>
</tr>
<tr>
<td>DA</td>
<td>50(62.5%)</td>
</tr>
<tr>
<td>SXT</td>
<td>11(13.8%)</td>
</tr>
<tr>
<td>VAN</td>
<td>0</td>
</tr>
<tr>
<td>TEC</td>
<td>0</td>
</tr>
<tr>
<td>LZD</td>
<td>0</td>
</tr>
</tbody>
</table>

**[Table/Fig-2]: Drug resistance of the 80 HA-MRSA isolates**

<table>
<thead>
<tr>
<th>Resistance pattern</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOX-P</td>
<td>9</td>
</tr>
<tr>
<td>FOX-P-CIP-DX-SXT</td>
<td>40</td>
</tr>
<tr>
<td>FOX-P-CIP</td>
<td>2</td>
</tr>
<tr>
<td>FOX-P-CIP-DX</td>
<td>24</td>
</tr>
<tr>
<td>FOX-P-DX</td>
<td>1</td>
</tr>
<tr>
<td>FOX-P</td>
<td>4</td>
</tr>
</tbody>
</table>

**[Table/Fig-3]: Resistance patterns of the MRSA isolates**
was t437. MRSA isolated from children may be community acquired
the predominant
[25] and [26] reported that
the same results among the 80 HA-MRSA isolates (35.0%, 28/80).
Further investigation is required.
CA-MRSA were different and there may be different evolutionary
implied that the virulence characteristics between HA-MRSA and
Similar results were also observed in a previous study [22]. This
this study, we did not find the existence of sec or tst.
was t2460(28), t002(21.3%, 17/80), seq (21.3%, 17/80), sep (10.0%, 8/80)
and pvl (7.5%, 6/80) [Table/Fig-4]. But none of the investigated
isotopes carried the sec or tst.
The enterotoxin gene cluster is always present in MLST CC5, CC22,
and CC45 strains but not in CC8, CC12, CC15, and CC395 [20]. The
results that CC5 is the major MLST CC type (50%) showed that
distribution of the virulence gene cluster in our study is similar to that
of previous findings.

DISCUSSION
Virulence and resistance are two important pathogenic character-
distinctive. Strains with different virulence factors commonly display different
level of pathogenicity. Genetic background and virulence differs in different geographic regions. This study was conducted to investigate the virulence characteristics and the presence of virulent
genomes in HA-MRSA from China. Wu et al., reported that the SAg
genes presence of exfoliative toxin genes in CA-MRSA isolates
from Chinese children [21]. The common toxin gene combination was sec-sek-seq, with 92.6% found in CC59 [21]. Our
results displayed that the most common toxin gene combination was sea-seb-sed-sel-sen-seo-ser (100.0%, 80/80), with 50% found
in MLST CC5. Previous study showed that SEA and SEC tend to
trigger T-cell proliferation and induce higher inflammatory responses
resulting in host tissue damage than do other enterotoxins [18]. In
this study, we did not find the existence of sec in Suzhou isolates.
Similar results were also observed in a previous study [22]. This
implied that the virulence characteristics between HA-MRSA and
CA-MRSA were different and there may be different evolutionary
mechanism underlying this. Further investigation is required.

Researches based on spa typing exhibited that the predominant
HA-MRSA clone was t2460-MRSA in Asian countries besides
Japan and South Korea (MLST CC5) [23,24]. Our study displayed
the same results among the 80 HA-MRSA isolates (35.0%, 28/80).
Shipeng Li et al., [25] and Yanghong Qiao et al., [26] reported that the
predominant spa-type in MRSA isolated from Chinese children
was t437. MRSA isolated from children may be community acquired
MRSA (CA-MRSA). Hang Cheng et al., [27] found that the prevalent
spa-type was t030. However, only three strains were spa-type
437 and six strains were spa-type t030 in the study. This implied that the prevalent spa-types between HA-MRSA and CA-MRSA
may be different. It was previously reported that t002, t601, and
t2460 are linked to MLST CC5, and t037 is associated with CC8
[25]. In the study, the CC5 isolates accounted for 50% (40/80) of the representative strains [Table/Fig-4]. [Table/Fig-4] showed that
t2460(35%, 28/80), t002(11.3%, 9/80), t632(8.8%, 7/80) and
t030(7.5%, 6/80) were the common spa types in Suzhou isolates.
It was previously reported that the genetic background is closely related to virulence factors [28]. The enterotoxin gene cluster is always present in MLST CC5, CC22, and CC45 strains but not in
CC8, CC12, CC15, and CC395 [17]. Our study displayed CC5 was the major MLST CC type (50%). Therefore, the distribution of the virulence gene cluster in our study is similar to that of previous
findings.

CONCLUSION
In summary, Genotypic and virulence evaluation of the HA-MRSA
revealed that the isolates with CC5 and SCCmec II were the
predominant type and highly homological. The virulence profiles
mainly existed in the genes of sea, seb, sed, sel, sen, seo and ser.
The prevalence of t2460 was an outbreak and the predominant spa
type. The prevalence of enterotoxin genes and spa genotypes of
HA-MRSA explored in this study enhance our current knowledge of the pathogenicity and genetic characteristics of MRSA.
Moreover, investigating the prevalence of enterotoxin genes and
spa genotypes of HA-MRSA is crucial for infection control and
appropriate therapy.

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REFERENCES
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