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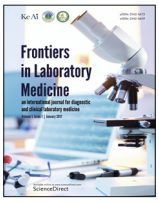

Molecular and virulence characteristics of methicillin-resistant *Staphylococcus aureus* in burn patients

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ABSTRACT

Objective: This work was to investigate the drug resistance, molecular and virulence characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) in burn patients and provide empiric antibiotic therapy for clinical treatment.

Methods: A total of 365 non duplicate *Staphylococcus* strains, including 85 MRSA and 280 methicillin-susceptible *Staphylococcus aureus* (MSSA), were collected from burn patients from 2008 to 2015. The susceptibility tests of antibacterial agents were performed by the Kirby-Bauer disk diffusion method. Polymerase chain reaction (PCR) and gel electrophoresis amplification technology were used to identify the *mecA*, *qacA/B*, and virulence genes, such as staphylococcal enterotoxin A (sea), staphylococcal enterotoxin B (seb), panton-valentine leukocidin (PVL), hemolysin A (hla), fibronectin-binding protein A (fnbA) of MRSA. Pulsed field gel electrophoresis (PFGE) was performed to analysis nucleotide homology of MRSA isolates.

Results: The resistance rates of MRSA to commonly-used antimicrobial agents were significantly higher than that of MSSA. The PCR assay results showed that all MRSA were *mecA*-positive and *qacA/B*-positive strains. The prevalence of virulence gene sea, seb, PVL, hla and fnbA were 70%, 60%, 90%, 85%, 5% in MRSA strains, respectively. Also, the prevalence of virulence genes sea, seb, pvl, hla and fnbA were 40%, 33.3%, 93.3%, 100%, and 20% in MSSA strains, respectively. According to the PFGE analysis, sixteen of MRSA isolates were classified into A, B, and C types and corresponding amounts were 13 (81.25%), 2 (12.5), and 1 (6.25%), respectively.

Conclusions: The burn patients who infected MRSA have a higher drug resistance, and further recognition the molecular characteristics of MRSA is necessary to find better treatment options.

Introduction

Staphylococcus aureus (MRSA), which always exhibits multiple antibiotic resistance in clinic manifestation, is one of the most important pathogenic species that may result in severe shock to death. It has been reported that the number of the dead MRSA infection in the USA is higher than that derived from AIDS in the past 10 years.[1] To our knowledge, severe burn patients are more susceptible to MRSA infection because of losing the protective skin barrier and immunological variations.[2] The outbreak of MRSA infection in burn patients has been widely reported, and the persistence of MRSA in burn units has been proved by more and more evidences.[3] In the last few years, the prevalence of MRSA isolated from clinical isolates, especially in burn patients, increased sharply in China.[4]

Antimicrobial resistance (AMR) has been recognized as a serious worldwide public health issue.[5,6] Previous studies showed that the MRSA colonization could be more inclined to cause infection with respect to MSSA colonization. Furthermore, the treatment and recovery of MRSA infection is more difficult than MSSA infection. Thus the presence of MRSA in burn patients could be a serious matter of concern.[7,8] MRSA strains isolated from burn patients were proved to exhibit resistance to many antibiotics. The complex and diverse mechanisms of MRSA resistance to different types of antimicrobial agents were investigated by similar studies.[9] As the

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explicit gene of MRSA, *mecA* gene is carried on the *SCCmec* cassette and encoded an additional penicillin-binding protein (PBP2A), which reduced affinity of β-lactam antibiotics.[10] In order to prevent pathogen infection, chlorhexidine has been widely used in clinic, resulting in the appearance of resistance gene. According to the reported results, the *qacA/B* gene may give main contribution to the resistance to chlorhexidine of MRSA.[11]

Possessing strong virulence and invasiveness, the pathogenic toxins of MRSA could be toxins, toxic shock toxins, hemolytic toxins, kill IL, plasma coagulase, et al. It is noted that these toxins could not only induce suppurative inflammation, but also resulting in food poisoning and other problems.[12] Until now, the characteristics conferring virulence of MRSA isolated from burn patients have not been clearly reported yet, especially in Gansu province of China. Also, these unreported information could be significant importance for solving the problem of MRSA infection in burn patients through finding new methods.

Herein, we analyzed the multidrug-resistant patterns of MRSA in burn patients and examined the possible genetic origin of methicillin resistance of these MRSA isolates. More specially, the virulence characteristics of these MRSA strains were given in this study.

Materials and methods

Isolated strains

There were 365 non-duplicate *staphylococcus aureus* strains derived from burn patients from January 2008 to December 2014. The specimens were mainly collected from six anatomical sites, including wound secretions, burn wounds, sputum, central venous catheters, and blood samples of patients in burn wards in Gansu Provincial Hospital. All samples were immediately taken to the laboratory and cultured on 5% sheep blood agar plates after collected from burn patients in aseptic way. All plates were incubated in 35 °C for 24 h. The strains were believed as *Staphylococcus aureus* according to the morphological characteristics on the plates and the related biochemical reactions (including tube coagulase, catalase and oxidase).

Antimicrobial susceptibility testing

The antibacterial agent susceptibility tests were performed by the Kirby-Bauer (K-B) disk diffusion method, which was recommended by Clinical and Laboratory Standards Institute (CISI). The sensitivity of *staphylococcus aureus* isolates to Penicillin, Oxacillin, Cefazolin, Cefuroxime, Cefotaxime, Cefoxitin, Amikacin, Gentamycin, Rifampicin, Ciprofloxacin, Levofloxacin, Co-trimoxazole, Trimethoprim, Fosfomycin, Clindamycin, Azithromycin, Erythromycin, Linezolid, Vancomycin, and Teicoplanin and Quinupristin/Dafoeleptin were tested. 5% sheep blood agar culture media were provided by Oxoid Ltd. (Basingstoke, UK). Moreover, the inhibition zones for the oxacillin (1 mg) disk with diameters of 17 mm for coagulase-negative staphylococci (CoNS) and 10 mm for *S. aureus* were considered to be resistant. Vancomycin was regarded as resistant when the minimal inhibitory concentration (MIC) was $\geq$ 500 μg/ml. All susceptibility tests were performed based on the CLSI criteria, 2015. All dates were analyzed by the software of WHO Antimicrobial Resistance Monitoring System (WHONET), version 5.6.

PCR identification of mecA gene, genes for virulence factors and homology analysis by PFGE

The encoding genes of *mecA* was detected after extracting genomic DNA from 85 MRSA strains and the quality-control strains were *Staphylococcus aureus* (MSSA) ATCC25923, *Staphylococcus aureus* (MRSA) ATCC43300, and mecA-positive *Staphylococcus aureus* CCUG35601. These quality-control strains were provided by the National Center for Clinical Laboratories in China. Primers were summarized in Table 1. Genes for virulence factors, namely Enterotoxin A (sea), Enterotoxin B (seb), α-hemolysin (hla), Fibronectin A (fnbA) and Panton valentine leukocidin (PVL).[13–15] PFGE typing was performed to analysis the genotypes of each strain and the results were interpreted according to previous reported.[16]

Statistical analysis

All dates were analyzed by the software of WHO Antimicrobial Resistance Monitoring system (WHONET), version 5.6. SPSS version 17.0 was used to statistical analysis. Frequencies of MSSA and MRSA in different anatomical sites were compared by using the Fish exact test. A p-value less than 0.05 could be considered as statistically significant.

Results

Distribution of MSSA and MRSA in burn patients and the multidrug-resistant pattern

There were 365 *Staphylococcus aureus* strains derived from burn patients. The numbers of MRSA and MSSA were 85 (23.9%) and 280 (76.1%), respectively. None of the MRSA isolates could be resistant to vancomycin, teicoplanin or linezolid in this study. The resistance rates of MRSA to penicillin, oxacillin, and cefoxitin were all 100%, while the resistance rate to Co-trimoxazole and Quinupristin/Dafoeleptin were 15.10% and 1.60%, respectively. Recently, the resistance rates to other antibiotics have been all higher than 30.00%. The multidrug resistance rate of MRSA was higher than that of MSSA (Table 2).

mecA, qacA/B gene and SCCmec types of MRSA isolates

All the 85 MRSA isolates harbored the *mecA* gene and the result was in accordance with PBP2a agglutination test (Fig. 1). 85 (100%) qacA/B gene positive isolates were found in the study (Fig. 2), which indicated a strong association between SCCmec type ccrAB3 and methicillin-resistance.

Table 1
Primers of PCR and fragment length of PCR production.

Target gene	Primer sequence (5′→3′)	Product size (bp)
mecA –R	TCC AGA TTA CAA CTT CAC CAG G	162
mecA-F	CCA CTT CAT ATC TTG TAA CG	
crAB1-F	ACC ACA AAC ACA CTT AAA GAT G	150
crAB1-R	CAA TTTCAAGTA TTT GGT CCA TAA C	
crAB 2-F	AGT TTC TCA GAA TTC GAA CG	311
crAB 2-R	CCG ATA TAG AAT GGG TTA GC	
crAB 3-F	AAC ACA ACG AAC ACA TTG AAA G	130
crAB 3-R	CGT ATT TCT CAA TCA CAT CAG C	
crAB 4-F	CGA AGT ATA GAC ACT GGA GCG ATA	134
crAB 4-R	GCG ACT CTC TTG GCG TTT A	
ccrC-F	GTA CTC GTT ACA ATG TTT GG	449
ccrC-R	ATA ATG GCT TCA TGC TTA CC	
qacA/B-F	TGG CTT TAC CGG AAT TAG TAA GAG	800
qacA/B-R	GTC TTA CGT CTA ACA TTG CAT CAG	
sea	13	122
seb	13	164
hla	14	229
fnbA	15	806
PVL	15	433

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