



Coagulase Gene Polymorphism of *Staphylococcus Aureus* Isolates Collected From Human Clinical Samples in Southeastern Iran

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Abstract

Background: *Staphylococcus aureus* is one of the most important pathogens in the development of hospital infections. The production of coagulase is directly related to the pathogenicity of *S. aureus*. The present study was conducted in order to investigate the polymorphism of the coagulase gene (*coa*) as a coagulase-encoding gene.

Methods: 120 clinical samples were collected from patients admitted to Imam Khomeini and Amirmomenin hospitals in Zabol, southeastern Iran, during 2014 and screened for the presence of *S. aureus*. Genetic diversity was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of *coa* gene and digested by *AluI* enzyme.

Results: Out of 120 clinical samples, 56 isolates were diagnosed as *S. aureus* and all of these isolates were positive for the 3' end of the *coa* gene by PCR. All isolates produced a single band on *coa* PCR, with molecular sizes ranging from 600 to 850 bp, with the highest frequency of 700 bp (39%). RFLP analysis of *coa* gene using *AluI* revealed P1-P4 band patterns with the highest frequency of P1.

Conclusions: The results of this study showed that P1 pattern was the most frequent pattern and lowest frequency was observed in P4 pattern. The results of this study suggest that the RFLP of *coa* gene can be used as a fairly suitable method for determining the genetic differences between *S. aureus* isolates.

Keywords: *Staphylococcus aureus*, *coa* gene, RFLP



Background

The high risk of staphylococcal infections in pediatric, surgical, chemotherapy and ICU wards is indicative of the need for research in regard to bacterial placement in the skin and various organs of healthy individuals and patients according to the method of transmission of a bacterium (1). Given the placement of bacteria in the nose and skin of healthy people, staphylococcal infections can spread due to direct contact between hospital staff and patients in hospitals (2). Prevention and control of *Staphylococcus aureus* infections are initially dependent on the risk of infection (3). In addition, the analysis of isolates by means of separable DNA typing methods and understanding the transmission of bacterial infections is one of the factors contributing to the prevention and control of *S. aureus* infections (4). As approaches for detecting the origin of infection, previously conducted research has shown that isolated genotypic characteristics can work properly to find

the source of infection as a step forward in controlling the infection (5). The *coa* gene, which codes for coagulase, is polymorphic. Coagulase is an important criterion for identifying *S. aureus* isolates (6).

Although pulsed field gel electrophoresis (PFGE) is used as a standard method for genotyping bacteria, the addition of *coa* gene has more advantages than the mentioned method due to lower cost, not needing specific skill and expertise, high speed and high repeatability; it, additionally, is used more frequently in researches (7,8). In Iran, especially Zabol, southeastern Iran, there is no information on the genetic differences between *S. aureus* isolates collected from different hospitals and patients with skin lesions and urine samples, especially on the basis of *coa* gene polymorphism.

Objectives

The main objective of this study was to classify isolates

collected clinically from 2 hospitals in Zabol (Imam Khomeini and Amiralmomenin) using *coa* gene and compare them with other regions of Iran. In addition, in this study, the *coa* gene polymorphism in *S. aureus* isolates collected from skin lesions and urine samples was investigated using restriction fragment length polymorphism (RFLP) method.

Materials and Methods

Bacterial Isolates

In this study, 120 samples were collected from skin lesions and urine samples belonging to patients hospitalized in Zabol from May to November 2014. The samples were then transferred to the Microbiology Department of the Medical School of Zabol University of Medical Sciences and were identified as *S. aureus* by different biochemical tests such as gram staining, catalase, coagulase, DNase and mannitol fermentation.

All *S. aureus* isolates were DNase and coagulase positive and fermented mannitol. *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 12228 were used as positive and negative controls respectively. Genomic DNA was extracted by boiling method and polymerase chain reaction (PCR) was performed on *coa* gene.

DNA Extraction

All isolates were swabbed on Trypticase Soy Agar (TSA) (BD, Germany), while the surface of the agar medium was covered with standard vancomycin disks and incubated overnight. The bacterial colonies from the edges of the inhibition zone were then resuspended in sterile distilled water and matched to 0.5 McFarland standards (approximately 10^8 CFU/mL). The bacterial suspension was heated at 95°C for 15 minutes and cooled at room temperature. The cured lysis (2 µL) was used as a DNA template for all isolates when PCR tests were carried out (9).

Polymerase Chain Reaction

PCR of the *coa* gene was carried out using primers forward: ATA GAG ATG CTG GTA CAG G and reverse: GCT TCC GAT TGT TCG ATG C. Reactions were prepared in a final volume of 15 µL, including 2 µL of pattern DNA, 7.5 µL of 2×MasterMix, 1 µL of each primer and 3.5 µL of distilled water. The amplification program included an initial denaturation step of 3 minutes at 94°C followed by 30 cycles of 20 seconds at 94°C, 15 seconds at 55°C and 15 seconds at 72°C and a final extension step of 2 minutes at 72°C.

Restriction Enzyme Digestion

Generally, 10 µL of PCR product was incubated with 1 µL of *AluI* endonuclease (Fermentas, USA), 2 µL of restriction buffer and 17 µL of distilled water for 16 hours at 37°C.

Agarose Gel Electrophoresis

Digested fragments and PCR products were separated in 2% agarose gels (Gibco, USA). A 100-bp ladder (Fermentas, USA) was used as a standard molecular marker for the calculation of the sizes of the *coa* and *AluI*-generated *coa* fragments. Gels were visualized under UV light after staining with ethidium bromide.

The data were analyzed using chi-square and Fisher exact test in SPSS 16.0, with a significance level of 0.05.

Results

A total of 120 clinical samples from 2 major hospitals were used in this study. Fifty-six specimens (46.6%) from these samples were identified as infected with *S. aureus* in microbiological studies. From the total of 56 *S. aureus* isolated in this study, 30 (53.5%) and 26 (46.4%) isolates recovered from skin lesions and urine samples, respectively.

RFLP was used to determine the polymorphism of the coagulase gene from all the isolates of *S. aureus* collected in this study. Five different sizes, ranging from approximately 600 to 850 bp were observed. Twenty-two isolates contained 700 bp and 14, 8, 6 and 6 isolates contained 650 bp, 600 bp, 800 bp and 850 bp fragment relevant to *coa* gene in the PCR (Figure 1).

Among the isolates recovered from skin lesions, PCR product of 650 bp was the most common one seen in 10 isolates, followed by 700 bp (nine isolates), 600 bp (five isolates), 800 and 850 bp (each one with 3 isolates). After enzymatic digestion with *AluI* in the PCR, 4 types of *coa* patterns ranging from 100 to 400 bp were observed. These types were designated as P1-P4 and the P1 type was the most predominantly noticed product sizes, accounting altogether for 35.71% of the total *coa*-positive isolates. As shown in Figure 2, the isolates with patterns P1 and P2 had DNA fragments of 250 and 400 bp, 250 and 350 bp, respectively. *AluI* digestion of the PCR products also yielded 3 PCR fragments in P3 type and 1 PCR fragment in P4 type.



Figure 1. PCR Products of *S. aureus* *coa* Gene Isolated From Clinical Samples. Lane 1-9, PCR products and lane 10, 100-bp ladder.

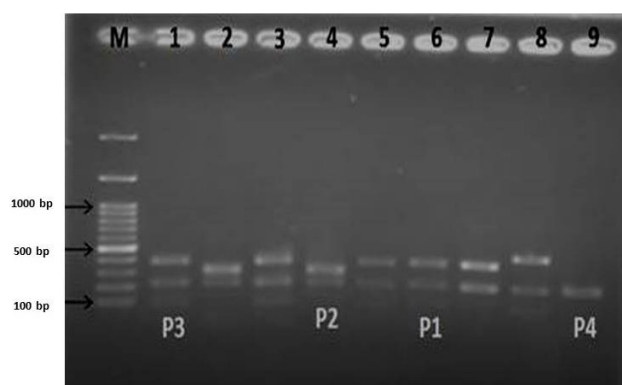


Figure 2. The PCR product of each isolate was digested, electrophoresed along with a DNA ladder (lane M), and stained with ethidium bromide and lanes 5, 6 and 7 (type P1), lanes 2 and 4 (type P2), lanes 1, 3, and 8 (type P3) and lane 9 (type P4).

As one can clearly observe in Figure 2, 20 isolates with 2 PCR fragments of 250 and 400 bp (P1 type), 14 isolates with 2 fragments of 250 and 350 bp (P2 type), 19 isolates with 3 PCR fragments of 100, 220 and 380 bp (P3 type) and 3 isolates with 1 PCR fragment of 220 bp genotype (P4 type) were noticed product sizes in all isolates (Figure 2 and Table 1).

Of 56 *S. aureus* isolates, type P1 was the most common type and was seen in 35.71% of the isolate and P4 type was seen in 5.35% of the isolates.

In isolates recovered from skin lesions, P1 type with 13 isolates (43.33%) was the most frequent and the lowest frequency belonged to P2 type with 8 isolates (26.66%). P4 type was not observed in any isolates of skin lesions. P3 type predominated in 10 isolates of urine sample (38.46%) and P4 type was observed only in 3 isolates (11.53%) of urine samples (Table 1).

Discussion

The *coa* gene in *S. aureus* isolates has various numbers of degenerate repeats, which are clearly polymorphic in both number and sequence (10). Therefore, the *coa* typing method has been reported to be a rapid, inexpensive and appropriate method for genotyping *S. aureus* strains in epidemiological studies (11).

In our study, PCR amplification of the *coa* gene resulted in the identification of five different amplicons (600, 650, 700, 800 and 850 bp) and an amplicon with 700 bp length as the most frequent type. The polymorphism of this gene is due to repetitions of 3' elements of the

coa gene in various strains (12). Previously published data from Iran have shown the presence of different *coa* types (13,14). Talebi-Satlou et al conducted a similar study on *S. aureus* isolates associated with skin and urinary tract infections in Urmia, Iran, and showed four *coa* types with 410, 530, 700 and 790 bp length (13). They also reported the *coa* type with 700 bp as the most common type, which is consistent with our results. However, in contrast to their study that determined 2 RFLP patterns for the dominant *coa* type, we could classify the predominant *coa* type in four subtypes (P1-P4), which indicate great heterogeneity among our isolates. It is noteworthy that the *coa* type with 700 bp length also was reported as a dominant type in another study from Iran (15). Considering this finding, it may be suggested that a specific subset of *S. aureus* strain is well-adapted in various parts of the human body in different regions of Iran. However, expanded genetic analyses are necessary to generate more evidence for this finding. The results of a study by Afrough et al, which was conducted on *S. aureus* collected from hospitals of Ahwaz, indicated that six PCR products were identified with lengths of 650, 750, 800, 850, 900 and 1000 bp (15) which is consistent with our results. In addition, Ramesh Babu et al showed the amplification of the *coa* gene from 60 isolates (30%) collected from the respiratory tract of patients in Shahrekord, which ranged from approximately 730 to 970 bp (16). In contrary to the results of our research, Khoshkharam-Roodmajani et al showed that several coagulase gene types (567, 648, 729, 810, 891 and 972 bp) were responsible for the majority of *S. aureus* infection in 3 main hospitals in Shiraz (17). Ramesh Babo et al showed identical restriction banding patterns of *S. aureus* isolates using PCR-RFLP of *coa* gene, which is similar to our results (600 and 700 bp) indicating the genetic relatedness of the isolates (16). In a study which was conducted in India, Himabindu et al distinguished 3 PCR products with 2 most common genotypes reported for 73.8% of the isolates (18). Tiwari et al tested 125 *S. aureus* isolates from different clinical samples and found 3 PCR products of 1456, 1150 and 710 bp with 710 bp being the predominant product (19).

The variation of the PCR products found by the amplification of the 3' end of *coa* gene reflects variations in sequence of the *coa* gene of *S. aureus* isolates. The presence of 5 different sizes in PCR products can indicate the variation in the repeat sequence of 81 bp in the isolates of the present study. This diversity is likely to be related to the presence of immigrants from neighboring countries in Zabol, southeastern Iran. A similar study by other researchers confirms the variation in the base pair size of the *coa* gene (20,21). Probably, the elimination or replacement of nucleotides within the *coa* gene will result in such a variety, which may also change the antigenic properties of the enzyme at the 3' end of *coa* gene (22). This is probably one of the reasons for the antigenic

Table 1. Pattern of *coa* Gene Diversity Among *Staphylococcus aureus* Isolates

| Samples (N) | P Type | | | |
|-------------------|---------------|---------------|---------------|---------------|
| | P1 No. (%) | P2 No. (%) | P3 No. (%) | P4 No. (%) |
| Skin lesion (30) | 13 (43.33%) | 8 (26.66%) | 9 (30%) | 0 (0%) |
| Urine sample (26) | 7 (26.92%) | 6 (23.07%) | 10 (38.46%) | 3 (11.53%) |
| Total (56) | 20 (35.71%) | 14 (25%) | 19 (33.92%) | 3 (5.35%) |

diversity and the persistence of *S. aureus* infections. The *coa* gene at 3' end sequence contains a sequence of repetitive element with 81 bp in length, varying among different strains (8). In some studies, the size difference of 10 to 20 bp for the PCR products of the *coa* gene has been proposed (17). The results of this study showed that the 650 bp and 700 bp were predominant in the isolates of skin lesions and urine samples respectively, indicating that some genotypes are unique for a particular site of infection. Considering this finding, it may be suggested that a specific subset of *S. aureus* strain is well-adapted in various parts of human body in different region of Iran. However, expanded genetic analyses are necessary to generate more evidence for this finding. Based on the results of a study conducted on *coa* gene polymorphism of *S. aureus* isolates collected from buffalo milk, four PCR products of 600, 700, 760 and 850 bp were observed, 3 of which were similar to the results of the present research (22). In a study which was conducted on 21 of *S. aureus* isolates recovered from cow's breast inflammation by Sanjiv et al, in India, 3 PCR products of 600, 680 and 850 bp (8) were found, 2 of which were similar to those of the current study.

This distribution might be explained by the coevolution of the hosts and the pathogens, and also differences in reservoirs and imply that the successful transfer of bacteria between bovine mastitis milk, raw meat and human is not a frequent occurrence (20, 23).

These characteristics show the necessity of comprehensive studies on epidemiological and ecological profiles of a specific origin before applying control programs.

The results of the present study showed that *coa* typing can be used along with other molecular methods as an appropriate method in epidemiological researches to control and monitor hospitals and community-acquired infections, in distinguishing *S. aureus* isolates collected from clinical samples.

Ethical Approval

We hereby declare that all ethical standards have been respected in the preparation of the article.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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