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Original Article

Microbiology Section

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

YANMENG LI[1], RUIKE ZHAO[2], XIANFENG ZHANG[3], QINGZHEN HAN[4], XUEFENG QIAN[5],GUOHAO GU[6], JINFANG SHI[7], JIE XU[8]

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 differs 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 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.

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, SED, SEE, SEG, SEH, SEI, 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 cefoxitin (FOX, 30μg), penicillin (P, 10μg), ciprofloxacin (CIP, 5μg), clindamycin (DA, 30μg), sulfamethoxazole (SXT, 25μg), vancomycin (VAN, 30μg), teicoplanin (TEC, 30μg) and linezolide (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^{0}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

Spa types	CCs	SCC mec	No. of positive strains												
			sea	seb	sec	sed	sel	sen	seo	sep	seq	ser	seu	cna	pvl	tst
t2460(28)	5	II	28	28	0	28	28	28	28	1	2	24	26	2	2	0
t002(9)	5	II	9	9	0	9	9	9	9	0	0	9	9	1	1	0
t632(7)	-	II	7	7	0	7	7	7	7	2	2	7	1	7	0	0
t030(6)	8	II	6	6	0	6	6	6	6	0	5	4	1	2	0	0
t437(3)	59	II	3	3	0	3	3	3	3	1	2	2	0	0	2	0
t211(2)	8	II	2	2	0	2	2	2	2	0	0	2	0	1	0	0
t4549(2)	-	II	2	2	0	2	2	2	2	0	1	2	1	0	0	0
t299(1)	-	II	1	1	0	1	1	1	1	0	0	1	1	0	0	0
t189(1)	-	II	1	1	0	1	1	1	1	1	0	1	1	0	0	0
t311(3)	5	V	3	3	0	3	3	3	3	0	2	3	3	1	0	0
t163(2)	-	V	2	2	0	2	2	2	2	0	2	1	2	2	0	0
t2310(2)	-	V	2	2	0	2	2	2	2	2	0	2	1	0	0	0
t164(2)	-	V	2	2	0	2	2	2	2	0	0	2	2	1	0	0
t377(1)	-	V	1	1	0	1	1	1	1	1	0	1	1	0	0	0
t037(5)	8	III	5	5	0	5	5	5	5	0	0	3	2	1	1	0
t264(2)	-	III	2	2	0	2	2	2	2	0	0	2	1	1	0	0
t279(2)	-	III	2	2	0	2	2	2	2	0	0	2	1	0	0	0
t459(1)	-	III	1	1	0	1	1	1	1	0	1	1	1	1	0	0
t034(1)	-	III	1	1	0	1	1	1	1	0	0	1	1	1	0	0
Total(80)			80 (100.0%)	80 (100.0%)	0	80 (100.0%)	80 (100.0%)	80 (100.0%)	80 (100.0%)	8 (10.0%)	17 (21.3%)	74 (92.5)	54 (67.5%)	21 (26.2%)	6 (7.5%)	0

[Table/Fig-4]: The SCCmec type, *spa* type, and virulence genes profile of the 80 HA-MRSA isolates

54/80), *cna* (26.2%, 21/80), *seq* (21.3%, 17/80), *sep* (10.0%, 8/80) and *pvl* (7.5%, 6/80) [Table/Fig-4]. But none of the investigated isolates 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 characteristics. 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 genes in HA-MRSA from China. Wu et al., reported that the SAg genes presence of exfoliative toxin genes in CA-MRSA isolates collected from Chinese children [21]. The common toxin gene combination was *seb-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 underling 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 t437 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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PARTICULARS OF CONTRIBUTORS:
1. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou, P.R. of China.
2. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou, P.R. of China.
3. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou, P.R. of China.
4. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou, P.R. of China.
5. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou, P.R. of China.
6. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou, P.R. of China.
7. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou,P.R. of China.
8. Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University, Suzhou,P.R. of China.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Jie Xu,
Faculty, Department of Clinical Laboratory, the First Affiliated Hospital of Soochow University,
No. 188, Shizijie, Canglang District, Suzhou, 215006, P.R. China.
E-mail : xuj2007@lzu.edu.cn

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