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Characterisation of SCCmec elements in methicillin-resistant *Staphylococcus aureus* isolated from burn patients

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ABSTRACT

Staphylococcus aureus is an important pathogen, especially in burn units all around the world. Because of the emergence of the β-lactam antibiotic-resistant strains since 1961, concern about the prevalence of methicillin-resistant *S. aureus* (MRSA) has increased in these units. Resistance to methicillin is mediated by penicillin-binding proteins (PBPs) that have enough affinity for binding to the β-lactam ring, but another kind of protein (PBP2α), which is encoded by the mecA gene, has a lower affinity for binding to these antibiotics. The mecA gene is transferred by SCCmec (staphylococcal cassette chromosome *mec*) as a mobile genetic element, exclusively found in the *Staphylococcus* genus. Identification of the frequency of the mecA gene, different SCCmec types and also its incidence may have benefit in surveillance prevention and control of MRSA strains in burn units. In this study, 40 *S. aureus* isolates were collected from patients hospitalised in Motahari burn center of Tehran, during 2012–2013. Conventional microbiological methods were applied and the confirmed isolates were stored at −20 °C for molecular polymerase chain reaction (PCR) tests. The antibiotic resistance pattern was performed by disc diffusion method and finally the different SCCmec types were determined by specific primers. During this research, 40 isolates of *S. aureus* were collected from burn patients, of which (37.5%) of the specimens belonged to female patients and 62.5% to male patients. The aetiology of the burn was classified as follows: open flame (35%), liquid (32.5%), chemical (5%) and other (27.5%). By a disc diffusion method, no resistance pattern was observed to vancomycin and fosfomycin. Based on a multiplex PCR assay, the five different SCCmec types were detected as: 47.5% type III, 25% type IV, 10% type V, 10% type II and 7.5% type I.

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1. Introduction

In recent years, the high rate of *Staphylococcus aureus* infections in burn units has been considered as a serious threat to patients. Loss of the functional skin barrier and depression of immune responses, which is caused by burns, have increased the incidence of various infections [1,2]. Due to *S. aureus* playing a significant role as an important pathogen especially in burn units all around the world and also the indiscriminate use of antibiotics, the issue of antibiotic resistance has become extremely critical [3]. A broad spectrum of synthetic and semi-synthetic penicillin productions, such as methicillin which was introduced in 1959, could overcome the problem of penicillin-resistant *S. aureus*; thereafter, the resistant strains emerged [4,5]. The first strain of methicillin-resistant *S. aureus* (MRSA) was isolated in Europe in 1961. In later years, more resistant strains showed a wide pattern of resistance not only to β-lactams but also to other antimicrobial groups such as 'aminoglycosides' and 'macrolides' [6]. β-Lactam antibiotics, for example methicillin, have enough ability to activate penicillin-binding proteins (PBPs) with acylation reaction. These proteins contain an enzymatic role in bacterial peptidoglycan synthesis [7]. Usually, PBPs have a high affinity for binding to the β-lactam ring, whereas in (MRSA) strains because of the mecA gene present another kind of protein (PBP2α) is generated which has a lower affinity for binding to these antibiotics. Subsequently, some antibiotics such as methicillin will lose their ability for cell wall destruction [8]. It is noticeable that the mecA gene is transferred by SCCmec as a mobile genetic element with the size of 21–67 kbp that is exclusively found in the *Staphylococcus* genus. A complex of the SCCmec gene contains the mec complex, the ccr complex and the Junkyard area. Due to different allotypes and complex classes, five (I–V) main types of SCCmec elements are classified [9]. SCCmec type I was determined in UK for the first time in 1961, SCCmec type II was identified in Japan and types III, IV and V were distinguished from New Zealand. According to different types of SCCmec elements, hospital acquired-MRSA (HA-MRSA) and community acquired-MRSA (CA-MRSA) are known as an independent deviation [10]. CA-MRSA strains are susceptible to most widespread antibiotics in comparison with HA-MRSA and also antibiotical resistances are detected in type III of SCCmec [11]. Concerning about MRSA infections which are reported from many countries, since emerging is still increasing. This microorganism is one of the widespread reasons of bacteraemia and wound infection in burn units. Thus, the antimicrobial resistance pattern, the frequency of the mecA gene and determination of the different SCCmec types in epidemiologic research among clinical isolates is an important stage in the treatment of these patients. Hence, the multiplex polymerase chain reaction (PCR) method, which is divided into rapid and feasible methods, may serve as a functional tool for elucidating the various structures of SCCmec elements and mecA identification in epidemiological research in health-care units; however, this process may have benefit for surveillance prevention and control programmes for clinicians in burn units [12].

2. Materials and methods

2.1. Setting

This study was conducted in Motahari Hospital, one of the most important teaching hospitals in Tehran, Iran. This hospital is exclusive for burn patients with three separated units including men, women and children. In these units, daily health care such as bathing the burn surface area (BSA), using silver sulphadiazine and mupirocin topical creams for cleaning and washing, is common. Motahari Hospital has strict guidelines for decontamination and horizontal, vertical, internal and external concurrent cleaning on all surfaces also occurs frequently. The patients are routinely screened for all of the bacteria isolated from burn wounds.

2.2. Swab sampling procedure

In our research, only patients with documented clinical evidence were studied. Swab sticks and scalpel blades were used for isolating burn wounds with pus and haemorrhaging tissues. In the next step, all collected specimens were transported to the laboratory for culturing and microbiological diagnosis tests.

2.3. Bacterial isolates

A total of 85 swabs of burn wounds were collected from the hospitalised patients in Motahari burn centre, Tehran, during the sixth month and referred to an antimicrobial resistance research centre in Tehran, Iran. From the 85 gathered swabs, other Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* were isolated. The 55 isolates were distinguished by conventional microbiological methods such as growth on blood agar, mannitol salt agar, coagulase and DNase tests as *S. aureus* primary identification. In the next step, the API-20-Staph system kit (bioMerieux, France) was used for final confirmation. Subsequently, 40 strains were confirmed mecA positive by a PCR molecular test [13] and stored at −20 °C for SCCmec typing.

2.4. Antimicrobial susceptibility testing

The antibiotic resistance pattern was performed by a disc diffusion method on Mueller–Hinton agar using vancomycin, rifampicin, fosfomycin, ciprofloxacin, oxacillin, cefepime, gentamicin, trimethoprim-sulfamethoxazole and erythromycin (MAST, Merseyside, England), according to Clinical and Laboratory Standards Institute (CLSI) 2011. *S. aureus* ATCC25923 was used as the control strain.

2.5. Preparation of DNA

The bacterial genomic DNA of *S. aureus* strains were extracted with a QIAGEN plasmid Minikit (QIAGEN, Hilden, Germany) as recommended.