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Phylogenetic relationships among *Staphylococcus aureus* isolated from clinical samples in Mashhad, Iran

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Summary The *spa* gene occurs in all strains of *Staphylococcus aureus* (*S. aureus*), can function as a genetic marker and might be used distinguish strains at the species level. Hence, due to these advantages, we used *spa* typing and the Based Upon Repeat Pattern (BURP) to assign the clonal and phylogenetic relationships of *S. aureus* strains. The sensitivity of *S. aureus* strains to methicillin was determined using agar disk diffusion. The extracted DNA from 56 isolates of *S. aureus* was subjected to PCR to detect the *spa* gene with specific primers. The *spa* typing method was performed for each of the isolates, and then, BURP was used to cluster *spa* types (*spa*-CCs). Finally, using relevant software, the phylogenic tree was drawn. The results of this study showed that 25 out of 56 (44.6%) isolates were resistant to

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methicillin. The typing of *S. aureus* isolates revealed 24 different *spa* types among 56 isolates, and BURP analysis clustered the 24 *spa* types into 5 *spa* clonal complexes (CCs) and 12 singletons. The process of *spa* typing, in combination with BURP analysis, provides an efficient method for investigating phylogenetic and clonal relationships among clinical isolates and can be useful for monitoring bacterial spread between hospitals and communities as well as between and within hospitals.

Introduction

Staphylococcus aureus (*S. aureus*) is among the most prevalent human pathogens and is responsible for many community and nosocomial acquired infections, thus increasing public health concerns worldwide [1]. The most prominent pathogenic species in the *Staphylococcus* genus is *S. aureus*, several strains of which cause a variety of diseases [2]. Genotyping methods for microbial pathogens can be used for two major objectives: (1) outbreak investigations and (2) assigning phylogenetic relationships and clonal relatedness to strains [3]. To investigate local epidemiologic situations or outbreaks, the pulsed-field gel electrophoresis (PFGE) method is required, although for many years multi-locus enzyme electrophoresis (MLEE) and multi-locus sequence typing (MLST) methods have been used to study the global epidemiology of pathogens, such as bacterial lineages [4]. Recently, Koreen et al. proposed *spa* typing as a useful method for assigning phylogenetic relationships to strains [3]. Protein A (*spa*) is a 42 kDa protein and an important virulence factor involved in the pathogenesis of *S. aureus* [5,6]. The *Spa* gene contains several regions with different functions, including (1) the N-terminal region, which comprises an S region (signal sequence) divided into four or five immunoglobulin G binding domains (A, B, C, E, and D) and (2) the C-terminal region (X region), which is divided into the X_R and X_C domains. The X_R region consists of variable repeats, which are used for *spa* typing, and the X_C region consists of an LPXTG-binding motif that confers binding to the cell wall [7]. Previous studies have shown that the *spa* gene can function as a genetic marker that can distinguish strains at fine resolution and that the discriminatory power of *spa* typing can be comparable to PFGE and whole-genome DNA microarrays for certain collections as used [8]. This study aimed to evaluate *spa* typing in combination with BURP to assign the clonal and phylogenetic relationships of *S. aureus* strains isolated from different clinical samples in Mashhad.

Materials and methods

Bacterial strains and susceptibility test

In this study, we collected a total of 56 isolates of *S. aureus* from different clinical samples of patients who had been referred to Mashhad hospitals (Iran) from December 2013 to May 2014. *S. aureus* isolates were obtained from blood, sputum, urine, wound, tissue and other specimens (swab, CSF, peritonitis) and identified based on standard methods such as gram staining, catalase, DNase, mannitol fermentation, and coagulase tests. Using agar disk diffusion, the sensitivity of *S. aureus* strains to methicillin was determined by cefoxitin disk (30 μg, Himedia-India). *S. aureus* ATCC 25923 was used as a control strain for antibiotic susceptibility testing.

PCR amplification, sequencing of *spa* gene and *Spa* typing

DNA was extracted from all *S. aureus* strains, which were grown overnight in nutrient agar (Merck/Germany) at 37 °C using the Fermentas DNA kit, according to the manufacturer's instruction (Fermentas, USA). The primers used for amplification of the X region of the *spa* gene in this study were as follows: *spa*-1113f (5-TAAAGACGATCCTTCGGTGAGC-3) and *spa*-1514r (5-CAGCAGTAGTGCCGTTTGCTT-3) [9]. For the characterization of nucleotide sequences based on the strands, the PCR products were run on 1.5% agarose gels, then sent for sequencing (Bioneer, Korea). The *spa* typing and evaluation of *spa* types from these *S. aureus* strains were performed using the *spa* database website (http://www.ridom.de/spaserver). The BURP algorithm was used to determine *spa* clonal complexes (CCs) using the StaphType software v. 1.5 (Ridom GmbH, Würzburg, Germany). When the relationship of various *spa* types was calculated, insertions, deletions and any recombination in repeats were accounted for. Two default parameters are offered by BURP for clustering analysis: (1) *spa* types that