

Investigation of *coa* Gene Polymorphism in Clinical Isolates of *Staphylococcus aureus* in North of Iran

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Abstract

Staphylococcus aureus is a common pathogen potentially able to cause a wide range of infectious diseases in human and animals and coagulase enzyme is one of the important virulence factors of this bacterium. Polymorphism of the coagulase encoding gene (*coa*) is one of the molecular-based typing methods of *S. aureus* isolates. In this study, the polymorphism of the coagulase gene among MRSA and MSSA isolates were investigated using PCR-RFLP analysis. To perform coagulase gene typing, the repeated units encoding hypervariable regions of coagulase gene of 30 clinical isolates of *S. aureus* were amplified by the PCR technique; this was followed by *AluI* restriction enzyme digestion and analysis of restriction fragment length polymorphism (RFLP) patterns. In total two amplicons (680 bp and 750 bp) and four distinct RFLP banding patterns (280+400, 340+340, 280+470, and no digested amplicon of 750 bp) were observed. Genotype with PCR-RFLP patterns of 280+400 bp was predominated. The results indicated polymorphism in the investigated regions of coagulase gene. This polymorphism can be used for identification of *S. aureus* isolates and showing the epidemiological relationship among them.

Keywords: *Staphylococcus aureus*, *coa* gene, PCR-RFLP, Polymorphism

Introduction

Staphylococcus aureus is a common pathogen potentially able to cause a wide range of infectious diseases, including skin and soft tissue infections, urinary tract infections, sepsis, endocarditis, pneumonia, deep abscess formation and osteomyelitis in both community and hospitals (Daum, 2007, Muder et al., 2006). In addition, *S. aureus* is responsible for toxin-mediated diseases, such as toxic shock syndrome (Dinges et al., 2000). The emergence of hypervirulent strains and the rise in antibiotic resistance increased public health concern of *S. aureus* (Shorr et al., 2006). Several cell wall associated components and extracellular proteins of *S. aureus* act as pathogenicity factors (Foster, 2005). Staphylocoagulase is an important virulence factor during *S. aureus* infection process, causes the clotting of host plasma and is required for abscess formation, bacterial persistence in host tissues and *S. aureus* lethal bacteremia (Cheng et al., 2010, McAdow et al., 2012). The biological activity of coagulase is caused by prothrombin binding domain. At the 3' end of coagulase encoding gene, 81 bp heterogenic tandem repeats encoding repeated 27 amino acid sequences have been recognized which are polymorphic in both number and sequence. Clotting occurs independently of the

repeats but polymorphism of this region can be used for differentiation of *S. aureus* isolates and investigating the epidemiological relationship among them (Mahmoudi et al., 2017, McAdow et al., 2012). Because of diversity in size and sequence, PCR amplification of 3' end of the *coa* gene from different *S. aureus* species may yield amplicons of different sizes and different restriction sites. PCR products of the *coa* gene can be digested by *AluI*, *CfoI*, *HaeIII* (Janwithayanuchit et al., 2006). *AluI* appears to yield the highest RFLP in restriction enzyme analysis and allows a greater discriminatory power than others. So *coa* gene PCR- and *AluI* RFLP-based typing can be used as a simple and effective method for typing of *S. aureus* clinical isolates (Schwarzkopf et al., 1994).

The present study was aimed to investigate *coa* gene polymorphism in clinical isolates of methicillin resistant and methicillin sensitive *Staphylococcus aureus* from various infection sources in Rasht, the north of Iran, using PCR and *AluI* restriction fragment length polymorphism.

Materials and Methods

Bacterial isolates

A total of 30 *S. aureus* isolated from various sources including urine (n=17), skin (n=6), blood

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(n=3) and synovial fluid (n=2) were identified using several tests such as gram staining, catalase test, growth onto MSA, haemolysis onto blood agar and tube coagulase test. A single isolate from individual patients was subjected to this study and all *S. aureus* isolates were screened for methicillin resistance by disc diffusion (6 µg/ml oxacillin) on Mueller Hinton agar with 2% NaCl.

DNA extraction and Ribotyping

The bacterial genomic DNA was extracted using Kit for the isolation of DNA from gram positive bacteria (Cinnagen, Iran). All the isolates were confirmed as *S. aureus* as described previously (Izadpanah & Asadpour, 2015).

Coa gene amplification

The *coa* gene was amplified by using Forward primer: 5' ATAGAGATGCTGGTACAGG 3' and reverse primer: 5' GCTTCCGATTGTTTCGATGC 3' as described previously (Anggraini et al., 2017) with a thermal cycling program of 94°C for 5 min, followed by 30 cycles of 95°C for 30 sec, 55°C for 45 sec and 72°C for 2 min. The final elongation step was 10 minutes at 72°C. PCR products were electrophoresed on a 1.5 % agarose gel, stained with gel red DNA stain and visualized under UV light. The 100-bp marker (MBI Fermentas) was used as a size standard for the calculation of the sizes of the *coa* gene.

RFLP of Coa gene amplicon

The *coa* gene restriction analysis was carried out by using *AluI* (MBI Fermentas). 10µl of *coa* gene PCR products were digested with 10U of *AluI* restriction enzyme in a 20 µL reaction mixture and incubated at 37°C for 16 hours.

PCR- RFLP reproducibility was tested by twice submitting PCR products to *AluI* digestion. The generated restricted fragments were separated on 1.5 % agarose gel.

Results

In total 30 *S. aureus* strains were isolated from clinical samples using conventional biochemical test. 21 isolates (70%) phenotypically recognized as MRSA. PCR amplification of *coa* gene of the isolates resulted in two different size fragments including 680 bp (17 isolates) and 750 bp (13 isolates). All the PCR products were digested with *AluI* enzyme and four different RFLP patterns were observed. Digestion of 680 bp amplicons in 13 isolates produced two fragments of 400 bp and 280 bp (pattern 1) and in 6 isolates only one fragment of

340bp was obtained (pattern 2). For 4 isolates *AluI* digestion produced two size fragments of 470 bp and 280 bp (pattern 3) and in 7 isolates *AluI* had no restriction site on the amplicon (pattern 4). Some of the obtained results are shown in figures 1 and 2.

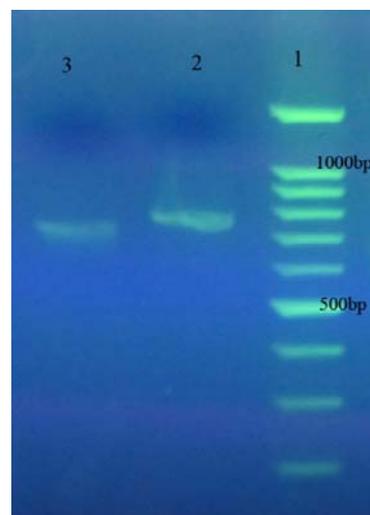


Figure 1. Polymorphism in *coa* gene PCR amplicons. Lane 1: 100bp DNA marker, Lane 2: 750 bp PCR amplicon, Lane 3: 680 bp amplicon.

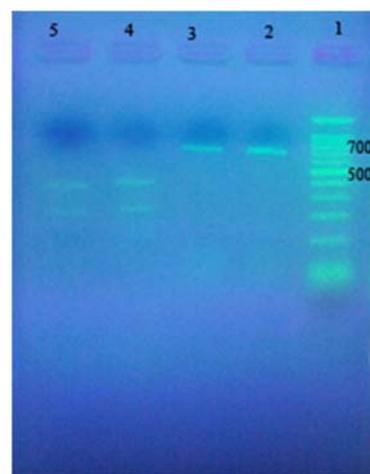


Figure 2. RFLP patterns obtained from *AluI* digestion of *coa* gene amplicons. Lane 1: 100bp DNA marker, Lane 2: pattern 4 (750 bp PCR product without digestion), Lane 3: pattern 1 (two size fragments of 400 bp and 280 bp), Lane 4: pattern 3 (two size fragments of 470 bp and 280 bp).

Out of 21, 7 MRSA isolates (33%) showed restriction pattern 1, 5 isolates (23%) showed restriction pattern 2. In addition, pattern 3 and 4 were observed in 4 (19%) and 5 (23%) isolates, respectively.

Discussion

In the present study, the polymorphism existing in *coa* gene of 30 *S. aureus* strains including MRSA and MSSA isolated from clinical samples in Rasht city, north of Iran, was investigated. The result of PCR-based RFLP on *coa* gene of the test bacteria showed 2 *coa* PCR type and 4 different RFLP pattern. In different studies, the *coa* gene polymorphism has been used as a simple and accurate method for molecular typing of *S. aureus* strains. In a study conducted by Babu et al, the PCR-RFLP results of 14 coagulase positive *S. aureus* gave two different sets of amplicons and 5 different *AluI* digestion patterns (Babu et al., 2014). Ishino et al (2007) classified 678 *S. aureus* isolates into 8 classes and the sizes of the PCR products of *coa* gene ranged from 350 to 917 bp. Sanjiv et al (2008) found three types of *coa* gene products of 600, 680 or 850 bp size and three distinct RFLP patterns in 20 *S. aureus* isolates. Talebi-Satlou et al (2012) determined 4 classes of PCR amplicons ranging from 410-790 bp and 6 distinct *HeaIII* RFLP patterns in the 3' end of *coa* gene in 26 *S. aureus* isolates associated with skin and urinary tract infections in Urmia, Iran.

In our research, amplified products with an approximate size of 680 bp and 750 bp were obtained. This variation in the size of amplicons indicates the presence of only one more 81 bp repeat in strains with 750 bp PCR product. *AluI* digestion of *coa* gene PCR products lead to 4 distinct patterns. Pattern 1, the most common pattern (43% isolates), includes two fragments of 400 bp and 280 bp. The pattern 2 with two fragments of 340 bp from 680 bp amplicon suggested that the amplicon was cut into two equal halves of 340 bp by *AluI*. Single fragment pattern of digestion was also obtained by Sanjiv et al (2008) when *S. aureus coa* gene amplicon of 600 bp was digested with *AluI*. *AluI* digestion of 750 bp amplicon in 4 isolates produced two size fragments 470 bp and 280 bp (pattern 3) and 750 bp amplicons without *AluI* digestion (in 7 isolates) designated as pattern 4. Lack of enzyme restriction sites amongst the amplicons has been previously reported by Sarvari et al, 2014. According to the results, MRSA isolates were found in each of the four patterns without discrimination. Similar results were obtained by Himabindu et al (2009) who found that *coa* PCR-RFLP typing method cannot be used to distinguish MRSA and MSSA. Also, we found no discriminative pattern to distinguish between various microbial sources including urine and skin. These results are contrary to those of Talebi-Satlou

et al (2012) who reported tissue-specific tendency of some genotypes.

The results of this study demonstrated genetic diversity in *S. aureus* clinical isolates in Rasht. The variation in the size of PCR amplicons indicates the variation in the *coa* gene lengths and differences in the RFLP patterns reflects the presence of VNTRs in the *coa* gene of *S. aureus* isolates. These *coa* gene variants are different from those reported in the previous studies in Iran (Afrough et al., 2013, Mahmoudi et al., 2017, Rezaee et al., 2016, Saei et al., 2009, Sajadi et al., 2017, Talebi-Satlou et al., 2012). So the presence of many variants of *S. aureus* in the country is concluded.

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