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Coagulase gene polymorphism of *Staphylococcus aureus* isolates: A study on dairy food products and other foods in Tehran, Iran

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Abstract

Coagulase is considered as a major determinant factor for the identification of *Staphylococcus aureus* strains. The 3′-end coding region of the coagulase (*coa*) gene contains a series of 81-bp tandem repeats, which differ in the number and location of enzymatic restriction sites among different isolates. *coa* PCR-RFLP has been used widely to type *S. aureus* isolates in epidemiological studies. The current study was conducted to investigate the coagulase gene polymorphisms in *S. aureus* isolated from various food samples using an *in house* PCR-RFLP method. A total of 100 strains of *S. aureus* were isolated from food samples. Isolates were typed by PCR-RFLP analysis using *Nde*I restriction digestion of the coagulase gene PCR products. Results showed that amplification of coagulase genes from *S. aureus* produced different PCR products. The isolates were grouped into 18 genotypes using RFLP analysis results of the genes. In this study, the *S. aureus* isolates have been shown to include more than one coagulase genotype, but only had a few *coa* genotypes predominated.

Keywords: *Staphylococcus aureus*; PCR-RFLP; Coagulase gene; Food samples

1. Introduction

Staphylococcus aureus is an important food-borne pathogen [1]. It is among the most significant pathogens that cause various diseases in humans and animals. In humans, nosocomial and community acquired infections are the most frequently reported problems caused by *S. aureus* [2,3]. The bacterium is one of the most significant pathogens causing intramammary infections (IMI) in dairy ruminants [4]. The primary reservoirs for *S. aureus* are the skin and mucous membranes, especially of the nasopharyngeal region of birds and mammals. This microorganism is found in 30%–80% of the human population, thus, unhygienic processing of foods has to be considered as a major risk of contamination [5–7]. Staphylococcal food poisoning (SFP) is considered to be one of the leading causes of all food-borne diseases [1]. In the last few decades, SFP has been reported as the third cause of food-borne infections in the world [5,8,9]. Milks, dairy products and meats, especially in traditional foods, play an important role in SFP; from which, *S. aureus* strains have been isolated frequently [1,10,11]. Genetic heterogeneity is considerable in natural population of *S. aureus* [12]. Relatively, many molecular techniques such as random amplified polymorphic

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DNA (RAPD), ribotyping, multilocus enzyme electrophoresis (MLEE), plasmid profiling and coagulase gene polymorphism have been used for the identification and characterization of *S. aureus* in epidemiological studies [13,14]. There is no information on genetic diversity of *S. aureus* isolated from foods in Iran since the bacterial routine identification is carried on by conventional methods such as Gram staining and catalase, clumping factor, DNase and mannitol fermentation tests [15]. However, use of molecular techniques as rapid tools in microbiology research and diagnosis has been increased recently. One of these molecular techniques, PCR-based coagulase genotyping by RFLP analysis (*coa*-RFLP) of the 3′ end of the gene encoding staphylococcal coagulase has been suggested as a simple and effective method for typing *S. aureus* isolates in epidemiological studies [16,17]. Numerous studies based on the coagulase gene polymorphism have been carried out for genotyping of *S. aureus* isolated from bovine mastitic milks and other foods [12,15,18,19]. The purpose of the current study was to identify *S. aureus* subtypes isolated from food samples, using *coa* gene polymorphism profile.

2. Materials and methods

2.1. Bacterial isolates

A total number of 100 *S. aureus* strains was isolated from food samples (78 isolates from dairy products, 16 isolates from meat products and six isolates from other foods). Samples were diluted with normal saline and then homogenized and seeded onto *Staphylococcus* selective media (Merck, Germany) and Baird–Parker agar (Merck, Germany). Colonies showing typical aspect of coagulase-positive staphylococci were identified by conventional methods, including Gram staining and catalase, clumping factor, DNase and mannitol fermentation tests. Isolates identified as *S. aureus* were frozen in skimmed mild containing 15% (v/v) glycerol at −20 °C until use.

2.2. DNA extraction

DNA was extracted using QIAamp DNA mini kit (Qiagen, Germany), according to the manufacturer's instructions for Gram-positive bacteria.

2.3. PCR amplification

PCR of the *coa* gene was carried out using primers $COAG_2$: 5′-CGAGACCAAGATTCAACAAG-3′ and $COAG_3$: 5′-AAAGAAAACCACTCACATCA-3′ described by Raimundo et al. in 1999 [20]. Reactions were prepared in a final volume of 25 μL using HotStarTaq Plus master mix kit (Qiagen, Germany), containing 12.5 μL of HotStarTaq Plus master mix, 2.5 μL of 10X buffer, 5 μL of RNase-free water, 20 pmol of each primer and 3 μL of the template. The amplification program included an initial denaturation step of 3 min at 94 °C followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C and a final extension step of 5 min at 72 °C. The amplicon size varied in various strains. *Staphylococcus epidermidis* ATCC 12228 and *S. aureus* COL were used as controls.

2.4. Restriction enzyme digestion

Generally, 8.5 μL of the PCR product was incubated with 10 U of *Nde*I endonuclease enzyme (Fermentas, USA), 2.5 μL of restriction buffer and 13 μL of distilled water for 3 h at 37 °C.

2.5. Agarose gel electrophoresis

Digested fragments and PCR products were separated in 1% and 2% agarose gels (Gibco, USA), respectively. A 100-bp ladder (Fermentas, USA) was used as a standard molecular marker for the calculation of the sizes of the *coa* and *Nde*I-generated *coa* fragments. Gels were visualized under UV light after staining with fluorescent dyes.

2.6. Specificity testing

For the specificity of the primer pair test, the DNA of *S. epidermidis* ATCC 12228 and *S. aureus* COL strain was analyzed.

2.7. Reproducibility testing

For the reproducibility of PCR, 5 selected isolates were chosen randomly and tested by twice submitting 3 different PCR products to *Nde*I digestion.

2.8. Data analysis

The software was used for the size of PCR and RFLP products. Numeric codes were assigned to the PCR genotypes and RFLP patterns.

3. Results and discussion

All isolates produced PCR amplicon with the COAG2 and COAG3 primers (Fig. 1). The agarose gel analysis of the digestion products showed ten different sizes, ranging from approximately 500 to 1000 bp. The product sizes of 800, 900 and 850 ± 20 bp were the most frequent sizes, reported for 24, 20 and 19% of the isolates, respectively. There was no amplification product of the DNA from *S. epidermidis*. As summarized in Table 1, *Nde*I restriction enzyme digestion of the PCR products generated 18 different *Nde*I restriction patterns for all isolates. Except for the products of 500, 600 and 1000 bp, amplicons of the same size generated different patterns, with the number of fragment varying from two to three and molecular sizes from approx. 80 to 330 bp (Fig. 2). Types 9, 11 and 14 were the most common patterns and seen in 50% of the isolates (Table 2). Type 1 was the most frequent pattern and reported in 31.25% of meat product samples, while Type 9 was the most frequent pattern in other foods (50%).

Coagulase protein is a main virulence factor in *S. aureus* [21]. The 3′ end of the coagulase gene contains a series of 81-bp tandem repeats, which is different between *S. aureus* strains [22].