



Restriction Fragment Length Polymorphism Genotyping of Human *Staphylococcus aureus* Isolates From Two Hospitals in Urmia Region of Iran Using the *coa* Gene

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ABSTRACT

Background: *Staphylococcus aureus* has become an emerging public health concern. Markers that differentiate tissue-specific lineages are needed to trace the sources of strains.

Objectives: The aims of this study were to determine the genotypic characteristics of *S. aureus* isolates that are associated with skin and urinary tract infections using polymorphisms in the coagulase gene.

Materials and Methods: Coagulase gene variants among 26 *S. aureus* isolates from human infected skin (n = 10) and urine (n = 16) samples were investigated by amplification of the repeat units encoding the hypervariable region of the coagulase gene. The amplicons ranged from 490-790 bp and were subjected to restriction fragment length polymorphism (RFLP) analysis with *HaeIII*.

Results: In total, 6 distinct RFLP banding patterns were observed, designated C1-C6. The C1 pattern predominated in skin and urine isolates. Notably, the C3, C5, and C6 patterns were present in isolates from urine, whereas the C2 and C4 genotypes were preferentially detected in skin sample isolates.

Conclusions: These data demonstrate the widespread prevalence of certain genotypes and tissue-specific tendency of other genotypes, suggesting the existence of lineage- and tissue-specific genes that mediate the development of tissue-specific pathogenicities of *S. aureus* isolates.

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► Implication for health policy/practice/research/medical education:

Results of the current study also showed that the repeat region of *coa* gene can be useful for typing and grouping of skin and urinary tract associated *S. aureus* isolates.

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1. Background

Staphylococcus aureus is a highly adaptable human pathogen that is responsible for many infections and fatalities worldwide. The interaction between *S. aureus* and its human host is complex and multifactorial, because it can colonize several niches in the host. This organism is the leading cause of skin and soft tissue infections (SS-

TIs), including furuncles, carbuncles, cellulitis, and abscesses, in most countries (1-3). Several studies of patients with *S. aureus* bacteriuria (SABU) have reported *S. aureus* to be the primary urinary pathogen and SABU as a potential reservoir of invasive infection (4, 5).

The prevention and control of *S. aureus* infections depend initially on identifying the risk factors of the exposed population with regard to acquiring *S. aureus*, and also analysis of isolates by discriminatory bacterial DNA typing, to understand the transmission of this infectious organism. In many countries, *S. aureus* genotyping has become a part of ongoing surveillance systems and an important tool in the study of strain origin, clonal

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relatedness, and the epidemiology of outbreaks (6, 7). An analysis of hospitalized patients with nosocomial *S. aureus* SSTIs showed that different clones were responsible for all cases of SSTIs (8). Genetic variation between isolates from human skin and urinary tract infections has also been observed (9-11).

Historically, typing of *S. aureus* has relied primarily on strain phenotype (for example, susceptibility to bacteriophages or antibiotics), but over the past 2 decades, a variety of molecular typing methods have been developed, including phage typing, pulsed field gel electrophoresis (PFGE), binary typing, multilocus sequence typing (MLST), and PCR amplification of variable regions combined with restriction endonuclease analysis (12-16). Genotypic methods have the advantage over phenotypic methods of being independent of the expression of specific genes under artificial assay conditions (eg, laboratory media), because genotypes are relatively stable in nature compared with phenotype (biotype, serotype, antibiogram). Genotypic methods yield reproducible results, regardless of variations in laboratory assay conditions; they are rapid and do not require in vitro culture, allowing one to identify fastidious or unculturable organisms. In contrast to phenotypic methods, chromosomal DNA-based typing methods achieve near 100% typability, because all bacteria contain DNA.

The discriminatory ability of genotypic versus phenotypic methods is also higher (17). Analyses of coagulase-encoding staphylococcal DNA genes have identified variable sequences in the 3'-end coding region of allelic gene forms (18). DNA sequence analysis of the 3'-end of the coagulase gene revealed heterogeneity in the region containing the 81 bp tandem repeats encoding repeated 27-amino-acid sequences in the C-terminal region. PCR amplification of this region generated DNA fragments of various sizes that could be further discriminated by digestion with *AluI* (19). Therefore, amplification of the coagulase gene is considered a simple and accurate method of typing *S. aureus* from various sources (15, 19-23) and can be included in routine infection control programs in hospitals and epidemiological studies (20).

2. Objectives

The aims of this study were to determine the genotypic characteristics of *S. aureus* isolates that are associated with skin and urinary tract infections based on coagulase gene polymorphisms.

3. Materials and Methods

3.1. Sample Collection and Bacterial Species Identification

A total of 26 clinical isolates of *S. aureus* (16 from urine samples and 10 from skin) were recovered from patients suffering from urinary tract and skin wound infections in Urmia region, West Azerbaijan province, Iran. Urine samples and wound cultures (obtained from skin infection swabs) were collected on admission, and primary

cultures were grown on mannitol salt agar plates (MSA, Merck, Germany), a selective medium for *S. aureus*. Each plate was examined for colonies that were morphologically consistent with *S. aureus*. Samples of putative *S. aureus* colonies were selected and reinoculated on blood agar plates to isolate pure colonies. These plates were confirmed visually to contain monotypic colonies on the following day. Monotypic isolates were stained by Gram's method; Gram-positive cocci were tested with 1% hydrogen peroxide for catalase activity. Catalase-positive isolates were subjected to tube coagulase test. Coagulase-positive isolates were frozen at -70°C in cryopreservation tubes. Species-specific identification was also performed for all preserved isolates by amplification of the *nuc* gene, as described (24). DNA was prepared using a genomic DNA purification kit (Fermentas, Germany) per the manufacturer's recommendations.

3.2. *coa* Polymerase Chain Reaction (PCR)

Amplification of the 3' end of the coagulase gene was performed using primers reported by Hookey et al. (25): 5'-ATA GAG ATG CTG GTA CAG G-3' (bases 1513 to 1531) and 5'-GCT TCC GAT TGT TCG ATG C-3' (bases 2188 to 2168). The total reaction volume was 25 µL, performed as described (22) on a thermal cycler (CORBETT thermocycler, Model CP2-003, Australia) with the following program: a 45-s precycle at 94°C, followed by 30 cycles (20 s at 94°C, 15 s at 57°C, and 15 s at 70°C). At the end of the program, the reaction mixture was maintained at 72°C for 2 minutes (26). The sizes of the PCR products (5-mL aliquot) were determined using the GeneRuler™ 100 bp DNA ladder plus marker (Fermentas, Germany) by electrophoresis at 80 V for 1 h on 1.2% (wt/vol) agarose gel with ethidium bromide (0.5 µg/mL). The reference strain ATCC 29213 was included as a positive control for the PCR assays and enzymatic digestion. For the negative control, sterile water was added instead of DNA.

3.3. *Hae* III Digestion

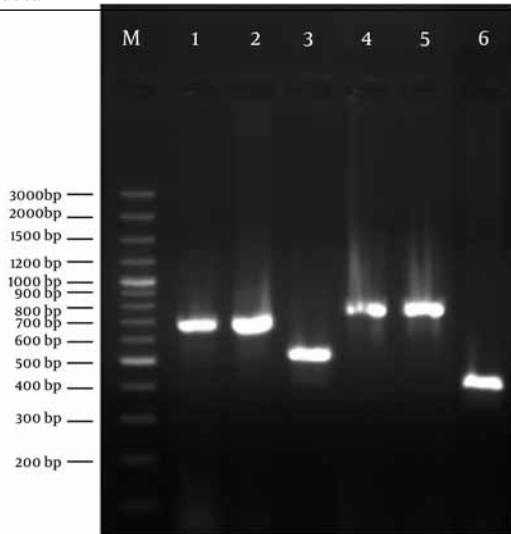
Ten microliters of PCR product was incubated with 6 U *Hae* III at 37°C for 1 h 45 min in a water bath.

4. Results

According to conventional tests, all 26 cultures were also identified as *S. aureus*, by PCR amplification of the thermonuclease gene (*nuc*) using primer pairs per Brakstad et al. (24) and performed per Saei et al. (27). Amplicons of the *nuc* gene were uniform in size—approximately 279 bp (data not shown).

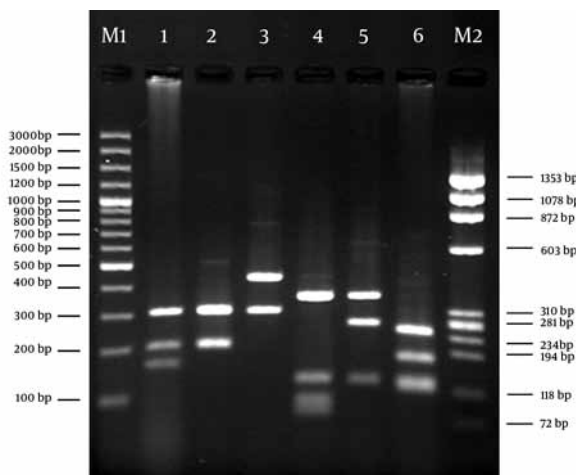
Amplification of 3' end region of *coa* generated 4 classes of bands, based on size, ranging from 410-790 bp (Figure 1). The 700-bp amplicon predominated in urine- and skin-origin isolates. *Hae*III digestion of the PCR products produced 6 distinct RFLP patterns, designated C1-C6 (Figure 2). Pattern C1 was the dominant pattern, occurring in the majority of isolates (69.2%). All *S. aureus* isolates were

Figure 1. Amplicons for the Demonstration of Four Different Sizes of *coa* PCR Product.



Lane M: size marker (GeneRuler™ 100 bp DNA ladder plus). Lanes 1-2: an approximately 700 bp-long *S. aureus* coagulase PCR product. Lane 3: 530 bp-long product. Lanes 4-5: 790 bp-long product. Lane 6: 410 bp-long product

Figure 2. RFLP Patterns of the *coa* amplicons After Digestion With Endonuclease *Hae*III.



Lane M1: GeneRuler™ 100 bp DNA Ladder Plus. Lanes 1-6: RFLP Patterns C1, C2, C3, C4, C5, and C6, Respectively. Lane M2: ΦX174 DNA/HaeIII Marker

typable using this technique. As shown in *Table 1*, the 700- and 790-bp amplicons formed 2 patterns after *Hae*III digestion, whereas the 410- and 530-bp fragments gener-

ated a unique patterns—C2 and C6, respectively. Several *coa* types were detected in urinary tract and skin tissue, but C1 was dominant and present in both tissues. Some types were detected only in skin (C2 and C4) or urine (C3, C5, and C6).

5. Discussion

A total of 26 *S. aureus* clinical isolates from skin and urine sources were studied to genotype the *coa* gene by PCR-RFLP. This efficient and reliable typing procedure is beneficial in developing efficient infection control measures in hospitals for staphylococcal infection (20). Molecular typing generated genetically distinct sets (C1-6) of *S. aureus* isolates. Yet, type C1 was responsible for 69.2% of all cases of skin and urinary tract infections, reflecting its prevalence. This finding suggests that a genetic subset of *S. aureus* isolates is particularly well adapted for causing infections in various parts of the human body. One explanation for this pattern relates to the genetic background, which differs substantially between strains, warranting further study of its function in effecting tissue adaptability and pathogenesis in skin and urinary tract infections. The existence of multiple virulence genes, such as adhesins, suggests that C1 isolates promotes infection and enhances their persistence in the urinary tract and skin.

Like antimicrobial peptides, fatty acids are induced in skin on injury or microbial stimulus through Toll-like receptor-dependent pathways (28, 29). In human sweat, secretory IgA, as well as IgG and IgE, have been detected (30). Secretory immunoglobulins of the skin cover surface structures of microorganisms and thus modify their adhesion and infectivity, resembling humoral immunity in mucous membranes (30). Bacteria, such as *S. aureus*, that colonize the skin and cause disease can resist host innate defenses through several mechanisms. Thus, the intractability of *coa* type C1 *S. aureus* to many effectors of innate defense in the skin explains its existence as the dominant etiological agent of infections in wounded skin in humans. Otsuka *et al.* (31) reported that the presence of bone-bound sialoprotein (*bbp*) confers a strong capacity for adherence (colonization) to MRSA, reflecting a pandemic spread via skin-to-skin contact.

Recently, a group has identified the contribution of iron-responsive surface determinant A (*IsdA*) expression to the biophysical properties of the cell surface of *S. aureus* (32). By reducing the overall hydrophobicity of

Table 1. The *coa* RFLP Patterns of *S. aureus* Isolates With Different *coa* amplicon Sizes and Their Distribution Among Urine and Skin Isolates

	Isolates, No.	3' End -Region of the <i>coa</i> Gene					
		410	530	700		790	
				C6	C2	C1	C4
Urine	16	2	-	12	-	1	1
Skin	10	-	2	6	2	-	-
Total	26	2	2	18	2	1	1

the bacteria, IsdA blocks the action of antibacterial molecules in normal skin, including cathelicidin, β -defensin peptides, human sebum, and its constituent hydrophobic fatty acids. Consequently, IsdA promotes *S. aureus* survival on human skin—the first molecular resistance mechanism of the pathogen to host fatty acid defenses to be identified. On the other hand, the predominance of type C1 among skin isolates is likely attributed to the finding that the transmission of infection is a dynamic phenomenon, caused by frequent person-to-person contact, contamination of surfaces, and the sharing of personal hygiene items.

Toshkova *et al.* (33) also indicated the importance of nasal carriage of *S. aureus* in the development of human skin infections. Children colonized with MRSA may also be an important reservoir and source of transmission of SSTIs in the household (34). In a study by Ellis *et al.* (35), MRSA nasal carriage was recognized as a risk for MRSA SSTIs, and individuals who have been colonized with MRSA are at increased risk of subsequent infections. Previous studies have demonstrated that individuals with *S. aureus* bacteremia (36) and surgical site infections (37) are colonized in their nares with the same isolates 80% to 90% of the time, as determined by pulsed-field gel electrophoresis.

As shown, the majority of urine-associated isolates possessed the same pattern (C1). Many virulence factors might have contributed to this predominance. For example, the intracellular adhesion (*icaA*) gene may enhance the adherence of *S. aureus* to host cells of the urinary tract and play a pathogenic role in UTI (38). Also, hematogenous spread of *S. aureus* from other areas, such as skin, which may harbor the C1 type, is likely possible. Earlier studies documented *S. aureus* bacteriuria and bacteremia in 8.3% to 15% of the patient population (5, 39, 40). Choi *et al.* (41) also found that *S. aureus* bacteriuria is frequently concomitant with *S. aureus* bacteremia. However, it is unknown whether *S. aureus* bacteriuria is caused via a hematogenous route from SAB or whether *S. aureus* bacteriuria itself leads to subsequent invasive infections. Additional large-scale studies using different molecular typing methods are necessary to confirm their association.

The C3, C5, and C6 genotypes were only detected in urine isolates, whereas C2 and C4 were only in skin, suggesting that specific selection has occurred in the skin and urinary tract. The environments of the skin and urinary tract differ markedly and might account for the degree of genetic divergence between the isolates studied. It has been reported that some clones of *S. aureus* have a greater tendency toward skin carriage, possibly due to the arginine catabolic mobile element, which might allow the strain to survive better at the low pH of the skin (42). The presence of combinations of virulence factors plays an important role in host and tissue specificity of *S. aureus* infections (43). Expanded genetic analyses are necessary to generate evidence of tissue specialization

among *S. aureus* clonal groups associated with human infections.

In conclusion, we observed heterogeneity among skin- and urine-associated *S. aureus* isolates using *coa* gene polymorphisms, but only one type was dominant in both skin and urine samples. Our study also revealed the existence of tissue specialization among isolates. As a consequence, more emphasis might be placed on skin and urine samples when trying to detect colonization of dominant or rare types. Additional studies of *S. aureus* genotypes that are commonly associated with human infections are recommended to determine the role of genetic features in contributing to the tropism of certain clones in infecting specific tissues and causing a particular disease. Further studies on these aspects from different regions of the country must be performed to gain insight into the epidemiology of *S. aureus* in hospitals and develop preventive strategies. Finally, clinicians who treat patients with *S. aureus* urinary tract infections are recommended to inquire about recent urinary tract instrumentation and recent hospitalization with intravascular device use.

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