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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

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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

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 *Spa*Server software (http://*spa*.ridom.de/index.shtml).

Primers	Oligonucleotide sequence (5'–3')	Sizes (bp)	Specificity	Reference
Type I -F	GCTTTAAAGAGTGTCGTTACAGG	613	SCCmec I	[13]
Type I-R	GTTCTCTCATAGTATGACGTCC			
Type II-F	CGTTGAAGATGATGAAGCG	398	SCCmec II	[13]
Type II-R	CGAAATCAATGGTTAATGGACC			
Type III-F	CCATATTGTGTACGATGCG	280	SCCmec III	[13]
Type III-R	CCTTAGTTGTCGTAACAGATCG			
Type IVa-F	GCCTTATTCGAAGAAACCG	776	SCCmec IVa	[13]
Type IVa-R	CTACTCTTCTGAAAAGCGTCG			
Type IVb-F	TCTGGAATTACTTCAGCTGC	493	SCCmec IVb	[13]
Type IVb-R	AAACAATATTGCTCTCCCTC			
Type IVc-F	ACAATATTTGTATTATCGGAGAGC	200	SCCmec IVc	[13]
Type IVc-R	TTGGTATGAGGTATTGCTGG			
Type IVd-F	CTCAAAATACGGACCCCAATACA	881	SCCmec IVd	[13]
Type IVd-R	TGCTCCAGTAATTGCTAAAG			
Type V-F	GAACATTGTTACTTAAATGAGCG	325	SCCmec V	[13]
Type V-R	TGAAAGTTGTACCCTTGACACC			
1095F	AGACGATCCTTCGGTGAGC		*spa* typing	[13]
1517R	GCTTTTGCAATGTCATTTACTG			
pvl-F	ATCATTAGGTAAAATGTCTG GACATGATCCA	433	pvl	[14]
pvl-R	GCATCAASTGTATTGGATA GCAAAAGC			
tst-F	ACCCCTGTTCCCTTATCATC	326	tst	[14]
tst-R	TTTTCAGTATTTGTAACGCC			
cna-F	GTCAAGCAGTTATTAACA CCAGAC	423	cna	[15]
cna-R	AATCAGTAATTGCACTTTG TCCACTG			
sea-F	GGTTATCAATGTGCGGGTGG	102	sea	[10]
sea-R	CGGCACTTTTTTCTCTTCGG			
seb-F	GTATGGTGGTGTAACTGAGC	164	seb	[10]
seb-R	CCAAATAGTGACGAGTTAGG			
sec-F	AGGTTTTTTCACAGGTCATCC	209	sec	[10]
sec-R	CTTTTTTTTCTTCGGTCAATC			
sed-F	CCAATAATAGGAGAAAATAAAAG	278	sed	[10]
sed-R	ATTGGTATTTTTTTTCGTTC			
selR-F	GGATAAAGCGGTAATAGCAG	166	selR	[16]
selR-R	GTATTCCAAACACATCTAAC			
sen-F	CTTCTTGTTGGACACCATCTT	135	sen	[17]
sen-R	GAAATAAATGTGTAGGCTT			
seo-F	AAATTCAGCAGATATTCCAT	172	seo	[17]
seo-R	TTTGTGTAAGAAGTCAAGTGTAG			
sep-F	ATCATAACCAACCGAATCAC	148	sep	[17]
sep-R	AGAAGTAACTGTTCAGGAGCTA			
seq-F	TCAGGTCTTTGTAATACAAAA	359	seq	[17]
seq-R	TCTGCTTGACCAGTTCCGGT			
ser-F	AGATGTGTTTGGAATACCCTAT	123	ser	[17]
ser-R	CTATCAGCTGTGGAGTGCAT			
seu-F	ATTTGCTTTTATCTTCAT	167	seu	[17]
seu-R	GGACTTTAATGTTTGTTTCTGAT			
mecA-F	ACTGCTATCCACCCTCAAAC	147	mecA	[10]
mecA-R	CTGGTGAAGTTGTAATCTGG			

[Table/Fig-1]: Primers used for amplification of *spa*, SCCmec and Virulence genes

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 linezolide 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 SCCmec 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, t002, t632, t030, t437, t211, t4549, t299, t189, t311, t163, t2310, t164, t377, t037, 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 Suzhou.

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, sed, sel, sen* and *seo* (100.0%, 80/80), followed by *ser* (92.5 %, 74/80), *seu* (67.5%,

Antibiotics	Resistant (%)
FOX	80(100%)
P	80(100%)
CIP	75(93.8%)
DA	50(62.5%)
SXT	11(13.8%)
VAN	0
TEC	0
LZD	0

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

Resistance pattern	No. of isolates
FOX-P-CIP-DA-SXT	9
FOX-P-CIP-DA	40
FOX-P-CIP-SXT	2
FOX-P-CIP	24
FOX-P-DA	1
FOX-P	4

[Table/Fig-3]: Resistance patterns of the MRSA isolates

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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REFERENCES

[1] Johnson AP, Aucken HM, Cavendish S, Ganner M, Wale MC, Warner M, et al. Dominance of EMRSA-15 and-16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J Antimicrob Chaemother*. 2001;48(1):143-44.

[2] Oliveira GA, Faria JB, Levyand CE, Mamizuka EM. Characterization of the Brazilian endemic clone of methicillin-resistant *Staphylococcus aureus* (MRSA) from hospitals throughout Brazil. *Brazilian Braz J Infect Dis*. 2001;5(4):163-70.
[3] Stefani S, Varaldo P. Epidemiology of methicillin resistant staphylococci in Europe. *Clin Microbiol Infec*. 2003;9(12):1179-86.
[4] Wang F, Zhu D, Hu F, Jiang X, Hu Z. 2012 CHINET surveillance of bacterial resistance in China. *Chin J Infect Chaemother*. 2013;13(05):321-30.
[5] Kluytmans-Vandenbergh MF, Kluytmans JA. Community-acquired methicillin-resistant *Staphylococcus aureus*: current perspectives. *Clin Microbiol Infec*. 2006;12 (Suppl 1):9-15.
[6] Ito T, Okuma K, Ma XX, Yuzawa H, Hiramatsu K. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist Update*. 2003;6(1):41-52.
[7] Frenay HM, Bunschoten AE, Schouls LM, van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, et al. Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. European journal of clinical microbiology & infectious diseases : official publication of the *European Society of Clinical Microbiology*. 1996;15(1):60-64.
[8] Nübel U, Strommenger B, Layer F, Witte W. From types to trees: reconstructing the *spa*tial spread of *Staphylococcus aureus* based on DNA variation. *International Journal of Medical Microbiology.* 2011;301(8):614-18.
[9] Udo EE, Al-Mufti S, Albert MJ. The prevalence of antimicrobial resistance and carriage of virulence genes in *Staphylococcus aureus* isolated from food handlers in Kuwait City restaurants. *BMC research notes*. 2009;2(1):108.
[10] Mehrotra M, Wang G, Johnson WM. Multiplex PCR for Detection of Genes for*Staphylococcus aureus* Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1, and Methicillin Resistance. *J Clin Microbiol*. 2000;38(3):1032-35.
[11] Cockerill FR. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. *Clinical and Laboratory Standards Institute (CLSI)*; 2011:68-86.
[12] Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, et al. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. *J Clin Microbiol*. 2003;41(12):5442-48.
[13] Zhang K, McClure JA, Elsayed S, Louie T, Conly JM. Novel multiplex PCR assay for characterization and concomitant subtyping of *staphylococcal cassette* chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol*. 2005;43(10):5026-33.
[14] Sapri HF, Sani NA, Neoh HM, Hussin S. Epidemiological Study on *Staphylococcus aureus* Isolates Reveals Inverse Relationship between Antibiotic Resistance and Virulence Repertoire. *Indian J Microbiol*. 2013;53(3):321-22.
[15] Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G. Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. *J Clin Microbiol*. 2003;41(9):4465-67.
[16] Omoe K, Hu DL, Takahashi-Omoe H, Nakane A, Shinagawa K. Comprehensive analysis of classical and newly described staphylococcal superantigenic toxin genes in *Staphylococcus aureus* isolates. *FEMS Microbiol Lett*. 2005;246(2):191-98.
[17] Chiang YC, Liao WW, Fan CM, Pai WY, Chiou CS, Tsen HY. PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. *Int J Food Microbiol*. 2008;121(1):66-73.
[18] Hu DL, Omoe K, Inoue F, Kasai T, Yasujima M, Shinagawa K, et al. Comparative prevalence of superantigenic toxin genes in meticillin-resistant and meticillin-susceptible *Staphylococcus aureus* isolates. *J. Med. Microbiol*. 2008;57(Pt 9):1106-12.
[19] Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. *Infect Genet Evol*. 2008;8(6):747-63.
[20] Holtfreter S, Grumann D, Schmudde M, Nguyen HT, Eichler P, Strommenger B, et al. Clonal distribution of superantigen genes in clinical *Staphylococcus aureus* isolates. *J Clin Microbiol*. 2007;45(8):2669-80.
[21] Wu D, Li X, Yang Y, Zheng Y, Wang C, Deng L, et al. Superantigen gene profiles and presence of exfoliative toxin genes in community-acquired meticillin-resistant *Staphylococcus aureus* isolated from Chinese children. *J Med Microbiol*. 2011;60(Pt 1):35-45.
[22] Liu Q, Han L, Li B, Sun J, Ni Y. Virulence characteristic and MLST-agr genetic background of high-level mupirocin-resistant, MRSA isolates from Shanghai and Wenzhou, *China. PloS one*. 2012;7(5):e37005.
[23] Kim T, Yi J, Hong KH, Park J-S, Kim E-C. Distribution of virulence genes in *spa* types of methicillin-resistant *Staphylococcus aureus* isolated from patients in intensive care units. *Korean J Lab Med*. 2011;31(1):30-36.
[24] Kwak YG, Truong-Bolduc QC, Bin Kim H, Song KH, Kim ES, Hooper DC. Association of norB overexpression and fluoroquinolone resistance in clinical isolates of *Staphylococcus aureus* from Korea. *J Antimicrob Chaemother*. 2013;68(12):2766-72.
[25] Li S, Sun J, Zhang J, Li X, Tao X, Wang L, et al. Comparative analysis of the virulence characteristics of epidemic methicillin–resistant *Staphylococcus aureus* (MRSA) strains isolated from Chinese children: ST59 MRSA highly expresses core gene–encoded toxin. *APMIS*. 2014;122(2):101-14.
[26] Qiao Y, Ning X, Chen Q, Zhao R, Song W, Zheng Y, et al. Clinical and molecular characteristics of invasive community-acquired *Staphylococcus aureus* infections in Chinese children. *BMC Infect Dis*. 2014;14(1):582.
[27] Cheng H, Yuan W, Zeng F, Hu Q, Shang W, Tang D, et al. Molecular and phenotypic evidence for the spread of three major methicillin-resistant *Staphylococcus aureus* clones associated with two characteristic antimicrobial resistance profiles in China. *J Antimicrob Chaemother*. 2013;68(11):2453-57.
[28] De Sousa MA, Conceicao T, Simas C, De Lencastre H. Comparison of genetic backgrounds of methicillin-resistant and-susceptible *Staphylococcus aureus* isolates from Portuguese hospitals and the community. *J Clin Microbiol*. 2005;43(10):5150-57.

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