Full Length Research Paper

Presence and antimicrobial susceptibility of methicillin-resistant *Staphylococcus aureus* in raw and pasteurized milk and ice cream in Tabriz by culture and PCR techniques

Hamid Mirzaei[1]*, Hosain Farhoudi[2], Hesam Tavassoli[2], Mahdi Farajli[2] and Alireza Monadi[3]

[1]Member of Veterinary Scientific Association, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[2]Graduate of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran.
[3]Department of Microbiology, Tabriz Branch, Islamic Azad University, Tabriz, Iran.

Reports of methicillin-resistant *Staphylococcus aureus* (MRSA) in food have become more frequent in recent years. The aim of this study was to estimate the presence of MRSA in raw milk, pasteurized milk and ice cream in Tabriz by culture and polymerase chain reaction (PCR) techniques, as well as the determination of their antimicrobial susceptibility. For this purpose, 300 samples of raw milk, pasteurized milk and ice cream were collected from different sellers in Tabriz. They were first evaluated for contamination by coagulase positive *S. aureus* through culturing method, and then the isolates were subjected to PCR method according to *nuc gene* in order to confirm *S. aureus* and methicillin resistance gene (*mecA*). Finally the antimicrobial resistance trend of the isolates was investigated by disc diffusion agar method. The cultured samples indicated that 50 (50%) of raw milk samples, 2 (2%) of pasteurized milk samples and 26 (26%) of ice cream samples were contaminated by *S.aureus*. Forty five, 1 and 23 of these isolates, respectively were confirmed to be *S.aureus* based on PCR using *nuc* gene primer. From the total of 69 isolates containing *nuc* gene, 20 isolates had the resistant *mecA* gene. Of the 69 isolates, 17 isolates showed simultaneous resistance against 4 antibiotics, 18 against 5 antibiotics, 8 against 6 antibiotics, 8 against 7 antibiotics, 7 against 8 antibiotics, 6 against 9 antibiotics, 2 against 10 antibiotics and 3 isolates against 11 antibiotics. In other words 100% of the isolates showed simultaneous resistance to more than 3 antibiotics. The results provide evidence that the presence of antimicrobial resistant strains of *S. aureus* has become remarkably widespread in food samples. This calls for better control of the sources of food contamination and as well as the spread of antimicrobial-resistance organisms.

Key words: Methicillin-resistant *Staphylococcus aureus*, milk, ice cream, polymerase chain reaction (PCR).

INTRODUCTION

Staphylococcus aureus is one of the major bacterial pathogens which cause clinical infection and food poisoning cases (Yu-Cheng et al., 2008). The primary reservoirs for *S. aureus* are the skin and the mucous membranes, especially of the nasopharyngeal region of birds and mammals. The pathogen is found in 30 to 80%

of the human population and one-third to two-third of these carriers harbour enterotoxigenic strains. Thus, unhygienic treatment of food has to be considered as a major risk of contamination, and staphylococcal food poisoning is often associated with highly manually handled food (Viktoria et al., 2001). Milk is a good substrate for *S. aureus* growth, and dairy products are common sources of intoxication (Morandi et al., 2007; Scherrer et al., 2004). *S. aureus* can gain access to milk either by direct excretion from udders with clinical or

---

*Corresponding author. E-mail: hmirzaei@iaut.ac.ir.

subclinical staphylococcal mastitis or by contamination from the environment during handling and processing raw milk (Peles et al., 2007).

Antimicrobial resistance is an important public health concern worldwide. The development of resistance both in human and animal bacterial pathogens has been associated with the extensive therapeutic use of antimicrobials or with their administration as growth promoters in animal production (Normanno et al., 2007). Methicillin-resistant *S. aureus* (MRSA) was first reported in the United Kingdom (UK) in 1961, and by mid-1990s, it had become a major problem worldwide (Mahony et al., 2005). Methicillin resistance is of particular relevance because it is conferred by the presence of mecA gene, which encodes production of an altered penicillin binding protein (PBP) (PBP2a or PBP2') that has a low affinity for all beta-lactam antimicrobials (penicillins, cephalosporins, carbapenems) (Kwon et al., 2006). Therefore, methicillin-resistant staphylococci are resistant to this broad range of important antimicrobials. The mecA gene resides on a staphylococcal chromosomal cassette (Scott et al., 2010).

Standardized methods of susceptibility test have been used for the detection of MRSA strains. However, phenotypic express of methicillin-resistance can be heterogenous. In addition, methicillin resistance is influenced by culture conditions such as temperature, pH and sodium chloride (NaCl) content in the medium. These factors complicate the detection of methicillin-resistance, especially for strains with low level resistance. The polymerase chain reaction (PCR) methods have high sensitivity and specificity and are independent of the physical and chemical conditions of the culture (Japoni et al., 2004; Zamani et al., 2007). The rate of infection with this kind of strain among Iranian patients has been studied, but no records were found of studying the rate of contamination and antimicrobial resistance patterns of MRSA strain in food including dairy, especially for PCR method. Therefore, the purpose of this study was to determine the presence and antimicrobial susceptibility of methicillin-resistant *S. aureus* in raw and pasteurized milk and ice cream in Tabriz by culture and PCR techniques.

MATERIALS AND METHODS

Sampling

In the present study, a total of 300 samples (100 raw milk, 100 pasteurized milk and 100 ice cream samples) were collected from different sellers across Tabriz during the summer in 2010. In order to collect the raw milk samples, first 25 raw milk sellers in different parts of the city were identified and then one sample of the each raw milk with 20 days interval was collected from every one of the sellers. For collecting samples of the traditional ice cream (50 samples), we collected one each sample of the ice cream from every one of the 50 sellers. In order to collect pasteurized milk (100 samples) and industrial ice creams (50 samples), based on the factory in which it was made, we collected every one of the pasteurized milk and industrial ice cream sample from the sellers

with the interval of the 10 days. The samples were transported to laboratory in sterile and cold containers (4°C) and preserved at this temperature. The time span between sampling and microbiological analysis was not more than 12 h.

Bacterial isolation and identification

For this purpose, 25 ml/g of raw milk, pasteurised milk or ice cream samples were homogenized for 2 min with 225 ml of physiological sterile saline, in a stomacher, Lab-Blender (PBI International, Milan, Italy). Then 1 ml of this suspension was added to 10 ml of cooked meat broth, and was incubated for 24 h at a temperature of 37°C. Then 0.5 ml of the produced culture was spread over Baird-Parker agar supplemented with egg yolk and potassium tellurite (Oxoid) and was incubated for 35 to 48 h at a temperature of 37°C (Tamagnini et al., 2006). Typical coagulase-positive *S. aureus* colonies are black, grey, or white and are surrounded by an opaque halo of precipitation which signifies the coagulase reaction (O'Brien et al., 2009). Characteristic colonies were identified by conventional methods, including Gram stain, catalase test, anaerobic utilization of glucose and manitol, and coagulase test (Miriam and Rosalia, 2004).

DNA isolation for PCR

DNA was extracted according to the method of Cremonesi et al. (2005) from 1 ml of overnight grown bacterial culture, incubated in BHI broth at 37°C.

Antimicrobial susceptibility testing

Antimicrobial drug susceptibility testing of the isolates was performed on Mueller–Hinton agar (Oxoid), by the disk diffusion method using commercial discs (Patanteb Company, Iran) in accordance with Clinical Laboratory Standards Institute guidelines (CLSI, 2007). The antimicrobial agents tested included methicillin (5 µg/disk), ciprofloxacin (5 µg/disk), erythromycin (15 µg/disk), tetracycline (30 µg/disk), gentamicin (10 µg/disk), sulfamethoxazole/trimethoprim (23.75/1.25 µg/disk), vancomycin (30 µg/disk), cephalothin (30 µg/disk), ceftazidime (30 µg/disk), cloxacillin (5 µg/disk), rifampin (30 µg/disk) and penicillin (10 U/disk). *S. aureus* ATCC 25923 was the control strain in every test run.

PCR for *nuc gene* detection

The nuc gene was amplified with the following two oligonucleotides: forward primer (5′- GCG ATT GAT GGT GAT ACG GTT - 3′) and backward primer (5′- CCAAGCCTTGACGAACTAAAGC - 3′) which gave a PCR product of 255 bp. The PCR was performed with an initial denaturation step of 3 min 94°C, followed by 40 cycles of 60 s 94°C, 60 s 55°C, 60 s 72°C, and the extension step of 10 min 72°C. Agarose gels were prepared with TAE buffer (Tris, glacial acetic acid, EDTA, pH 8) and added with ethidium bromide (1 µg/15 ml gel). PCR product (5 µl) of each sample was mixed with 1 µl of sample buffer (6X: 0.25% bromophenol, 0.25% xylen cyanol, 15% ficol 400) and loaded on 1.5% agarose and electrophoresed in 80 volt for 60 min. The band of fragment was observed by UV transilluminator and was later documented by gel analyser machine (Kim et al., 2001).

PCR for *mec-A gene* detection

The *mec-A* gene was amplified with the following two

Table 1. The frequency of *S.aureus* in the samples based on culture method and *nuc* and *mecA* genes in isolates.

Sample	Number	A	B	C
Raw milk	100	50	45	14
Pasteurised milk	100	2	1	0
Ice cream	100	26	23	6
Total	300	78	69	20

A,The number of samples contaminated with *coagulase-positive S.aureus* based on culture method. B,The number of *S. aureus* isolates containing *nuc* gene. C, The number of *S. aureus* isolates containing *MecA* gene.
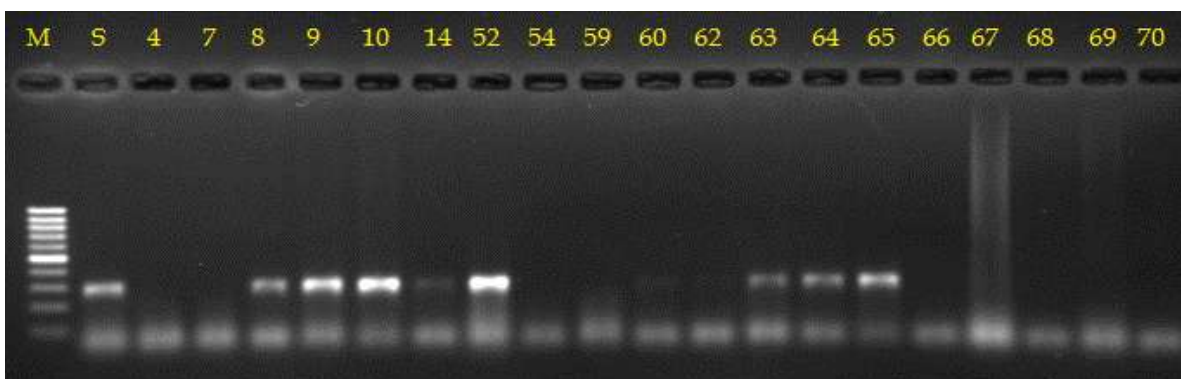

Figure 1. Agarose gel electrophoresis analysis for the *nuc* gene in *S. aureus* isolates. Lane 1, Molecular size markers; lane M, *S. aureus* PTCC 1112 *(*positive control); lanes 4, 7, 8, 9, 10, 14, 52, 54, 59, 59, 60, 62, 63, 64, 65, 66, 67, 68, 69 and 8 *S. aureus* isolates. Arrow indicates the 255 bp amplicon.

forward primer 1276 (5′-AAA ATC GAT GGT AAA GGT TGG C - 3′) and backward primer 1787 (5′-AGT TCT GGA GTA CCG GAT TTG C - 3′) which gave a PCR product of 533 bp (Merlino et al., 2002). The PCR was performed with an initial denaturation step of 3 min 94°C, followed by 40 cycles of 60 s 94°C, 30 s 50°C, 90 s 72°C, and the extension step of 10 min 72°C. Agarose gels were prepared with TAE buffer (Tris, glacial acetic acid, EDTA, pH 8) and added ethidium bromide (1 µg/15 ml gel). PCR product (5 µl) of each sample was mixed with 1 µl of sample buffer (6X: 0.25% bromophenol, 0.25% xylen cyanol, 15% ficol 400) and loaded on 1.5% agarose and electrophoresed in 75 volt for 60 min. The band of fragment was observed by ultraviolet (UV) transilluminator and was documented by gel analyser machine (Zamani et al., 2007).

RESULTS AND DISCUSSION

Using culturation method, 50% of raw milk samples, 2% of pasteurized milk samples, and 26% of ice cream samples were contaminated with coagulase-positive *S. aureus*. According to PCR using *nuc* gene primer, 45 (90%) of raw milk isolates, 1 (50%) of pasteurized milk isolates, and 23 (88.5%) of ice cream isolates contained *nuc* gene (Table 1, Figures 1 and 2). It was demonstrated that the potentially pathogenic role of *S. aureus* as a food-borne pathogen should not be neglected (Pereira et al., 2009). Algero et al. (2007), who analyzed 172 food samples including milk, soft cheese, hard cheese, ice cream, yoghurt, and fast food like sandwiches in Botucitu

market in Brazil, reported that 26 samples (15.1%) of the food were contaminated with *S. aureus.* Dastmalchi Saee et al. (2009) evaluated 370 cow milk samples in West Azerbijan and East Azerbijan in Iran, using culture and PCR methods. They reported that 58 samples were contaminated with coagulase-positive *S. aureus*. Akineden et al. (2008) collected and tested 181 goat milk samples in Hesse, Germany and reported that 14 samples (17.7%) were contaminated with *S. aureus*. Normano et al. (2007) examined 1634 food samples including 641 samples of dairy products and 993 samples from meat products and reported that 109 samples (17%) of dairy products, 100 samples (10%) of meat products were contaminated with *staphylococcus aureus*. We found that from a total of 69 isolates of *S. aureus* confirmed by PCR method, 20 isolates (28.99%) contained methicillin resistant gene (*Mec-A*) (Table 1).

Pereira et al. (2009) assessed 147 coagulase-positive *S. aureus* strains isolated by culture method using PCR method based on 16 s recombinant deoxyribonucleic acid (rDNA) and *nuc* genes. They reported 100% agreement rate between culture and PCR methods. Mahony et al. (2005) used *mecA* gene and disc agar diffusion method to determine the prevalence of infection with MRSA strains in domesticated animals and the people dealing with veterinarian careers infected with MRSA strains in
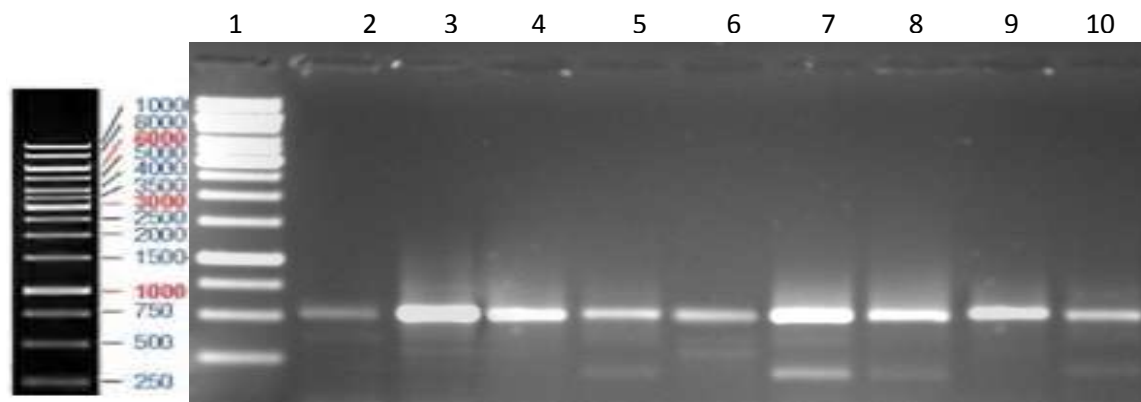
Figure 2. Agarose gel electrophoresis analysis for the *mec-A* gene in *Staphylococcus aureus* isolates. Lane 1, Molecular size markers; lane 2, Methicillin- Resistant *S. aureus* (ATCC 43300, positive control); lanes 4, 5, 6, 7, 8, 9 1nd 10 *S. aureus* isolates. Arrow indicates the 533 bp amplicon. Presence of the 533 bp shows the *mec-A* gene existence in the isolates.

Table 2. Antimicrobial susceptibility of S*. aureus* isolates.

Antibiotic	No (%) of coagulase-positive *S. aureus* isolates (n = 69)		
	S (%)	I (%)	R (%)
Methicillin	38	8	54
Ciprofloxacin	75	35	0
Erythromycin	12	12	76
Tetracycline	4	73	23
Gentamicin	100	0	0
Sulfamethoxazole/trimethoprim	85	12	3
Vancomycin	89	4	7
Rifampin	12	53	35
Penicillin	0	0	100
Cephalothin	8	12	80
Ceftazidime	88	12	0
Cloxacillin	0	0	100

S, Sensitive; I, Intermediate; R, resistant.

Ireland. They reported that 25 animals including 14 dogs, 8 horses, one cat, one rabit, and one seal, as well as 10 people working in veterinary career were infected with MRSA strains. Neeling et al. (2007) who studied on 540 pigs in 9 slaughterhouses in Holland referred to a high prevalence (39 %) MRSA strains in pigs. The results of the present study indicated that from a total of 69 isolates confirmed in PCR, 17 isolates showed simultaneous resistance against 4 antibiotics, 18 against 5 antibiotics, 8 against 6 antibiotics, 8 against 7 antibiotics, 7 against 8 antibiotics, 6 against 9 antibiotics, 2 against 10 antibiotics and 3 isolates against 11 antibiotics. In other words 100% of the isolates showed simultaneous resistance to more than 3 antibiotics (Table 2).

Food is an important source for the transfer of antibiotic resistance. Such transfer can occur by means of anti-biotic residues in food, through the transfer of resistant food-borne pathogens, or through the ingestion of resistant strains of the original food microflora and resis-tance transfer to pathogenic microorganisms (Pereira et al., 2009; Pesavento et al., 2007). *S. aureus* strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within microabscesses, which limit the action of drugs (Pereira et al., 2009; Gundogan et al., 2006). According to CLSI (2007), it was possible to conclude that 38% of the strains were potentially MRSA (MIC 6 mg/ml for oxacillin). They also reported that these results were significantly different from those obtained by PCR, in which the gene mecA was detected in only 16.95% of the isolates (CLSI, 2007). According to the report from CFSPH (2006), the definition

of MRSA can be overestimated when determining the methicillin resistance. Despite the standardized recommendations for the susceptibility testing of MRSA, Lee et al. (2004) demonstrated that some of the isolates that did not carry *mecA* gene were considered phenotypically resistant to oxacillin (MRSA) and that the phenotypic expression of resistance can vary depending on the cellular growth conditions.

There have been divergent reports of the frequency of *mecA* gene among *S. aureus* strains in Iran and the other parts of the world. This difference can be attributed to differential distribution of the mentioned gene in different places or to the method of determining them. However, the common theme of all reports is the large distribution of *mecA* gene in the world. In an Italian survey of 1634 foodstuff MRSA strains were isolated from 6 bovine milk and cheese (0.37%) (Normanno et al., 2007). Kitai et al. (2005) isolated two MRSA strains (0.45%) from 444 raw chicken meat products sampled in supermarkets in Japan. A study in Korea, including 930 slaughterhouse and retail meat samples showed the presence of MRSA in two chicken meat samples (0.22%) but not in any pork or beef sample (Kwon et al., 2006). Van Loo et al. (2007) found 2 (2.53%) MRSA strains in 79 samples of raw pork and beef. In another research, Zamani et al. (2007) showed that out of a total of 70, *Staphylococcus aureus* isolates obtained from the patients who consulted with the clinical centers of Hamedan Medical Science University and a private laboratory in Iran, 50% of the strains (35 cases) in PCR method, and 31.4% (22 cases) in antibiotic resistance patterns with disc agar diffusion method were resistant to methicillin.

Nafisi et al. (2008) researched on 52 isolates of coagulase positive *S. aureus* among 204 clinical staff workers of the different departments of Hajar Educational Institute, a subpart of Shahre Kord Medical Science University, using Agar Screen and Duplex PCR methods and reported that phenotypically 23 cases (44%) of the isolates, and genotypically (mecA) 27 cases of the isolates (52 %) are resistant to methicillin. The results of this study show a high presence of *S. aureus* resistant to a variety of antibiotics, most importantly methicillin resistant *S. aureus* in the raw milk and ice cream. Therefore, food handlers should take appropriate measures to prevent the spread of MRSA by contaminated raw foods and to prevent the occurrence, growth and survival of MRSA in prepared food. Better knowledge on transmission routes of MRSA in the food chain to provide the tools for preventing the spread of MRSA is highly needed and a proper risk assessment should be conducted to further clarify the possible health hazard for consumers related to the presence of MRSA in foods.

ACKNOWLEDGEMENT

REFERENCES

Akineden O, Ahmed HA, Schneider E, Usleber E (2008). Enterotoxigenic properties of *Staphylococcus aureus* isolated from goats' milk cheese. Int. J. Food. Microbiol. 124:211-216.

Aragon-Alegro LC, Konta EM, Suzuki K, Silva MG, Júnior AF, Rall R, Mores RVL (2007). Occurrence of coagulase-positive *Staphylococcus* in various food products commercialized in Botucatu, SP, Brazil and detection of toxins from food and isolated strains. Food Control 18:630-634.

CFSPH Center of Food Security and Public Health (2006). Methicillin Resistant *Staphylococcus aureus*. Institute for International Cooperation in Animal Biologics.www.cfsph.iastate.edu.

CLSI Clinical and Laboratory Standards Institute (2007). Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI document M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.

Cremonesi P, Luzzana M, Brasca M, Morandi S, Lodi R, Vimercati C, Agnellini D, Caramenti G, Moroni P, Castiglioni B (2005). Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. Mol. Cell. Probes 19:299-305.

Dastmalchi H, Ahmadi M, Mardani K, Batavani RA (2009). Molecular typing of *Staphylococcus aureus* isdated from bovine mastitis based on polymorphism of the coagulase gene in the north west of Iran. Vet. Microbiol. 137:202-206.

Gundogan N, Citak S, Turan E (2006). Slime production, DNAse activity and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk, pasteurised milk and ice cream samples. Food Control 17:389-392.

Japoni A, Alborzi A, Rasouli M, Pourabbas B (2004). Modification DNA extraction for rapid PCR detection of methicillin-resistant Staphylococci. Iran. Biomed. J. 8(3):161-165.

Kim CH, Khant M, Morin DE, Hurleys WL, Tripathy DN, Kehrli M, Oluocht AO, Kakomat I 92001). Optimization of the PCR for detection of *Staphylococcus aureus* nuc gene in bovine milk. J. Dairy Sci. 84:74-83.

Kitai S, Shimizu A, Kawano J, Sato E, Nakano C, Uji T, Kitagawa H (2005). Characterization of methicillin-resistant *Staphylococcus aureus* isolated from retail raw chicken meat in Japan. J. Vet. Sci. 67:107-110.

Kwon N, Park K, Jung W, Youn H, Lee Y, Kim S, Bae W, Lim J, Kim J, Kim J, Hong S, Park Y 2006. Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet. Microbiol. 117:304-312.

Lee JH, Jeong J, Park Y, Choi S, Kim Y, Chae J, Moon J, Park H, Kim S, Eo S (2004). Evaluation of the methicillin-resistant *Staphylococcus aureus* (MRSA)- screen latex agglutination test for detection of MRSA of animal origin. J. Clin. Microbiol. 42:2780-2782.

Mahony RO, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Fanning S, Rossney AS (2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Vet. Microbiol. 109:285-296.

Merlino J, Watson J, Rose B, Beard-Pegler M, Gottlieb T, Bradbury R, Harbour C (2002). Detection and expression of methicillin/oxacillin resistance in multidrug-resistant and non-multidrug-resistant *Staphylococcus aureus* in Central Sydney, Australia. J. Antimicrob. Chemother. 49(5):793-801.

Miriam OI, Rosalia F (2004). Microbiological quality of Port Salute Argentina cheese stored at two temprature treatments, Lebensm. Wiss. Technol. 37:739-748.

Morandi S, Brasca M, Lodi R, Cremonesi P, Castiglioni B (2007). Detection of classical enterotoxins and identification of enterotoxin genes in *Staphylococcus aureus* from milk and dairy products. Vet. Microbiol. 124:66-72.

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 Shahre-kord. A. M.U.J. 11:94-100.

Neeling AJ, Broek MJM, Spalburg EC, Santen-Verheuvel MG, Dam-

Deisz WDC, Boshuizen HC, Giessen AW, Duijkeren E, Huijsdens XW (2007). High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. Vet. Microbiol. 122:366-372.

Normanno G, La Salandra G, Dambrosio A, Quaglia NC, Corrente M, Parisi A, Santagada G, Firinu A, Crisetti E, Celano GV (2007). Occurrence, characterization and antimicrobial resistance of enterotoxigenic *Staphylococcus aureus* isolated from meat and dairy products. Int. J. Food Microbiol. 115:290-296.

O'Brien M, Hunt K, McSweeney S, Jordan K (2009). Occurrence of foodborne pathogens in Irish farmhouse cheese. Food Microbiol. 26: 910-914.

Peles F, Wagner M, Varga L, Hein I, Rieck P, Gutser K, Keresztúri P, Kardos G, Turcsányi I, Béri B, Szabo A (2007). Characterization of *Staphylococcus aureus* strains isolated from bovine milk in Hungary. Int. J. Food Microbiol. 118:186-193.

Pereira V, Lopes C, Castro A, Silva J, Gibbs P, Teixeira P (2009). characterization for enterotoxin production. Virulence factors, and antibiotic susceptibility of *staphylococcus aureus* isolates from various foods in Portugal. Food Microbiol. 26:278-282.

Pesavento G, Ducci B, Comodo N, Nostro AL (2007). Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw meat: a research for methicillin resistant *Staphylococcus aureus* (MRSA). Food Control 18:196-200.

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. Vet. Microbiol. 101:101-107.

Scott WJ, Van Duijkeren E (2010). Methicillin-resistant *Staphylococcus aureus* and *Staphylococcus pseudintermedius* in veterinary medicine. Vet. Microbiol. 140(3-4):418-429.

Tamagnini LM, Sousa GB, Gonzalez RD, Bude CE (2006). Microbiological characteristics of Crottin goat cheese made in different seasons. Small Rumin. Res. 66:175-180.

Van Loo, IHM, Diederen BMW, Savelkoul PHM,Woudenberg JHC, Roosendaal R, van Belkum A, Lemmens-den Toom, N, Verhulst C, van Keulen PHJ, Kluytmans JAJW (2007). Methicillin-resistant *Staphylococcus aureus* in meat products, the Netherlands. Emerg. Infect. Dis. 13:1753-1755.

Viktoria A, Alexandra M, Christian R (2001). Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham-a comparison of classical culturing detection and RFLP-PCR. Int. J. Food Microbiol. 68:105-113.

Yu-Cheng CH, Wan-Wen L, Chin-Ming F, Wan-Yu P, Chien-Shun CH, Hau-Yang T (2008). PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. Int. J. Food Microbiol. 121:66-73.

Zamani AR, Sadaghian S, Najafiemosleh M, Goudarzi MT, Yoysefi-Moshouf R, Ghaderkhani J (2007). Detection of methicillin-resistance (mec-A) gene in *Staphylococcus aureus* strains by PCR and determination of antibiotic susceptibility. Ann. Microbiol. 57:273-276.