Ann Microbiol (2010) 60:189–196
DOI 10.1007/s13213-010-0025-4

ORIGINAL ARTICLE

Occurrence and molecular characterization of *Staphylococcus aureus* strains isolated from meat and dairy products by PCR-RFLP

Mohammad Mehdi Soltan Dallal · Zohreh Salehipour · Saeed Eshraghi · Jalil Fallah Mehrabadi · Ronak Bakhtiari

Received: 21 August 2009 / Accepted: 13 January 2010 / Published online: 14 April 2010

Abstract A total of 100 *Staphylococcus aureus* strains were isolated from 1,047 food samples. In addition to biotyping all isolates, the occurrence of the *tst* gene and protein were evaluated by PCR and the RPLA test, respectively. Moreover, polymorphism of the X-region of the protein A gene was analyzed by PCR-RFLP. $TSST_1$ was detected in 12 strains and production of $TSST_1$ in all strains was confirmed by RPLA assay. It was noteworthy that 66.7% of $TSST_1$-producing *S. aureus* strains belonged to the human biotype. The result of genotyping amplification of the *spa* gene displayed size polymorphisms and revealed seven different clusters ranging from 1,200 bp to 1,600 bp. Digestion of amplicons with *Hind*III and *Hae*II resulted in six distinct patterns for each of them. An amplicon of 1,450 bp in size was the predominant type of Spa-PCR product. The accuracy of the Spa typing system by PCR-RFLP was 100%, thus confirming this method as a reliable tool in routine epidemiological investigation for *S. aureus*.

Keywords *Staphylococcus aureus* · Food · Molecular typing · *tsst*$_1$ · Biotyping

M. M. Soltan Dallal (✉) · Z. Salehipour · S. Eshraghi · R. Bakhtiari
Microbiology Division, Pathobiology Department,
School of Public Health and Institute of Public Health Research,
Tehran University of Medical Science,
P.O. Box 6446-14155, Tehran, Iran
e-mail: soltanda@tums.ac.ir

J. Fallah Mehrabadi
MARS Bioinformatics Institute,
Tehran, Iran

Introduction

Foodborne illness is a serious public health problem, and is associated with reduced economic growth. *Staphylococcus aureus* is the second or third most important cause of these illnesses throughout the world (Normanno et al. 2005). *S. aureus* strains produce several toxins and have virulence factors that are responsible for pathogenicity. Enterotoxins and toxic shock syndrome toxin 1 ($TSST_1$) are members of the pyrogenic toxin superantigen (PTSAg) family. SAgs act through major histocompatibility complex (MHC) class II molecules and the specific V_B chain of the T cell receptor (TCR), which leads to extensive T cell proliferation and release of proinflammatory cytokines (Normanno et al. 2007). $TSST_1$ is the causative agent in the severe and life threatening toxic shock syndrome (TSS) in humans. Increasing rates of non-menstrual TSS, and horizontal transfer of these strains with mobile genetic elements (Hwang et al. 2007), especially in high risk people such as the elderly, young children and immunocompromised people, are among the worrying issues surrounding this toxin. Typing of *S. aureus* is an important tool in the evaluation of strain origin, infection rout, reservoir detection, surveillance of major infections, epidemiological outbreak investigation and setting up preventive strategies (Manfreda et al. 2005). During the last few years, phenotypic methods have been replaced with molecular approaches for typing of *S. aureus*. However, biotyping using the simplified system published by Devriese (1984), indicates sources of *S. aureus* strains in animal foods. These methods could complement genotyping methods (Hata et al. 2005). Numerous molecular typing methods can be used for characterization of *S. aureus* strains. Among these techniques, pulse field gel electrophoresis (PFGF) became the

gold standard, with excellent discriminatory power for genotyping of *S. aureus* strains (Strommenger et al. 2008). However, it has disadvantages, e.g., it is time-consuming, labor intensive and difficult to interpret and standardize (Hallin et al. 2007). *spa* typing based on the polymorphic X region of the protein A gene (*spa*), provides an easy, inexpensive, rapid, simple to interpret, accurate and reproducible type analysis for both local and global epidemiological studies (Wichelhaus et al. 2001). Food safety rules in the industrialized world differ from those in developing countries. Furthermore, the findings of epidemiological investigation varies from one locality to another. To date, there is no data published on the characterization of *S. aureus* strains in foods in Iran. The aims of the current study were: (1) to evaluate the prevalence of *S. aureus* in dairy and meat products in Iran; (2) to characterize the isolated strains based on production of $TSST_1$ using PCR and immunoassay methods; and (3) to perform *spa* typing of the isolated strains by determination of different patterns of the variable regions of the protein A gene based on PCR followed by restriction fragment length polymorphism (RFLP) patterns.

Materials and methods

Sampling

A total of 1,047 food samples including 455 dairy products, 458 meat products and 134 other products were purchased and were collected from July 2006 to November 2007, in Tehran, Iran (Table 1).

Identification of *Staphylococcus* isolates

Baird Parker agar containing egg-yolk tellurite emulsion (Merck, Darmstadt, Germany) was used for isolation. Isolates were identified using the following criteria: production of coagulase, DNase, catalase, mannitol fermentation, hemolytic zone on 5% sheep blood agar (Merck), VP test and Gram staining (Hata et al. 2005).

Detection of *tst* gene by PCR and immunoassay

For each strain, an overnight culture in Brain heart infusion Agar (Merck) was centrifuged and treated with 2 µl lysostaphin (10 mg/ml; Sigma, St. Louis, MO) for 1 h at 37°C (Normanno et al. 2007). DNA extraction was carried out by using the QIA amp DNA mini kit (Qiagen, Hilden, Germany).

The presence of the Staphylococcal *tst* gene was assessed by PCR. The sequence of the primers used was: 5′-CATCTA CAAACGATAATATAAAGG-3′, and 5′-CATTGTTATT TTCCAATAACCACCCG-3′ forward and reverse, respectively (Fueyo et al. 2005b). PCR reactions were performed by using HotStar *Taq* plus Master Mix Kit (Qiagen). For amplification, the reaction mixture (25 µl) contained 1 µl of each primer (20 pmol/µl), 12.5 µl Hotstar *Taq* Plus master mix (DNA polymerase, $MgCl_2$,dNTP), 2.5 µl 10x Coralload buffer (Buffer, dyes and gel loading reagent), 5 µl RNase-

Table 1 Occurrence of *Staphylococcus aureus* strains in analyzed samples

Analyzed samples	Foods stuff	Samples (*n*)	Samples positive for *S. aureus*	
			(*n*)	(%)
Dairy products	Traditional ice cream French rollet	455	77	17.1
	Banana milk			
	Cream cake			
	Ice cream in cone			
	Chocolate ice cream			
	Cream bread			
Meat products	Cooked kebab Raw kebab	458	16	3.5
	Cooked chicken			
	Retail raw chicken			
	Hotdog			
	Sheep cooked liver			
	Fresh and minced meat			
Other food products	Fruit juice Garden salad	134	7	4.5
	Cooked rice			
	Cooked macaroni			
Total		1,047	100	9.5

Table 2 Classification of *Staphylococcus aureus* non-host specific (NHS) biotypes (Devriese 1984)

NHS Biotype	Numbered
K – β + CV: C	NHS1
K – β + CV: A	NHS2
K + β - CV: A	NHS3
K + β + CV: A	NHS4
K – β - CV: C	NHS5

free water and 3 µl DNA (50 ng/µl). Reactions cycles were are follows: 94°C for 3 min, followed by denaturation at 94°C for 45 s, annealing at 55°C for 30 s, then extension at 72°C for 1.5 min. After 35 cycles, a final 10 min extension at 72°C was performed. Amplicons were assessed by electrophoresis in 1% agarose gels. *Staphylococcus aureus* RN 8465 and *Staphylococcus epidermidis* ATCC 12228 were used as positive and negative controls, respectively.

The production of $TSST_1$ was determined using a commercial reverse passive latex agglutination kit (TST-RPLA, Oxoid, Wesel, Germany) according to the manufacturer's instructions.

Biotyping

Biotyping was performed according to the simplified system described by Devriese (1984), using four tests including staphylokinase production with bovine fibrinogen (Sigma), β-haemolysis on sheep blood agar (Merck), coagulation of bovine plasma for a duration of 6 h, and crystal violet growth types on Tryptose agar (Merck). All strains were classified to host specific ecovars and non-host specific (NHS) biotypes (Table 2).

Molecular typing by *spa* gene restriction profile analysis

The X region of the *spa* gene was amplified by PCR using the primers SPA1 (5′-ATCTGGTGGCGTAACAC CTG-3′) and SPA2 (5′-CGCTGCACCTAACGCTAATG-3′; Wichelhaus et al. 2001). All PCR series included *S. aureus* RN 8465 and *S. epidermidis* ATCC 12228 as positive and negative amplification controls, respectively. Prior to RFLP, a visible amount of PCR products of the *spa* gene were separated completely on a 2% agarose gel and DNA was purified thoroughly using a DNA extraction kit (Fermentas, St. Leon-Rot , Germany) according to the manufacturer's instructions. Purified amplicons were digested with 50 U *Hind*III (Fermentas) at 37°C for 16 h and 5 U *Hae*II (Roche, Mannheim, Germany) at 37°C for 4 h, in separate reactions. All digested fragments were assessed by electrophoretic separation in 2% (w/v) agarose gels.

Typeability is defined as the number of strains that are located in a type by a typing system (Hallin et al. 2007).

Statistical analysis

Data were analyzed by means of chi-square (χ^2) test and Fisher's exact test.

Results

On the basis of the results obtained from the cultural and biochemical properties of the 1,047 food samples analyzed, 100 (9.5%) were contaminated with *S. aureus*, of which 45 isolates showed β-haemolysis, 30 α-hemolysis and 25 non-haemolysis. Sixteen (3.5%) of the 458 meat product samples, and 77 (17.1%) of the 455 dairy products were contaminated with *S. aureus*. The contamination rate of dairy products was significantly higher than that of other foods ($P<0.001$; Table 1). Of the 100 strains isolated, 12 (12%) isolates harbored the *tst* gene as shown by PCR amplification. Eight (10.4%) of the 77 dairy products and 3 (18.8%) of the 16 meat products were contaminated with $TSST_1$-producing *S. aureus* strains. Figure 1 shows the presence of the *tst* gene.

All of the *tst*-containing *S. aureus* strains detected by PCR produced $TSST_1$ by RPLA immunoassay detection. Conversely, RPLA confirmed the PCR results for all 12 strains. Of the 100 analyzed strains by the biotyping method, 29 belonged to the human ecovar, 47 to the NHS ecovar, 11 to the poultry ecovar, 9 to the bovine ecovar and 4 were not allocated (Table 3). Among the NHS strains, 8 (8%) were K-β + CV:A, 10 (10%) K + β-CV:A, 22 (22%) K + β + CV: A and 7 (7%) K-β-CV: C. Non ovine ecovar was found (Table 4). Notably, 66.7% of $TSST_1$-producing *S. aureus* strains belonged to the human biotype.

Amplification of the X region of the *spa* gene revealed a single amplicon for each isolate. The sizes of the PCR products ranged from approximately 1,200 to 1,600 bp. The

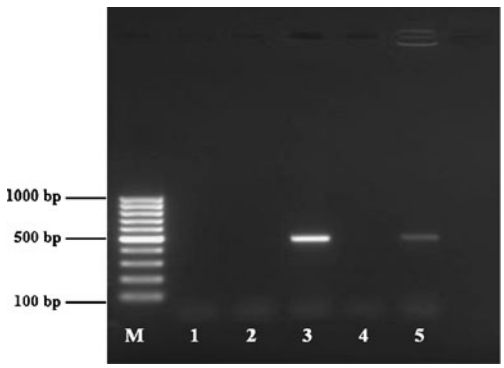

Fig. 1 PCR amplification products for the *Staphylococcus aureus tst* gene. Lanes: *M* DNA marker (100 bp ladder); *1* negative control; *2*, *4* negative products; *3* positive control *S. aureus* strain RN8465; *5* positive product

Table 3 Distribution of the *tst* gene and biotypes among the *S. aureus* strains isolated from different foods

Food	Isolates (*n*)	*tst* gene (*n*)	Biotype
Dairy products	77	8	NHS (40)
-Traditional ice cream			Human (21)
-French rollet			Poultry (6)
-cream cake			Bovine (8)
-Ice cream in cone			Not allocated (2)
-Chocolate ice cream			
-Cream bread			
Meat products	16	3	NHS (5)
-Cooked kebab			Human (7)
-Fresh and minced meat			Poultry (2)
-Raw kebab			Bovine (0)
-Cooked chicken			Not allocated (2)
-Retail raw chicken			
-Hot dog			
-Cooked sheep liver			
-Hamburger			
Other food products	7	1	NHS (2)
-Fruit juice			Human (1)
-Garden salad			Poultry (3)
-Macaroni			Bovine (1)
-Cooked rice			
Total	100	12	100

100 strains were divided into seven different clusters (A–G) with seven different sizes of approximately 1,200, 1,250, 1,300, 1,450, 1,500, 1,550 and 1,600 bp. Gene polymorphisms of the X region of protein A are shown in Fig. 2. Among the seven types, type D with an amplicon of 1,450 bp was predominant (27%) followed by type C (18%), type G (17%), type E (16%), type F (14%), type A (5%) and type B (3%). Restriction with the enzyme *Hin*dIII resulted in six distinct RFLP patterns among the 100 strains, which were classified as $HindS_1$–$HindS_6$ (Fig. 3). The results revealed that RFLP-type $HinSd_4$ predominated, with 28% isolates (Table 4). All the types obtained from PCR-RFLP with *Hin*dIII subdivide into several subtypes. Thus, 22 genotypes in total were identified (Table 5). Genotypes C_1 and D_4 were most prevalent (13%). Furthermore, the 100 isolates generated six RFLP different restriction patterns (HaeS1–HaeS6) using the restriction enzyme *Hae*II (Fig. 4). Sixteen genotypes were obtained with *Hae*II (Table 5). RFLP type $HaeS_3$ and genotype D_1 were predominant using *Hae*II digestion (31% and 21%, respectively). Typeability of *spa* typing using the PCR-RFLP system on the 100 strains analyzed was 100%, and all of the 100 strains could be typed using this method.

Discussion

Foodborne illnesses are caused by consuming contaminated foods. *Staphylococcus aureus* can grow easily in every environment and can contaminate different foods (Oh et al. 2007). In our survey, the rate of occurrence of *S. aureus*

Table 4 Biotypes of *S. aureus* isolated from foods

Food	Isolates (*n*)[a]	Biotype						
		Human	Poultry	Bovine	K-β + CV:A	K + β-CV:A	K + β + CV:A	K-B-CV:C
Dairy products	77	21	6	8	6	10	19	5
Meat products	16	7	2	0	1	0	2	2
Other products	7	1	3	1	1	0	1	0
Total	100	29 (29%)	11 (11%)	9 (9%)	8 (8%)	10 (10%)	22 (22%)	7 (7%)

[a] Four strains were not allocated

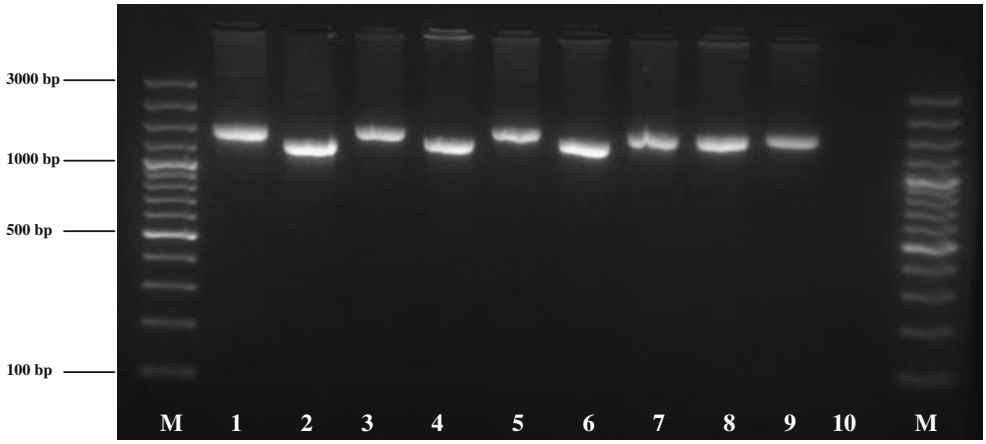

Fig. 2 Polymorphisms of the gene encoding the X region of protein A in *S. aureus* strains isolated from foods. Lanes: *M* 100 bp plus DNA marker, *1* positive control *S. aureus* COL strain, *2–9* PCR products, *10* negative control *S. epidermidis* ATCC12228

in the foods analyzed was 9.5%, with a significant higher rate in dairy products (17.1%) vs meat product (3%). In comparison with other studies throughout the world, the presence of *S. aureus* is lower than on meat and dairy products in Italy (12.5%; Normanno et al. 2007), on bovine and caprine bulk milk and in raw milk products (17%; Jorgensen et al. 2005), and on raw and cooked meat products (57.1%; Atanassova et al. 2001). Nevertheless, in their study on ready-to-eat food in Korea, Oh et al. (2007) reported that 8.6% of samples were contaminated by *S. aureus*. Thus, the higher contamination rate of dairy products in our study is agreement with some other reports.

We found also the presence of $TSST_1$-producing *S. aureus* in foods. The results showed that 12% of strains were carriers of the *tst* gene. Jung et al. (2005) suggested a need for $TSST_1$ testing in food following the isolation of *S. aureus* from handled foods. The present study found a good correlation between RPLA and PCR results for detection of $TSST_1$-producing *S. aureus*. However, PCR detects only the presence of the genes, whereas monitoring production of $TSST_1$ is dependent on immunoassay methods (Zschock et al. 2000). The prevalence of $TSST_1$ by *S. aureus* strain (12%) was lower than that associated with foods and bovine mastitis (42.2, 15.5, 27.1, 13.5, 58.4, and 34.8%) reported in previous studies by Chapaval et al. (2006), Akineden et al. (2001), Adesiyun et al. (1992), Oh et al. (2007), Hata et al. (2005) and Nagase et al. (2002), respectively. However, in some other reports this rate was lower than in the present study (Fueyo et al. 2005a; El-Ghodban et al. 2006).

Numerous typing techniques to characterize *S. aureus* strains include genotypic and phenotypic methods (Wu and Della-Latta 2002). We chose the biotyping technique established by Devriese (1984) as a conventional typing method. This simplified biotyping system has been useful in tracing the origin of *S. aureus* strains in animal feed and in the food industry (Kitai et al. 2005). In the present study, of the 100 strains biotyped, 47% belonged to the NHS biotype, followed by 29% to human, 11% to poultry and 9% to bovine biotypes. In dairy products the NHS biotype was predominant (42%), while in meat products the human biotype had a higher rate (43.8%). In our study, the human biotype was more prevalent in comparison with some other studies on biotyping of *S. aureus* strains recovered from meat products (Capita et al. 2002; Rodríguez-Calleja et al. 2006). This supports a positive correlation between human biotype strains and handled meat foods and suggests that human contamination of meat products can be primary source

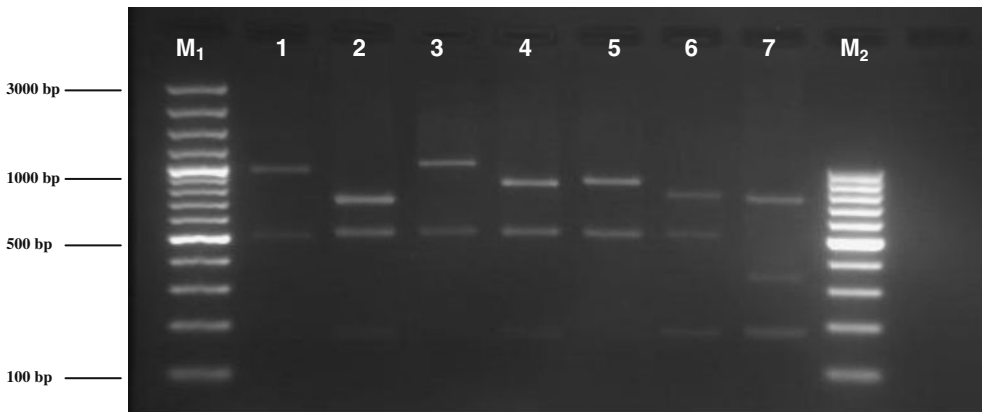

Fig. 3 PCR amplicons of the X region of protein A were digested with *Hin*dIII. Lanes: M_1 100 bp plus DNA marker; *1 S. aureus* COL strain; *2–7* different restriction patterns; M_2 100 bp DNA marker

Table 5 Occurrence of *S. aureus* types and subtypes based on the X region of the protein A gene

Spa-PCR type	RFLP Pattern (*Hin*dIII)	Genotypes	RFLP Pattern (*Hae*II)	Genotypes
A (1,200 bp)	HindS1 (26%)	A_1 (5%)	$HaeS_1$ (8%)	A_1 (4%)
B (1,250 bp)	HindS2 (22%)	B_1 (3%)	$HaeS_2$ (30%)	A_2 (1%)
C (1,300 bp)	HindS3 (17%)	C_1 (13%)	$HaeS_3$ (31%)	B_1 (3%)
D (1,450 bp)	HindS4 (28%)	C_2 (2%)	$HaeS_4$ (14%)	C_1 (8%)
E (1,500 bp)	HindS5 (4%)	C_3 (3%)	$HaeS_5$ (6%)	C_2 (1%)
F (1,550 bp)	HindS6 (3%)	D_1 (3%)	$HaeS_6$ (11%)	C_3 (4%)
G (1,600 bp)		D_2 (7%)		C_4 (5%)
		D_3 (1%)		D_1 (21%)
		D_4 (13%)		D_2 (6%)
		D_5 (3%)		E_1 (5%)
		E_1 (1%)		E_2 (11%)
		E_2 (6%)		F_1 (3%)
		E_3 (2%)		F_2 (9%)
		E_4 (6%)		F_3 (2%)
		E_5 (1%)		G_1 (5%)
		F_1 (5%)		G_2 (12%)
		F_2 (5%)		
		F_3 (4%)		
		G_1 (1%)		
		G_2 (4%)		
		G_3 (9%)		
		G_4 (3%)		
Total	100	100	100	100

of staphylococcal food poisoning, especially considering that 66.7% of $TSST_1$-producing *S. aureus* strains also belonged to the human biotype. Casciano et al. (2003) found that, in biotyping of *S. aureus* strains from raw milk, NHS biotype is the predominant type (66.6%). These results confirm our findings. In two studies carried out on the characterization of *S. aureus* strains isolated from meat and dairy product, in total, the human biotype occurred more frequently than other biotypes (Kérouanton et al. 2007; Normanno et al. 2007). These findings are in disagreement with the present study. However, strains that are NHS and human biotype may pose a potential risk for public health (Capita et al. 2002).

From among the available molecular typing methods for *S. aureus* strains, we performed spa typing by using PCR-RFLP. The polymorphic X region of the protein A (*spa*) contains a variable number of repeats of a 24-bp region that provides a suitable tool for epidemiological investigation (Hallin et al. 2007). In our study, amplification of the *spa* gene yielded a single amplicon with seven different sizes ranging from approximately 1,200 to 1,600 bp. An amplicon with a size of 1,450 bp was the most prevalent. Many reports using *spa* typing of *S. aureus* strains isolated from food as a genotyping method have been published (Annemüller et al. 1999; Jakubczak et al. 2007). Annemüller

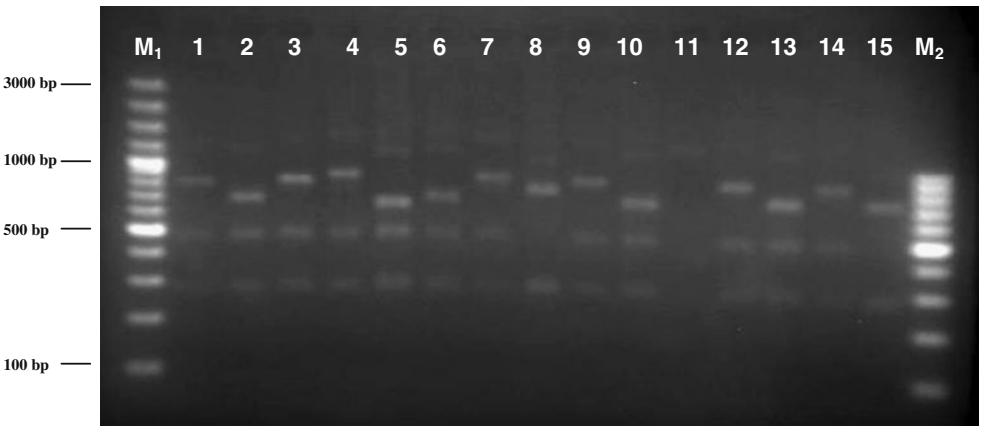

Fig. 4 PCR amplicons of the *spa* gene were digested with *Hae*II. Lane M_1 100 bp plus DNA marker; Lane M_2, 100 bp DNA ladder; Lane *1*, *S. aureus* COL strain; Lane *11*, negative control; Lanes *2–10* and *12–15*, different restriction patterns

et al. (1999) reported that spa typing results corresponded well to DNA fingerprinting patterns. Six different RFLP patterns ($HindS_1$–$HindS_6$) were observed among the 100 strains analyzed here after *Hin*dIII digestion. These strains were analyzed further with the restriction enzyme *Hae*II, yielding six distinct patterns. Unlike the *coa* gene, which has commonly been used for typing, no reports are available on spa typing of *S. aureus* strains isolated from food by PCR-RFLP. Only one study has been carried out on this subject in *S. aureus* strains isolated from bovine mastitis milk in Germany (Strommenger et al. 2008). Wichelhaus et al. (2001) identified PCR-RFLP as a new typing system for *S. aureus* with a discriminatory power corresponding effectively with PFGE. This method represents a powerful, rapid, reliable, easy and inexpensive system for epidemiological surveillance (Hallin et al. 2007). In our study, typeability using the PCR-RFLP system was 100%. We subdivided the types obtained from each digestion into several subtypes (Table 5). These types aimed for genotype definition similar to that already carried out for coagulase genotypes (Katsuda et al. 2005). Finally, 22 genotypes with *Hin*dIII and 16 genotypes with *Hae*II digestion were obtained.

In summary, the prevalence of *S. aureus* strains in different foods in Iran is no higher than in other countries. The NHS biotype is the predominant ecovar in our strains, although the pattern in meat and dairy products is different. Type D *spa* products, $HindS_4$ of PCR-RFLP and subsequently genotypes C_1 and D_4 with *Hin*dIII digestion and types $HaeS_3$ of PCR-RFLP and genotype D_1 with *Hae*II digestion were the most prevalent, respectively. Spa typing by PCR-RFLP was a reliable system for genotyping of *S. aureus* strains with 100% typeability in our study. This paper is the first report on characterization of *S. aureus* isolates in meat and dairy products in Iran.

Acknowledgments We would like to thank S. Agha Amin for kindly helping in samples collection; A. Azimian (PhD student of microbiology, Isfahan University of Medical Science) for preparing reference strains; I. Ashrafi (Bacteriology Laboratory of the Veterinary Medicine Faculty, Tehran University of Medical Science) for kindly helping with biotyping, and M. Shokuhi (Physiology Research Centre, Kerman University of Medical Science) for helping us with data analysis.

References

Adesiyun AA, Lenz W, Schaal KP (1992) Production of Toxic Shock Syndrome Toxin-1 (TSST-1) by *Staphylococcus aureus* strains isolated from humans, animals and foods in Nigeria. Microbiologica 15:125–133

Akineden O, Annemuller C, Hassan AA, Lammler C, Wolter W, Zschock M (2001) Toxin genes and other characteristics of *Staphylococcus aureus* isolates from milk of cows with mastitis. Clin Vac Immunol 8:959–964

Annemüller C, Lammler C, Zschock M (1999) Genotyping of *Staphylococcus aureus* isolated from bovine mastitis. Vet Microbiol 69:217–224

Atanassova V, Meindl A, Ring C (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

Capita R, Alonso-Calleja C, Garcia-Fernandez MC, Moreno B (2002) Characterization of S*taphylococcus aureus* isolated from poultry meat in Spain. Poultry Sci 81:414–421

Casciano R, Alberghini L, Peccio A, Serraino A, Rosmini R (2003) Typing of *Staphylococcus aureus* isolates from raw milk. Vet Res Commun 27:289–291

Chapaval L, Moon DH, Gomes JE, Duarte FR, Tsai SM (2006) Use of PCR to detect classical enterotoxins genes (ent) and toxic shock syndrome toxin-1 gene (tst) in *Staphylococcus aureus* isolated from crude milk and determination of toxin productivities of *S. aureus* isolates harboring these genes. Arq Inst Biol Sao Paulo 73:165–169

Devriese LA (1984) A simplified system for biotyping *Staphylococcus aureus* strains isolated from different animal species. J Appl Microbiol 56:215–220

El-Ghodban A, Ghenghesh KS, Marialigeti K, Esahli H, Tawil A (2006) PCR detection of toxic shock syndrome toxin of *Staphylococcus aureus* from Tripoli. Libya. J Med Microbiol 55:179–182

Fueyo JM, Mendoza MC, Martin MC (2005a) Enterotoxins and toxic shock syndrome toxin in *Staphylococcus aureus* recovered from human nasal carriers and manually handled foods: epidemiological and genetic findings. Microbes Infect 7:187–194

Fueyo JM, Mendoza MC, Rodicio MR, Muniz J, Alvarez MA, Martin MC (2005b) Cytotoxin and pyrogenic toxin superantigen gene profiles of *Staphylococcus aureus* associated with subclinical mastitis in dairy cows and relationships with macrorestriction genomic profiles. J Clin Microbiol 43:1278–1284

Hallin M, Deplano A, Denis O, De Mendonça R, De Ryck R, Struelens MJ (2007) Validation of pulsed-field gel electrophoresis and spa typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* infections. J Clin Microbiol 45:127–133

Hata E, Katsuda K, Kobayashi H, Ogawa T, Endo T, Eguchi M (2005) Characteristics and epidemiological genotyping of *Staphylococcus aureus* isolates from bovine mastitic milk in Hokkaido, Japan. J Vet Med Sci 68:165–170

Hwang SY, Kim SH, Jang EJ, Kwon NH, Park YK, Koo HC, Jung WK, Kim JM, Park YH (2007) Novel Multiplex PCR for the detection of the *Staphylococcus aureus* superantigen and its application to raw meat isolates in Korea. Int J Food Microbiol 117:99–105

Jakubczak A, Szweda P, Lukaszewska K, Kur J (2007) Molecular typing of *Staphylococcus aureus* isolated from cows with mastitis in the east of poland on the basis of polymorphism of genes coding protein A and coagulase. Pol J Vet Sci 10:199–205

Jorgensen HJ, Mork T, Hogasen HR, Rorvik LM (2005) Enterotoxigenic *Staphylococcus aureus* in bulk milk in Norway. J Appl Microbiol 99:158–166

Katsuda K, Hata E, Kobayashi H, Kohmoto M, Kawashima K, Tsunemitsu H, Eguchi M (2005) Molecular typing of *Staphylococcus aureus* isolated from bovine mastitic milk on the basis of toxin genes and coagulase gene polymorphisms. Vet Microbiol 105:301–305

Kérouanton A, Hennekinne JA, Letertre C, Petit L, Chesneau O, Brisabois A, De Buyser ML (2007) Characterization of *Staphylococcus aureus* strains associated with food poisoning outbreaks in France. Int J Food Microbiol 115:369–375

Kitai S, Shimizu A, Kawano J, Sato E, Nakano C, Kitagawa H, Fujio K, Matsumura K, Yasuda R, Inamoto T (2005) Prevalence and characterization of *Staphylococcus aureus* and enterotoxigenic *Staphylococcus aureus* in retail raw chicken meat throughout Japan. J Vet Med Sci 67:269–274

Jung HJ, Cho JI, Park SH, Ha SD, Lee KH, Kim CH, Song ES, Chung DH, Kim MG, Kim KY, Kim KS (2005) Genotypic and phenotypic characteristics of *Staphylococcus aureus* Isolates from lettuces and raw milk. Korean J Food Sci Technol 37:134–141

Manfreda G, Mioni R, Cesare A (2005) Surveillance and Characterization of enterotoxigenic staphylococci in foods of animal origin collected in the Veneto Region. Vet Res Comm 29:331–333

Nagase N, Shimizu A, Kawano J, Yamashita K, Yoshimura H, Ishimaru M, Kojima A (2002) Characterization of *Staphylococcus aureus* strains isolated from bovine mastitis in Japan. J Vet Med Sci 64:1169–1172

Normanno G, Firinu A, Virgilio S, Mula G, Dambrosio A, Poggiu A, Decastelli L, Mioni R, Scuota S, Bolzoni G (2005) Coagulase-positive staphylococci and *Staphylococcus aureus* in food products marketed in Italy. Int J Food Microbiol 98:73–79

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

Oh SUK, Lee N, Cho Y, Shin D, Choi SY, Koo M (2007) Occurrence of toxigenic *Staphylococcus aureus* in ready-to-eat food in Korea. J Food Protect 70:1153–1158

Rodríguez-Calleja JM, García-López I, Santos JA, Otero A, García-Lopez M-L (2006) Molecular and phenotypic typing of *Staphylococcus aureus* isolates from rabbit meat. Res Microbiol 157:496–502

Strommenger B, Braulke C, Heuck D, Schmidt C, Pasemann B, Nübel U, Witte W (2008) spa typing of *Staphylococcus aureus* as a frontline tool in epidemiological typing. J Clin Microbiol 46:574–581

Wichelhaus TA, Hunfeld KP, Boddinghaus B, Kraiczy P, Schafer V, Brade V (2001) Rapid molecular typing of methicillin resistant *Staphylococcus aureus* by PCR-RFLP. Infect Cont Hospital Epidemiol 22:294–298

Wu F, Della-Latta P (2002) Molecular typing strategies. Semin Perinatol 26:357–366

Zschock M, Botzler D, Blocher S, Sommerhouser J, Hamann HP (2000) Detection of genes for enterotoxins (ent) and toxic shock syndrome toxin-1 (tst) in mammary isolates of *Staphylococcus aureus* by polymerase-chain-reaction. Int Dairy J 10:569–574