Genotyping of Staphylococcus aureus Strains Isolated from Bovine Mastitis in Turkey by using ERIC-PCR Method

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ABSTRACT

Mastitis is an intramammary infection that leads to important economic losses in dairy industry such as increasing the amount of waste milk by affecting the quality and quantity of the milk, removing cows from herd or sending them for slaughter early. Staphylococcus aureus has an important place in the fight against mastitis. In this study, it is aimed to genotyping of the 98 S. aureus isolates that were isolated from bovine mastitis by the polymerase chain reaction of enterobacterial repetitive intergenic consensus (ERIC-PCR). ERIC primers generated DNA products in sizes ranging between ~8000 bp and 250 bp. According to the Nei homology, a dendrogram was obtained by the assessment of 28 bands in total. ERIC-PCR grouped 98 S. aureus isolates into this clusters (I-VI) showing 64 ERIC genotypes. This method, which enables the S. aureus strains to be described and classified successfully, could be used in forming a database by formulation of genotypes, determining the origin of the epidemic, studies of vaccine development and developing effective protection strategies against mastitis since it is easy, fast and reliable. At the same time, this study would contribute to the comprehension of S. aureus epidemiology and ecology in dairy herds.

INTRODUCTION

Mastitis is an inflammatory anomaly causing economic losses and milk safety problems in dairy industry (Arslan et al., 2009a; Ibarra-Velázquez et al., 2011; Qayyum et al., 2016). The infection threatens human health by consumption of raw milk and milk products. Mastitis, which is seen in all of the domestic animals in many countries of the world, is particularly known to be a significant problem in dairy cows (Gudding et al., 1984; Han et al., 2000).

Mastitis is generally caused by bacterial (Staphylococcus aureus, Escherichia coli, Streptococcusagalactiae, Streptococcus uberis, Streptococcus dysgalactiae, Streptococcus pyogenes, Corynebacterium pyogenes), mycotic (Candida, Cryptococcus, Trichosporon, Aspergillus, Penicillium) and viral pathogens (herpes-type viruses). Staphylococcus aureus (S. aureus) is the most prevalent pathogen among the bacterial factors (isolation rate from clinical mastitis: 60-65%; isolation rate from subclinical mastitis: 80-85%) causing mastitis in ruminants. But non-infectious (trauma, hot-cold, chemical agents) mastitis can be shaped depending on traumatic cause. S. aureus is in the first place among the bacterial factors (Singh and Singh, 1968; Erganiş et al., 1995). It was reported that phenotypic and genotyping characteristics of the factor need to be known for an active fight (Aarestrup et al., 1995). Moreover, it was stated that genotypic typing methods are more valuable than phenotypic typing methods in epidemiological studies (Olive and Bean, 1999). In recent two decades, phenotyping and genotyping methods have been improved and applied to study mastitis-causing bacteria of dairy cattle at species, subspecies and strain level. Many genotyping methods have existed to characterize bovine mastitis-causing pathogens (Tenover et al., 1995; Struelens, 1996; van Belkum et al., 2007; Zadoks et al., 2011). Comparative typing methods based on electrophoretic banding patterns are increasingly used in veterinary diagnostic laboratories, bringing the use of molecular epidemiology for outbreak- and farm- investigations within reach of dairy veterinarians and farm advisors (Zadoks et al., 2011).

Molecular methods have become an important tool in revealing origins of the infections concerning public health or hospital outbreaks. In order for the genotypic methods to be used in routine, they should be easy, cheap, fast, and reliable for application and should distinguish between microorganisms that are similar but unmatched microorganisms and provide more information than traditional methods (Tenover et al., 1994).

Precise and effective use of epidemiological typing system is required in limitation and monitoring the spread...
of intra-herd and inter-herd \textit{S. aureus} strains. DNA-based methods are used to compare \textit{S. aureus} isolates from staphylococci infections with human and animal origin and to describe epidemiologically (Kapur et al., 1995; Zadoks et al., 2000; Reinoso et al., 2004). Enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC) method has been determined to be reliable in identification of \textit{Staphylococcus} and \textit{Streptococcus} strains with human and animal origin (del Vecchio et al., 1995; van Belkum et al., 1996; Wieser and Busse, 2000; Reinoso et al., 2007). ERIC-PCR has become a powerful tool for molecular genetic analysis of bacteria and bacterial taxonomy since it allows individual fingerprints of genus, species and strains and helps to determine the phylogenetic relationships (de Bruijn, 1992).

Studies about epidemiology, ecology, pathogenesis and strain variations of important \textit{Staphylococcus} species significant udder pathogens in many countries and herds are limited. In this study, it is aimed to type advanced-level 98 \textit{S. aureus} isolates isolated from the bovine mastitis by ERIC-PCR method.

**MATERIALS AND METHODS**

**Bacterial strains**

A total of 98 isolates from Konya Region were isolated from bovine subclinical mastitis milk samples. All isolates were identified as \textit{S. aureus} by a standard microbiological procedure (Holt et al., 1994) and confirmed by use of identification test with the VITEK 2 system (bioMerieux).

**DNA isolation and PCR amplifications**

Bacterial strains were incubated in Brain Heart Infusion Broth at 37 °C for 18 hours. Genomic DNA isolation was made modifying the method described by Ausubel et al. (1991).

DNA samples were amplified by PCR for the repetitive element sequence using the primer ERIC-PCR: ERIC1R: 5'-ATGTAAGCTCCTGGGGATTAC-3', ERIC2: 5'-AAGTAAGTGACTGGGGATTCAC-3' (Louws et al., 1994; Versalovic et al., 1991).

Each 25µl of the reaction contained 50ng of genomic DNA, 10XPCR reaction buffer (50mM KCl, 10mM Tris-HCl, pH=9, %0.1 TritonX-100), 3mM MgCl\(_2\), 2.5mM dNTP, 75pmol of each primer and 2.5U of \textit{Taq} DNA polymerase (Fermentas). PCR was performed in a Mastercycler Gradient thermal cycler (Eppendorf). The initial step of 95°C for 7 min was followed by 30 cycles of 94°C for 1 min, annealing 52°C for 1 min, 65°C for 8 min, and a final cycle at 65°C for 16 min. The amplification products were separated by gel electrophoresis in 2% agarose gels, stained with ethidium bromide, photographed on a UV transilluminator. Gel image has been transferred to the computer with DNA imaging system (Vilber Lourmat).

**Data analysis**

Fingerprints were scored visually as present (1) or absent (0). Genetic similarity among \textit{S. aureus} strains was estimated based on Nei homology using Bio1D++ computer programme. Cluster analysis was performed using the UPGMA.

**RESULTS**

Genetic relationship within all \textit{S. aureus} isolates was analyzed by ERIC-PCR using the ERIC1R and ERIC2 primers. ERIC-PCR produced genomic profiles consisted of 8 to 14 bands, with a size of range of 8000 bp and 250 bp.

According to the dendrogram obtained by the assessment of 28 bands in total (Fig.1), a differentiation was formed by analyzing the dendrogram with a similarity coefficient of 68% and considered to define six clusters namely I to VI. ERIC-PCR grouped 98 \textit{S. aureus} isolates into these clusters I–VI showing 64 ERIC genotypes with discrimination indexes (D) of 0.96. While this result showed that genotypes were highly similar between \textit{S. aureus} isolates, the discriminatory power by ERIC-PCR method was determined to be high. In the entire \textit{S. aureus} isolates worked on, it was observed that two band patterns with sizes <6000 bp and 500 bp are common (monomorphic). In sizes 3000 bp, 1000 bp and 250 bp, common bands were observed in most isolates. It was found that the isolates in cluster VI exhibited their specific band profiles (2000 bp and 7500bp).

**DISCUSSION**

In identification of bacteria strains phenotypic characteristics such as biotyping, phage typing, serotyping and antibiotic resistance are used. However, more recently, molecular approaches are beneficial in genotypic distinction of bacteria strains. Restriction fragment length polymorphism (RFLP) (Owen, 1989), Pulsed field gel electrophoresis (PFGE) (Murray et al., 1990; Gardella et al., 2005), plasmid profiles (Litwin et al., 1991; Arslan et al., 2009a), repetitive extragenic palindromic–polymerase chain reaction (REP-PCR) (Versalovic et al., 1994; Louws et al., 1996; Arslan et al., 2009b; Aruna et al., 2009; Nordin et al., 2010; Manga and Vyletělová, 2012; Njage et al., 2013; Kang and Dunne, 2003) and random amplified polymorphic DNA-Polymerase chain reaction (RAPD-PCR) (Arslan et al., 2005; Gardella et al., 2005) can be given as examples of some molecular genotyping methods.
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ERIC-PCR have been successfully used by many researchers in distinction of gram (-) (Versolavic et al., 1991; Rodriguez-Barradas et al., 1995; Kerouanton et al., 1996; Appuhamy et al., 1997; Diab and Al-Turk, 2011) and gram (+) (van Belkum et al., 1993; Lipman et al., 1996; Kang and Dunne, 2003) bacteria strains. Lipman et al. (1996), by genotyping of S. aureus strains from isolated bovine mammary glands, determined origin of outbreak of S. aureus in herd. It was emphasized that ERIC-PCR allow the clear differentiation of Staphylococcus epidermidis and Staphylococcus hominis strains which have a problem in biochemical tests by exhibiting species specific banding patterns (Wieser and Busse, 2000). By separating to 11 types with ERIC-PCR of 67 S. aureus strains, it was shown that ERIC-PCR technique provides a reliable tool for investigating epidemiology and tracking the spread of S. aureus strains in the hospital environment (Candan et al., 2013). But in this study, ERIC-PCR showed better discrimination power with 64 genotypes of 98 S. aureus strains. The result of a previous study exhibiting 75 ERIC-types in 90 S. aureus was also in concordance with our data (Abdollahi et al., 2014). As similar with our discrimination indexes (D) of 0.96, Ye et al. (2012) classified 35 S. aureus isolates into 28 ERIC types with discrimination indexes (D) of 0.984. It was shown that PFGE allowed better discrimination of S. epidermidis isolates than REP-PCR and RAPD methods (Begović et al., 2013). Gardella et al. (2005) determined that ERIC method is as good as PFGE technique.

CONCLUSION

According to these studies, the ERIC-PCR method has been successfully applied for typing and distinguishing of Staphylococcus species and strains. In conclusion, it will be useful to create a fingerprint database through this rapid and reliable molecular method (ERIC-PCR) in finding the source of the epidemic causing the disease, determining the pathogen routinely and in vaccine development studies. In this way, rapid diagnosis will be possible and the treatment process will be shortened by providing useful data in developing effective protection strategies against the mastitis.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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Molecular Typing of Staphylococcus aureus Strains


