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polymorphism to characterize methicillin-
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Research Article

Use of restriction fragment length polymorphism to characterize methicillin-resistant *Staphylococcus aureus* in dairy products

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Abstract

Staphylococcus aureus is one of the frequent causes of animal and human infections. In the present study, methicillin-resistant *S. aureus* (MRSA) was investigated in dairy products in Iran. From 116 coagulase-positive *S. aureus* isolates, 7 samples had MRSA gene. Amplification of MRSA gene produced 1 polymerase chain reaction product with the size of 530bp. By analyzing the restriction fragment length polymorphism (RFLP) of MRSA gene with Msa1 restriction enzyme, there was no restriction pattern. The results showed that there was no genetic diversity in MRSA gene in *S. aureus* isolates from dairy products of different areas in East-Azerbaijan Province, Iran.

Keywords: RFLP; Methicillin-resistant; *Staphylococcus aureus*; Dairy products.

Introduction

Staphylococcus (S.) aureus is worldwide the most important pathogen in food poisoning and also is involved in another wide variety of infections found in human beings and animals. It can cause gastrointestinal symptoms like nausea, emesis, abdominal cramps, and diarrhea in humans (Scherrer *et al.* 2004; Morandi *et al.* 2010).

Also *S. aureus* is a major nosocomial pathogenic agent that causes endocarditis, osteomyelitis, pneumonia, toxic shock syndrome, carbuncles, and boils (Shopsin *et al.* 2001).

Food poisoning due to *S. aureus* is related to the production of enterotoxins by microorganism in food stuffs. *S. aureus* can access milk through direct excretion from udders suffering clinical and sub-clinical staphylococcal mastitis and by environmental contamination during the milk handling and processing (Morandi *et al.* 2010).

In 1959, methicillin, synthetic penicillin, was introduced. However, by 1960, methicillin-resistant *S. aureus* (MRSA) strains were identified, the direct result of *S. aureus* acquiring the *MecA* gene, which encodes for an altered penicillin-binding protein gene (*PBP_{2a}*) (Shopsin *et al.* 2001). MRSA is one of the important

pathogenic microorganisms in the hospital and community settings, with substantial rates in morbidity and mortality (Shittu *et al.* 2007). In the United States, MRSA is responsible for approximately 25% of the nosocomial infections, and reports of community acquired MRSA infection are increasing (Shopsin and Kreiswirth 2001).

Because published reports on the use of restriction fragment length polymorphism (RFLP) to characterize MRSA in dairy products in Iran are rare and little information is available in this regard, the present study was conducted as one of the first reports.

Materials and Methods

Bacterial strains

A group of 116 isolates were selected from dairy products in East-Azerbaijan province, Iran. Of these isolates, 42 were obtained from raw milk (Marand and Tabriz cities), 57 from traditionally produced cheese (Tasouj, Bonab, and Lighvan) and 17 from ice cream (Tabriz).

Biochemical profile

All the strains were subcultured three times in brain-heart infusion (BHI) broth at 37°C for 24 hr.

All the isolates were tested twice and retested in case of discrepancies.

Extraction of DNA

Bacterial DNA extraction was carried out according to Rodrigues da Silva and da Silva (2005).

PCR technique

PCR was performed in a 25- μ l reaction mixture containing 1 μ l of template DNA, 12.5 μ l of master kit, 10 μ l of H₂O, and 0.5 μ l of each primer (5'-AAA, ATC, GAT, GGT, AAA, GGT, TGG, C-3'; 5'-AGT, TCT, GGA, GTA, CCG, GAT, TTG, C-3'). The PCR reaction was performed according to the following cyclic condition:

Initial denaturation at 94°C for 4 min, 32 cycles of 1 min in each with denaturation at 94°C, 1 min annealing at 57°C, 1 min extension at 72°C, a final 10 min extension at 72°C.

Restriction fragment length polymorphism

The PCR products were digested with Sma1 for restriction analysis. Reaction mixture containing

5- μ l of PCR products, 1.5- μ l of enzyme, 7- μ l of H₂O and 1.5- μ l restriction buffer, and then incubated at 37°C overnight.

Agarose gel electrophoresis

The resulting fragments were separated by electrophoresis in 1% agarose gel. The gels were stained with ethidium bromide and visualized on an UV transilluminator. The size of the fragment was determined by comparison with the 100 bp marker.

Results

The findings showed that only there were 7 MRSA in 116 samples. The size of PCR products of 7 MRSA was 530 bp (Figure 1). Digestion of MRSA PCR products with Sma1 yielded no different restriction profile. So, it was observed that no restriction site is produced with Sma1 for methicillin-resistant genes (Figure 2) in examined dairy products.

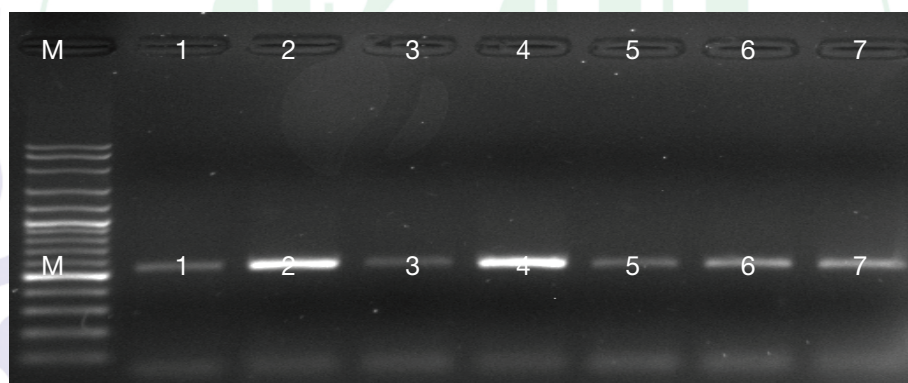


Figure 1: Agarose gel electrophoresis of MRSA gene of *Staphylococcus aureus* PCR Product. Lane M: 100 bp marker. Lanes 1–7: approximately 530 bp long *S. aureus* MRSA PCR product.

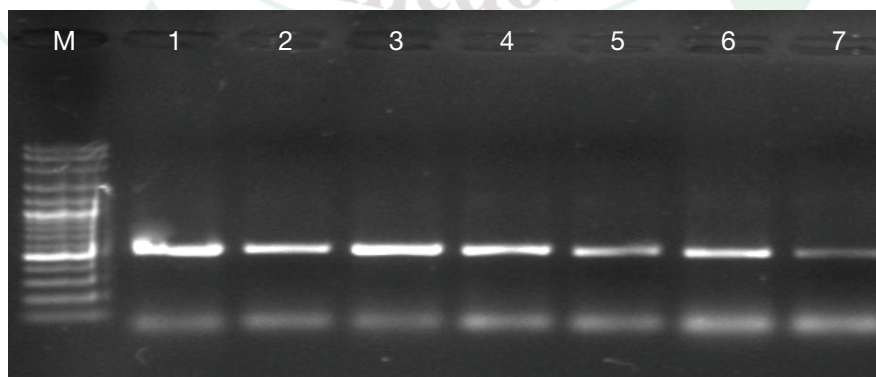


Figure 2: PCR amplified MRSA gene digested with the DNA restriction endonuclease of Sma1. Lane M: 100 bp marker. Lanes 1–7: have no RFLP pattern. After treatment of MRSA PCR product with Sma1 endonuclease.

Discussion

Methicillin-resistant *S. aureus* isolates were once confined largely to hospitals, other healthcare environments, and patients frequenting these facilities. Community-associated MRSA strains have rapidly disseminated among the general population in many countries (Chowdhury, 2011). Restriction endonuclease digestion analysis has been used to determine MRSA isolates.

The results of this study suggested that no RFLP pattern is produced in 7 isolates yielding methicillin-resistant *S. aureus*. But Wei *et al.* (1992) from 26 clinical isolates of MRSA collected from 6 Australian hospitals, reported 13–17 bands of 7–700 kb with Sma1 digestion. Nafisi *et al.* (2008) researched on the isolates of coagulase-positive *S. aureus* among 204 clinical staff from different areas of Shahrekord city, Iran, reported that phenotypically, 23 cases (44%) of the isolates and genotypically (*MecA*), 27 cases (52%) of the isolates were resistant to methicillin. As MRSA may be present in raw milk and traditional dairy products, insufficiently hygienic handling of these contaminated foods may lead to transmission of MRSA to human and possible colonization in nostrils, skin, and gastrointestinal tract Mirzaei *et al.* (2011).

Findings of Mirzaei *et al.* (2011) showed that 2 (50%) of the pasteurized milk isolates and 2 (22%) of the traditional cheese isolates collected from Sarab city, Iran, contained *MecA* gene (MRSA). Chu *et al.* (2012) reported the appearance of MRSA strains in mastitic goats for the first time in Taiwan. *MecA* gene of *Staphylococcus aureus* isolated from raw water buffalo milk and dairy products in Turkey were determined by Pamuk *et al.* (2012). All strains showed resistance to at least one antibiotic. Of the 97 *S. aureus* strains, 2 (5.7%) harbored *MecA* gene. Overall, 2.5% of the samples were contaminated with MRSA.

Study of Grady *et al.* (2000) in MRSA isolated with fluorescent amplified-fragment length polymorphism (FAFLP technique) showed another approach to the epidemiological study of MRSA. They found several clusters of strains and isolates with that technique. Hookey *et al.* (1999) reported that in typing 34 isolates and 4 reference strains of MRSA with FAFLP technique, there were from 40 to 70 fragments, 50–450 bp in size. Janwithayanuchit *et al.* (2006) from a total of 129 MRSA, achieved 4 different genotypes by PCR-RFLP pattern, however, these results are in contrast with our findings.

It is concluded that although no restriction pattern was seen in the present study in Iran, East-Azerbaijan province, laboratories are encouraged to investigate strains with restriction sites and RFLP pattern. This is the first report on the molecular typing of the MRSA based on RFLP technique. Measures to prevent contamination and growth of MRSA in food, including the use of microbiological criteria should be equal to those that are valid for *S. aureus* in general.

Conflict of Interests

There is no conflict of interest.

References

- Chowdhury A, 2011. Evolving antibiotic resistance: a great threat to medical practice. *Bangladesh Journal of Medical Science*, 11: 1–3.
- Chu C, Yu C, Lee Y, Su Y, 2012. Genetically divergent methicillin-resistant *Staphylococcus aureus* and second dependent mastitis of dairy goats in Taiwan. *BMC Veterinary Research*, 8: 1–10.
- Grady R, O'Neill G, Cookson B, Stanley J, 2000. Fluorescent amplified-fragment length polymorphism analysis of the MRSA epidemic. *FEMS Microbiology Letters*, 187: 27–30.
- Hookey JV, Edwards V, Patel S, Richardson JF, Cookson BD, 1999. Use of fluorescent amplified fragment length. *Journal of Microbiological Methods*, 37: 7–15.
- Janwithayanuchit I, Ngam-Ululert S, Paungmonung P, Rangsipanurath W, 2006. Epidemiologic study of methicillin-resistant *Staphylococcus aureus* by coagulase gene polymorphism. *Science Asia*, 32: 127–132.
- Mirzaei H, Tofghi A, Karimi Sarabi H, Farajli M, 2011. Prevalence of methicillin-resistant *Staphylococcus aureus* in raw milk and dairy products in Sarab by culture and PCR techniques. *Journal of Animal and Veterinary Advances*, 10: 3107–3111.
- Morandi S, Brasca M, Lodi R, Brusetti L, Andrighetto C, Lombardi A, 2010. Biochemical profiles, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and multilocus variable number tandem repeat analysis (MLVA) for typing *Staphylococcus aureus* isolated from dairy products. *Research in Veterinary Science*, 88: 427–435.

Nafisi MR, Kalhor H, Zamanzad B, Karimi A, Farokhi A, Validi M, 2008. Comparison of agar screen and duplex-PCR in determination of methicillin-resistant *Staphylococcus aureus* (MRSA) strains isolated from nose of personnel in Hajar hospital of Shahrekord. Arak University Medical Sciences Journal, 11: 94–101.

Pamuk S, Yildirim Y, Seker A, Gurler Z, Kara R, 2012. A survey of the occurrence and properties of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus intermedius* in water buffalo milk and dairy products in Turkey. International Journal of Dairy Technology, 65: 416–422.

Rodrigues da Silva E, da Silva N, 2005. Coagulase gene typing of *Staphylococcus aureus* isolated from cows with mastitis in Southern Brazil. Canadian Journal of Veterinary Research, 96: 260–264.

Scherrer D, Corti S, Muehlherr JE, Zweifel C, Stephan R, 2004. Phenotypic and genotypic characteristics of *Staphylococcus aureus* isolates from raw bulk-tank milk

samples of goats and sheep. Veterinary Microbiology, 101: 101–107.

Shittu A, Lin J, Morrison D, 2007. Molecular identification and characterization of manitol-negative methicillin-resistant *Staphylococcus aureus*. Diagnostic Microbiology and Infectious Disease, 57: 93–95.

Shopsin B, Kreiswirth BN, 2001. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus*. Emerging Infectious Diseases, 7: 323–326.

Wei MQ, Grubb WB, 1992. Typing of Australian methicillin-resistant *Staphylococcus aureus* strains by pulsed field gel electrophoresis. Journal of Medical Microbiology, 37: 187–191.

Wei MQ, Wang F, Grubb WB, 1992. Use of contour-clamped homogeneous electric field (CHEF) electrophoresis to methicillin-resistant *Staphylococcus aureus*. Journal of Medical Microbiology, 36: 172–176.