

Frequency of Adherence Genes *cna*, *fnbA* and *fnbB* in *Staphylococcus aureus* Isolates from Traditional Cheese

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ABSTRACT

Background and Objectives: Several virulence factors are involved in the pathogenesis of *Staphylococcus aureus*. Surface proteins such as collagen binding proteins (*Cna*) and fibronectin-binding proteins (FnBP) are important factors in adhesion and invasion of *S. aureus*. The aim of this study was to evaluate the frequency of adherence genes *cna*, *fnbA* and *fnbB* *S. aureus* isolates from traditional cheese.

Methods: All 22 isolates tested were identified as *S. aureus*. The isolates were tested for the presence of adherence genes *cna*, *fnbA* and *fnbB* using specific primers in polymerase chain reaction assay.

Results: Six isolates (27.27%) were positive for the *cna* gene. Of the 22 isolates studied, one isolate was positive for *fnbA* and one was positive for the *fnbB*. Co-presence of the genes examined was not observed in any of the isolates.

Conclusion: The results indicate the weak biofilm formation ability of the *S. aureus* isolates from traditional cheese.

Keywords: *Staphylococcus aureus*, Biofilm, Genes, Cheese.

INTRODUCTION

Milk and dairy products have an important role in the transmission of spoilage microorganisms and pathogens to consumers (1). *Staphylococcus aureus* is one of the most important pathogens that can contaminate milk and dairy products, and cause infection in humans. On the other hand, these bacteria can play an important role in the development of chronic mastitis through formation of biofilm (1). Virulence of these bacteria is mediated primarily by various virulence factors such as surface adhesion molecules. According to in vitro studies, adhesion of *S. aureus* to epithelial cells and mammary gland is of great importance for the development of mastitis (2). Adhesion to epithelial cells or extracellular matrix proteins is also effective for blocking outward flow of bacteria in milking (3). *S. aureus* contains specific adhesion factors that can be attached to a variety of host proteins, especially in the extracellular matrix (4). The adhesion of bacteria is mediated by a family of proteins known as microbial surface components recognizing adhesive matrix molecules. Proteins such as collagen-binding protein (Cna) and fibronectin-binding protein (FnBP) can covalently bind to bacterial cell wall peptidoglycan. Studies have shown that FnBP is essential for the invasion of eukaryotic cells (5). It has been shown that Cna, FnbA and FnbB are significantly important in development of various pathological conditions such as keratitis (6), osteomyelitis, septic arthritis (7) and medical device-related infections (8). *Staphylococcus* is one of the main pathogens that are involved in the infections associated with implants (injection or implant prosthetics) (9). Studies have also shown that FnBPs on the surface of *S. aureus* are required for adhesion and invasion of bovine mammary cells (10). Some evidence suggests that some *S. aureus* adherence proteins be associated with certain invasive infections (9). Therefore, we aimed to evaluate the frequency of adherence genes *cna*, *fnbA* and *fnbB* in *S. aureus* isolates from traditional cheese.

MATERIAL AND METHODS

Twenty-two coagulase-positive *S. aureus* strains were isolated from traditional cheese produced in the city of Maragheh, Iran. The bacteria were cultured in Brain Heart Infusion

agar (Merck Co., Germany). One ml of bacterial suspension was centrifuged at 5000 g for 5 minutes, and supernatant was discarded. Then, 1 mL of lysis buffer containing 1 M Tris (pH=7.5), 5 M sodium chloride, 0.5 M EDTA and 2% C-TAB was added to the sediment, and the mixture was placed in bain-marie at 85 °C for 30 minutes. The vials containing the lysed cells were centrifuged at 12000 g for 5 minutes, and supernatant was transferred to new vials, and then mixed with same volume of chloroform and isoamyl alcohol (ratio 24:1) and shaken gently. After formation of two liquid phases, surface layer was removed from the vials and transferred to another tube. After adding 0.5 ml of RNAase, the tube was placed in bain-marie for 30 minutes at 37 °C. After 30 minutes, content of the vials was mixed with equal volume of isopropanol and then placed at -20 °C for 15 minutes. After centrifugation at 12000 g, DNA sedimented was dried at room temperature. Finally, DNA was dissolved in 50 ml of double distilled water (11).

Polymerase chain reaction (PCR) was performed using a 25 µl solution prepared from 12.5 ml master mix containing 0.4 µM of specific primers (Table 1) and 1 µl of DNA extracted (50 ng). PCR was performed starting with predenaturation at 94 °C for 4 minutes, 32 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, extension at 72 °C for 1 minute and final extension at 72 °C for 10 minutes. PCR product was electrophoresed on 1.5% agarose gel and observed using gel doc. DNA from *S. aureus* PTCC1112 was used as positive control and double distilled water was used as negative control. Presence of a 275-bp band related to thermonuclease gene confirmed detection of *S. aureus*.

Amplification of the *cna* gene was carried out using specific primers starting with predenaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 52 °C for 30 seconds, extension at 72 °C for 30 seconds and final extension at 72 °C for 10 minutes (14). PCR product was electrophoresed on 1% agarose gel and imaged using gel doc. Detection of a 201 bp band confirmed the presence of the *cna* gene. Amplification of the *fnbA* and *fnbB* genes was carried out starting with predenaturation at 94°C for 5 minutes, 35 cycles of denaturation

at 94 °C for 1 minute, annealing at 55 °C for 50 seconds, extension at 72 °C for 1 minute and final extension at 72 °C for 5 minutes (15). PCR product was electrophoresed on 1%

agarose gel and imaged with gel doc. Detection of 191 bp and 201 bp bands confirmed the presence of the *fnbA* and *fnbB* genes, respectively.

Table 1- Sequence of specific primers used for each gene and PCR product size

Reference	Production size(bp)	Sequence	Gene
12	275	5'-GCGATTGATGGTGATAACGGTT-3' 5'-AGCCAAGCCTTGACGAACCTAAAGC	<i>nuc</i>
13	192	5'-AAAGCGTTGCCTAGTGGAGA-3' 5'-AGTCCTCCCAAACCTTT-3'	<i>cna</i>
13	191	5'-GATACAAACCCAGGTGGTGG-3' 5'-TGTGCTTGACCATGCTCTTC-3'	<i>fnbA</i>
13	201	5'-TGTGCTTGACCATGCTCTTC-3' 5'-AGTTGATGTCGCGCTGTATG-3'	<i>fnbB</i>

RESULTS

All bacterial isolates (22 isolates) were identified as *S. aureus* after standard biochemical testing and PCR (Figure 1). The most frequent gene was *cna*, which was detected in six *S. aureus* isolates (27.27%) (Figure 2).

Figure 1- Detection of 275 bp band in electrophoresis of the PCR products. No.1: Ladder (100 bp), No.2: negative control, No.3: positive control, No.4-14: *S. aureus* isolates.



Among the *S. aureus* isolates tested, only one isolate (4.54%) was positive for the *fnbA* gene. In addition, only one isolate (4.54%) was positive for the *fnbB* gene. None of the isolates contained more than one gene.

Figure 2- Detection of 192 bp band in electrophoresis of the PCR products. No.1: Ladder (100 bp), No.2: negative control, No.3: positive control. No. 4-9: *cna*-positive isolates.

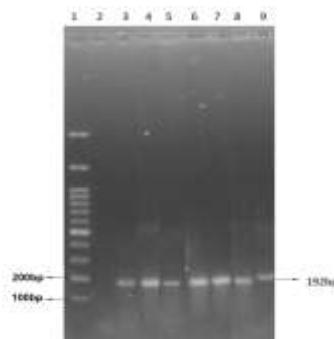


Figure 3- Detection of 192 bp bands in electrophoresis of the PCR products. No.1: Ladder (100 bp), No.2: negative control, No.3: positive control, No.4: *fnbB*-positive isolate

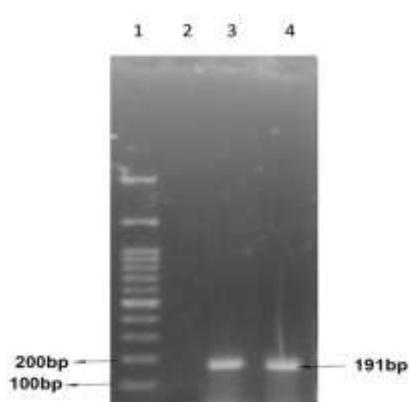
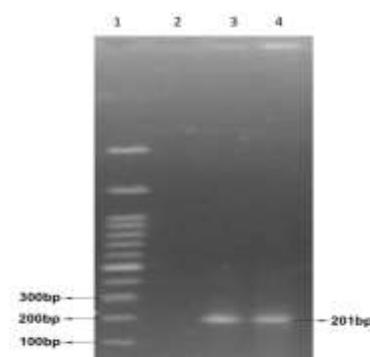


Figure 4-Detection of 201 bp bands in electrophoresis of the PCR products. No.1: Ladder (100 bp), No.2: negative control, No.3: positive control, No.4: *fnbB*-positive isolate



DISCUSSION

A wide range of pathogenic microorganisms on surfaces or in contact with food products could act as sources of infections. *S. aureus* is an important pathogen that can multiply in milk and dairy products, and cause infection in humans. On the other hand, it can cause chronic mastitis through biofilm formation (16), or cause resistance against antibiotics (17). *S. aureus* is also known for production of slime layer. Bacteria that are not able to produce slime layer, form a weaker biofilm (18). Evidence suggests that proteins including Cna and fnbpA, fnbpB are essential for adhesion and virulence of bacteria (7, 19). In this study, *cna* was the most prevalent gene in the *S. aureus* isolates tested, which is inconsistent with other studies suggesting that the *cna* gene has low abundance in isolates of human origin (21) and livestock (22). Further studies with larger sample size could help clarify the prevalence of the *cna* gene in *S. aureus* isolates from dairy products. The role of Cna has been well demonstrated in virulence and development of diseases such as septic arthritis and bone infections (23). In study of Dastmalchi Saei et al., 43 of 45 *S. aureus* isolates from sheep with mastitis contained the *cna* gene (24), which is inconsistent with our results. Thomas et al. analyzed 159 isolates from different regions and reported the prevalence of the *cna* gene as 67% and 44% in the UK and New Zealand, respectively (25). It is established that FnbpA and FnbpB play a crucial role in the assembly,

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adhesion and invasion of *S. aureus* to study, only one isolate (4.54%) contained the *fna* or *fnb* gene. In a similar study, 86.66% and 77.77% of the isolates contained the *fna* and *fnb* genes, respectively (24), which is inconsistent with our results. We also found that none of the isolates contained more than one gene. In study of Duran et al. on *S. aureus* isolates from ulcers, 78.4% of the isolates contained the *cna* gene, while 97.7% of the isolates contained the *fna* gene (28). Considering the low frequency of the genes tested and their co-presence, it can be concluded that such isolates have weaker ability to attach to mammary cells, and lower dominance and emissions than other isolates. In addition, study of Hesen et al. reported the difference in adhesion and invasion ability among *S. aureus* isolates (29).

CONCLUSION

Majority of the isolates tested in our study were negative for presence of the *cna*, *fna* and *fnb* genes. Therefore, it can be concluded that these isolates may have weak biofilm-forming ability in traditional cheese.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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