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

Mohammad Reza Izadpanah, Leila Asadpour*

Department of Biology, Rasht Branch, Islamic Azad University, Rasht, Iran

Received 3 January 2016  Accepted 24 September 2018

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

*Corresponding author E-mail: Asadpour@iaurasht.ac.ir

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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 \( S. \text{aureus} \) isolates were screened for methicillin resistance by disc diffusion (6 \( \mu \)g/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 \( S. \text{aureus} \) as described previously (Izadpanah & Asadpour, 2015).

**Coa gene amplification**

The \( coa \) gene was amplified by using Forward primer: 5’ ATAGAGATGCCTGATACAGG 3’ and reverse primer: 5’ GCTTCCGATTTGCTGATGC 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 \( S. \text{aureus} \) 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.](image)

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.](image)

Lane 1: 100bp DNA marker, Lane 2: 750 bp PCR product without digestion, Lane 4: pattern 1 (two size fragments of 400 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 HaeIII 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.

Acknowledgements

This manuscript is prepared from MSc thesis at Islamic Azad University, Rasht Branch, Rasht, Iran. We are grateful to the Islamic Azad University, Rasht Branch for support.

References


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