ORIGINAL ARTICLE

Molecular typing of *Staphylococcus aureus* of different origins based on the polymorphism of the *spa* gene: characterization of a novel *spa* type

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Abstract

The present study was conducted to determine the molecular diversity of *Staphylococcus aureus* strains isolated from human, bovine and food samples based on the polymorphism of the *spa* gene. A total of 208 *S. aureus* isolated from human, bovine raw milk and food samples were assessed using polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) and single locus sequence typing (SLST) methods, followed by determination of *spa* types using Ridom SpaServer. Altogether, 15 distinct RFLP patterns were recorded (I–XV). The highest heterogeneity was observed among *S. aureus* isolated from humans, whereas most of bovine and food *S. aureus* isolates indicated certain RFLP patterns. Although most of the isolates from patients showed RFLP pattern I, none of the *S. aureus* isolated from carriers had this *spa* pattern. Besides, the results of SLST led to the characterization of 16 *spa* types, and one of them was a novel *spa* type which has been registered in Ridom SpaServer for the first time and designated as type t16929. Determination of a high number of shared RFLP patterns between human and food *S. aureus* isolates indicated possible transmission of *S. aureus* and the source of food contamination. Thus, effective hygiene measures should be taken to break transmission routes. However, it seems that *S. aureus* isolated from patients, carriers and bovine should be considered in a different way, since some isolates had similar patterns, while the others showed their own specific pattern.

Keywords *Staphylococcus aureus* · Typing · PCR–RFLP · *spa* gene

Introduction

Although *Staphylococcus aureus* is present on the skin and mucous membrane of warm-blooded animals, it may cause multiple diseases in these hosts (Walther et al. 2008; Persoons et al. 2009). In humans, *S. aureus* is also an important food-borne pathogen and it is a major causative agent of hospital- and community-associated infections (Wertheim et al. 2004; Lee 2003). Animal infections may result in huge economic losses, in particular, in the case of industrial animal farms (Abebe et al. 2016; Tesfaye et al. 2010).

Hence, typing of *S. aureus* isolates can be helpful in prevention and control of *S. aureus* infections, as well as investigation of possible sources of infections. Several phenotypic and genotypic methods are available for this purpose. In the last few years, a variety of molecular methods including pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) have been used for discrimination of *S. aureus* isolates (Laplana et al. 2007; Larsen et al. 2012). However, these methods are time consuming, not widely available and expensive (Stranden et al. 2003). PCR-based typing methods such as polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) possess a high degree of reproducibility, good discriminatory power and comparable results (Acosta et al. 2014; Omar et al. 2014; Mehndiratta et al. 2009). One of these approaches is RFLP analysis of the *spa* gene which contains polymorphic repeat regions (21 bp) and can be targeted to type *S. aureus* strains (Mehndiratta et al. 2009).

In fact, among various molecular typing methods, *spa* typing (single locus sequence typing: SLST) has been shown to be the most effective and rapid method to differentiate *S. aureus* isolates. This method is based on the sequence

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variation and the number of tandem repeats in the region X of the *spa* gene (Frenay et al. 1996; Sabat et al. 2013). This region is highly polymorphic and consists of up to 12 tandem repeats with a length of 21–27 nucleotides. The result of *spa* typing is usually in good concordance with the result of PFGE and it has been shown that its discriminatory power is similar to PFGE. However, it is also suggested that simultaneous utilization of both methods may have better results (Hallin et al. 2009).

The aim of the present study was to investigate the molecular diversity and compare the sources of contamination and distribution of *S. aureus* isolated from different sources based on the PCR–RFLP and SLST of the *spa* gene.

Materials and methods

Bacterial isolates

A total of 208 *S. aureus* isolates which had been previously isolated from bovine raw milk samples (83 isolates), humans (40 isolates from patients, 20 isolates from healthy carriers) and food samples (45 isolates from pastries, 20 isolates from cheese) were included in the present study.

DNA extraction

DNA extraction was carried out from 10 ml of overnight cultures of the isolates in tryptic soy broth (TSB, Merck Germany) using phenol–chloroform method as previously described (Wilson 1987). All of these isolates had also been genetically confirmed to be *S. aureus* using a species-specific PCR which targeted the *fem*A gene (data are not shown).

PCR amplification of the *spa* gene

Amplification of the *spa* gene was done using previously introduced primers (Wichelhaus et al. 2001). The sequences of the primers were as follows: SPA1 (5′-ATCTGGTGGCGT AACACCTG-3′) and SPA2 (5′-CGCTGCACCTAACGC TAATG-3′). PCR reactions (50 µl) contained 30 ng of the extracted DNAs, 5 µl of 10 × PCR buffer (500 mM, KCl and Tris HCl, pH 8.4), 5 µl of 25 mM $MgCl_2$, 200 mM of dNTPs, 1 U of Taq DNA polymerase (all materials were prepared from SinaClon, Iran), and 50 pmol of each primer (BioNeer, Korea). PCR amplifications were performed under the following conditions: initial denaturation at 94 °C for 5 min, followed by denaturation at 94 °C for 45 s, annealing at 55 °C for 90 s and extension at 72 °C for 90 s (32 cycles) and a final extension at 72 °C for 10 min. The PCR products were analyzed using electrophoresis on 1% agarose gel (SinaClon, Iran) containing ethidium bromide (0.5 µg/ml). *S. aureus* ATCC 25923 and *S. epidermidis* ATCC 13499

were used as positive and negative controls of the *spa* gene, respectively. Prior to sequencing of the amplified DNA fragments and RFLP analysis, the PCR products were purified using a commercial gel purification kit (Vivantis, Malaysia) according to the manufacturer's instructions.

Restriction fragment length polymorphism (RFLP)

The PCR products of the *spa* gene were digested using two restriction enzymes, *Hin*dIII (Jena Bioscience, Germany) and *Hin*fI (Jena Bioscience, Germany). *Hin*dIII and *Hin*fI enzymes are type II site-specific deoxyribonuclease restriction enzymes which identify A′AGCTT and G′ANTC sequences, respectively, and cleave them from the specified position (Tang et al. 2000). Briefly, 10.5 µl of distilled water, 2.5 µl of 10 × enzyme buffer, and 7 µl of each of the PCR products were mixed with 2 U of each restriction enzyme and the mixtures were incubated at 37 °C for 1 h. Finally, the digested PCR products were analyzed using electrophoresis on 2% agarose gel containing ethidium bromide (Dallal et al. 2010).

Single locus sequence typing of the *spa* gene (*spa* SLST)

To investigate the diversity among the isolates, *spa* amplicons of each size (representative fragments of the determined RFLP patterns plus one indigestible DNA fragment) were submitted for sequencing (BioNeer, Korea). In the following, the resulting amplicon sequences were analyzed by DNASTAR software (version 14.1) to confirm the results of PCR–RFLP and to determine the *spa* types (Harmsen et al. 2003).

Statistical analysis

The relationships among *S. aureus* strains isolated from human, bovine and food samples were compared based on the *spa*-RFLP patterns using Chi square test and SPSS software (version 16), and $P < 0.05$ was considered to be statistically significant. Also, the numerical index for the discriminatory power of the *spa*-RFLP method was calculated using Simpson's index of diversity equation as follows:

$$D = \frac{1}{N(N-1)} \sum_{j=1}^{s} nj(nj-1),$$

where N is the total number of isolates, S the total number of types, and nj the number of strains belonging to the *j*th type (Hunter and Gaston 1998).

Results

PCR of the *spa* gene

In human isolates, PCR amplification of the *spa* gene was successfully performed for all of the isolates and resulted in the production of 7 DNA fragments with the sizes ranging from ~ 725 to 1450 bp. The most frequent PCR product among human *S. aureus* isolates (31.7%) was a DNA band of about 1170 bp (Fig. 1a).

Amplification of the *spa* gene in bovine *S. aureus* isolates resulted in the formation of three different DNA bands with the sizes of ~ 1200 to 1350 bp. However, two isolates were not positive in this PCR. A PCR product of 1350 bp was the most frequently observed DNA band in bovine *S. aureus* isolates (47%). Moreover, six isolates showed double bands, whereas the rest indicated a single DNA band (Fig. 1b).

As depicted in Fig. 1c, amplification of the *spa* gene among food *S. aureus* isolates showed that 98.5% (64 isolates) of them were positive for the *spa* gene and the PCR assay yielded DNA bands with molecular sizes of ~ 1250, 1300, and 1450 bp. An amplicon of ~ 1450 bp was the mostly observed DNA fragment for these isolates (59.5%).

RFLP

As presented in Table 1, digestion of the purified PCR products with *Hin*fI restriction enzyme yielded 8 distinguishable electrophoretic patterns, whereas 11 patterns were characterized when the same products were digested with *Hin*dIII

(Figs. 2, 3). However, both enzymes did not digest a DNA fragment of 725 bp. It should be noted that the presence of two DNA bands after digestion of the PCR products with *Hin*fI resulted from the creation of DNA fragments of the same sizes.

Altogether, the isolates were classified into 15 *spa*-RFLP patterns designated as I–XV. The highest heterogeneity was observed among *S. aureus* isolated from humans (especially patients), as they showed ten RFLP patterns; whereas bovine and food *S. aureus* isolates indicated much lower heterogeneity. The results of statistical analysis showed that distribution of *S. aureus* strains with certain *spa*-RFLP patterns was different in *S. aureus* isolated from each source. While *spa*-RFLP patterns V and XI were the most frequently observed patterns for bovine *S. aureus* isolates, the majority of pastry and cheese *S. aureus* isolates showed patterns XII and XIII and this distribution was statistically significant ($P < 0.05$). However, such concurrent distribution was not detected for human *S. aureus* isolates ($P > 0.05$).

The most prevalent patterns in human, bovine, pastry and cheese *S. aureus* isolates were patterns I, XI, XII and XIII, respectively. Moreover, RFLP patterns I, XII and XIII were common between human and food isolates, while pattern XI was shared between bovine and human isolates.

spa SLST

The sequences of the *spa* gene were analyzed using DNASTAR and the created restriction maps of *Hin*dIII and *Hin*fI enzymes confirmed the results obtained from *spa*-PCR–RFLP. As summarized in Table 2, sequencing of 16
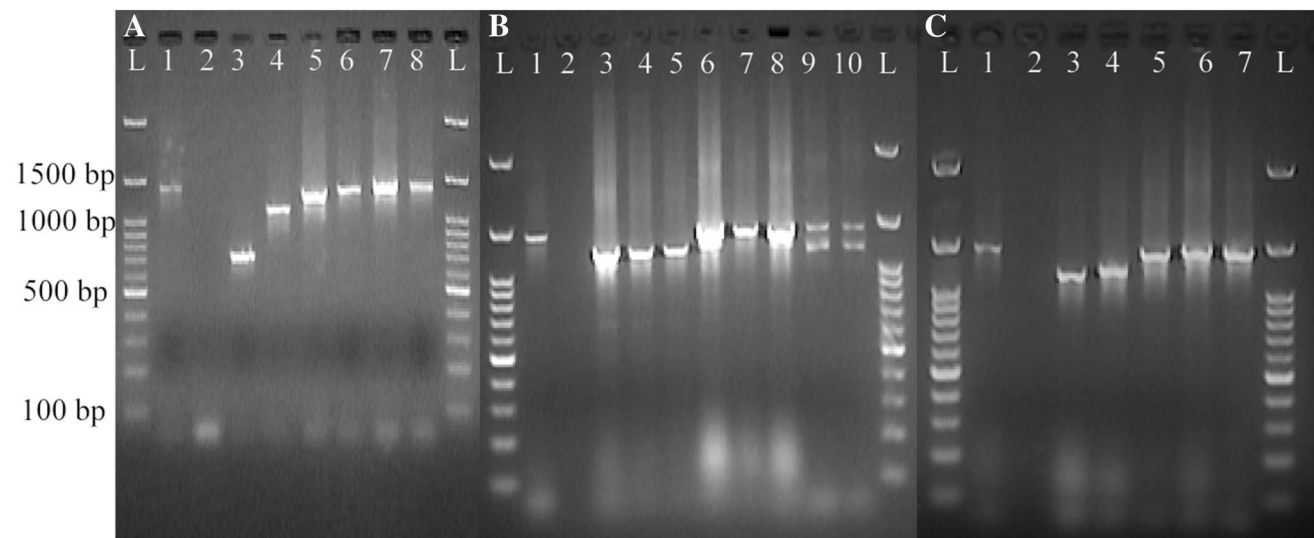

Fig. 1 The PCR products of the *spa* gene in human (**a**), bovine (**b**), and food (**c**) *S. aureus* isolates. Lane L: a 100 bp DNA ladder; lane 1: a reference *S. aureus* strain (*S. aureus* ATCC 25923), lane 2: negative control (*S. epidermidis* ATCC 13499). The other lanes represent various *S. aureus* isolates

Table 1 RFLP patterns of the *spa* gene among *S. aureus* isolates

Pattern	PCR product (bp)	Digested fragments (*Hin*dIII)[a]	Digested fragments (*Hin*fI)[a]	Source					Number (%)
				C	P	B	Ch	Pa	
No band	–	–	–	0	0	2	1	0	3 (1.4)
No digest	725	–	–	0	3	0	0	0	3 (1.4)
I	1170	180-300-680	180-180-800	0	14	0	2	0	16 (7.7)
II	1170	180-300-680	180-180-775	2	1	0	0	0	3 (1.4)
III	1170	180-300-680	180-180-750	2	0	0	0	0	2 (0.96)
IV	1170	450-720	170-170-825	0	0	0	1	0	1 (0.48)
V	1200	180-470-550	180-180-180-650	0	0	35	0	1	36 (17.3)
VI	1270	180-300-750	180-180-750	0	1	0	0	0	1 (0.48)
VII	1270	180-470-630	170-180-180-775	0	0	1	0	0	1 (0.48)
VIII	1300	180-470-680	170-180-180-700	0	0	1	0	0	1 (0.48)
IX	1300	470-830	170-180-180-700	0	2	0	0	0	2 (0.96)
X	1350	180-450-720	170-180-180-750	7	4	0	0	0	11 (5.3)
XI	1350	180-450-720	170-180-180-700	0	6	38	0	0	44 (21.2)
XII	1400	450-950	180-180-180-850	2	6	0	1	37	46 (22.1)
XIII	1450	180-450-820	170-170-170-950	5	2	0	15	7	29 (13.9)
XIV	1450	180-450-820	170-170-170-170-775	2	1	0	0	0	3 (1.4)
XV	1200, 1350	180-470-550-720	170-180-180-750	0	0	6	0	0	6 (9.2)
Total				20	40	83	20	45	208 (100)

C carrier, *P* patient, *b* bovine, *Ch* cheese, *Pa* pastry
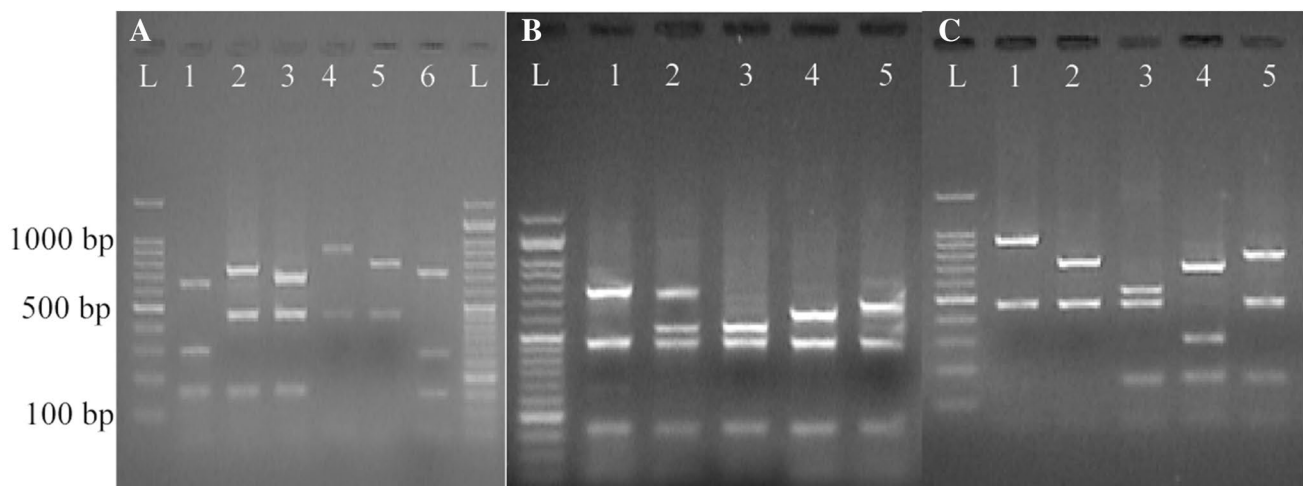
[a]Fragments < 50 bp are not shown

Fig. 2 *spa*-RFLP patterns obtained from *Hin*dIII digestion of the PCR products. **a** Human, **b** bovine and **c** food *S. aureus* isolates. Lane L: a 100 bp DNA ladder. The other lanes: RFLP patterns of *S. aureus* isolates

amplified DNA fragments of the *spa* gene led to the identification of 16 different *spa* types. Interestingly, one of these *spa* sequences was characterized in the world for the first time. This sequence was submitted to Ridom SpaServer (http://www.ridom.de/spaserver), a worldwide database for *S. aureus* *spa* types, and designated as a new *spa* type (t16929). The number of repeats within the *spa* gene varied between 4 and 11 among these sequences (http://www.ridom.de/spaserver).

Statistical analysis

The discrimination index (DI) for the *spa*-RFLP method using *Hin*dIII enzyme (0.8316) was higher than that of *Hin*fI enzyme (0.8027). Generally, the DI of *spa*-RFLP was found to be 0.8506 in this analysis.
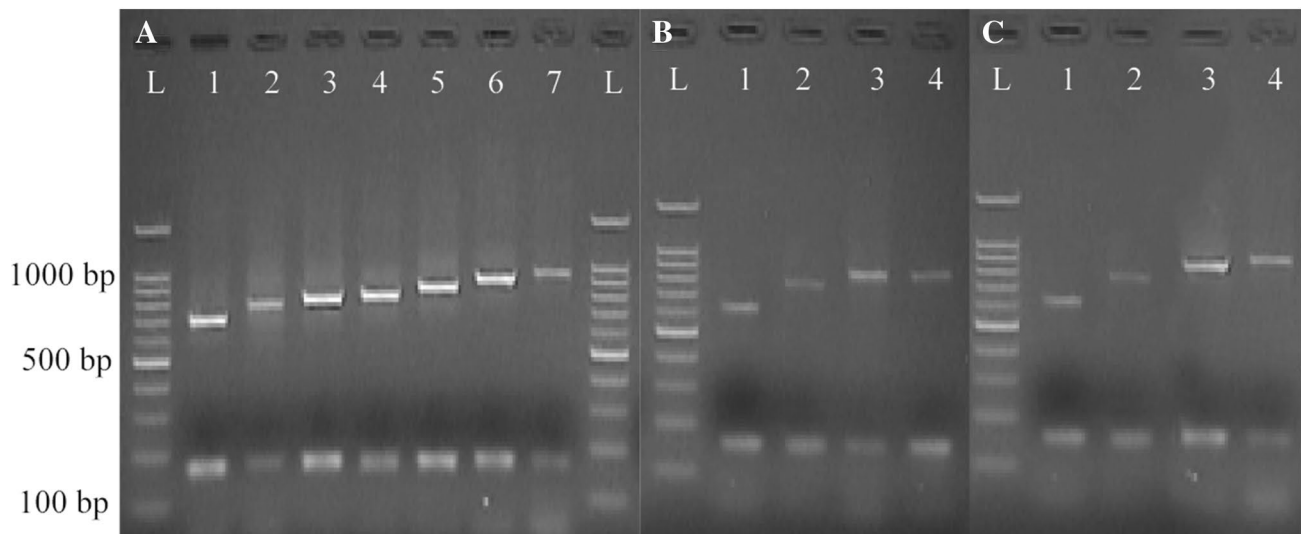
Fig. 3 *spa*-RFLP patterns obtained from *Hin*fI digestion of the PCR products. **a** Human, **b** bovine and **c** food *S. aureus* isolates. Lane L: a 100 bp DNA ladder. The other lanes: RFLP patterns of *S. aureus* isolates

Table 2 Representative *spa* type for each *spa*-RFLP pattern

spa type	*spa*-RFLP pattern	Repeat succession	Source
t701	No pattern	11-10-21-17-34-24-34-22-25-25	Human
t037	I	15-12-16-02-25-17-24	Cheese
t030	II	15-12-16-02-24-24	Human
t1149	III	08-16-34-24-34-17-17	Human
t1155	IV	15-12-16-02-25-17-16	Cheese
t162	V	14-44-12-17-17-23-18-17	Bovine
t5584	VI	14-44-13-12-17-17-23-17	Human
t1534	VII	04-31-17-25-17-17	Bovine
t16929[a]	VIII	26-13-33-34	Bovine
t4348	IX	26-16-25-16-28	Human
t189	X	07-23-12-21-17-34	Human
t359	XI	07-23-12-21-17-34-34-33-34	Bovine
t2062	XII	26-23-13-23-31-29-17-25-16-28	Human
t521	XIII	07-23-12-21-17-34-34-34-34-33-34	Cheese
t14870	XIV	11-10-13-25	Human
t13981	XV	07-12-21-17-34-13-34-13-33-34	Bovine

[a]New type

Discussion

As the *spa* gene is highly polymorphic at the 3′ end region, PCR amplification of this gene may yield amplicons of different sizes and this can help in the differentiation of *S. aureus* isolates to determine possible sources of

contamination and control infections caused by this bacterium (Strommenger et al. 2008).

In this study, the target sequence of the *spa* gene was successfully amplified from most of the *S. aureus* isolates (98.5%). However, some researchers have reported lower prevalence of *S. aureus* without the *spa* gene (Adesida et al. 2006; Shakeri et al. 2010). We also found a number of isolates (2.9%) with dual-band *spa*. This may have resulted from hybridization of the primers with more than one specific site, indicating that the sequence of the *spa* gene for such isolates may contain repeated annealing sites for the used primers. This phenomenon was also documented by Shakeri and Ghaemi (2014) who reported 10.6% frequency for isolates with double-band *spa* in PCR (Shakeri and Ghaemi 2014).

Thereafter, RFLP analysis of the *spa* gene was used to investigate the distribution and clonal relatedness of the examined *S. aureus* isolates. Although *Hin*dIII enzyme has been used in previous studies (Rohinishree and Negi 2011; Sato'o et al. 2013), here we also used *Hin*fI enzyme to type *S. aureus* for the first time.

Our findings indicated that the discriminatory power of *Hin*dIII enzyme was generally higher than that of *Hin*fI enzyme for all isolates and the RFLP patterns obtained from digestion with this enzyme were quite distinctive between human and bovine *S. aureus* isolates. However, *Hin*fI enzyme created more RFLP patterns among the human isolates, suggesting that combination of both enzymes is beneficial in typing of human *S. aureus* isolates.

According to the results of digestion, 15 different patterns were created by the restriction enzymes. As presented in Table 1, the majority of bovine and food *S. aureus* isolates

showed certain *spa*-RFLP patterns (V and XI for bovine and XII and XIII for food isolates), indicating that isolates of each type may originate from the same source of contamination, or some strains may be dominant because of their compatibility with the environment (Oliver et al. 2005). Nevertheless, a high diversity was observed among human isolates. In addition, there was little similarity between the RFLP patterns of human and bovine *S. aureus* strains, and only 6 out of 60 human isolates shared the same RFLP pattern with bovine isolates (RFLP pattern XI). As previously reported; a certain host specificity is usually seen between bovine and human isolates (Zadoks et al. 2000; Alni et al. 2017).

On the other hand, some strains of different origins showed similar RFLP pattern including I, V, XI, XII and XIII. This may be due to the circulation of these strains among various sources. These findings are in agreement with the results obtained by Lee (2003) who showed that *S. aureus* can be transmitted from food of animal origin to humans.

The results of RFLP also revealed that most of the food *S. aureus* isolates (62 out of 65 strains) shared similar RFLP patterns with human isolates (patterns I, XII and XIV). This possibly came from the fact that *S. aureus* may be present as normal flora on the skin and this can lead to contamination of food by workers handling them.

Meanwhile, 18 (90%) out of 20 strains isolated from carriers had similar RFLP patterns with isolates from patients, and only two isolates (10%) indicated a different *spa*-RFLP pattern (III). This high similarity reflected the fact that carriers of *S. aureus* are at increased risk to infect individuals. In this case, some researchers reported that nosocomial *S. aureus* bacteremia in carriers is indicated as an endogenous source and *S. aureus* nasal carriers are more susceptible to nosocomial infections (Wertheim et al. 2004). In another study, Toshkova et al. (2001) also indicated the importance of nasal carriage of *S. aureus* in causing skin disease in humans. Therefore, elimination of *S. aureus* from nasal carriers is a great help in controlling of such staphylococcal diseases. Conversely, although most of the isolates from patients showed RFLP pattern I, none of the *S. aureus* isolated from carriers had this *spa* pattern suggesting that more studies are needed to clarify the potentials of various *S. aureus* strains as causative agents of different diseases.

In the present study, to global comparison of *S. aureus* *spa* types, the corresponding DNA fragment of each *spa*-RFLP pattern was sequenced and the *spa* types were determined using the Ridom SpaServer. Among the examined isolates, some identified *spa* types such as t13981, t037, t030, t1149, t14870 and t701 have previously been reported from Iran (http://www.ridom.de/spaserver). However, here, we reported a panel of new *spa* types including t1155, t162, t1534, t189, t4348, t5584, t359, t521 and t2062 in the country. Besides, a novel *S. aureus* *spa* type, type t16929, was identified and has been registered in Ridom SpaServer in the present study for the first time.

According to Ridom SpaServer, *spa* type t037 has the highest frequency (23.1%) in the world and has been registered in 40 countries so far (http://www.ridom.de/spaserver); whereas *spa* type t16929 is the lowest observed (0%). The results of our study were also in agreement with these data, although not all of the *spa*-PCR products were sequenced. *spa* type t037 (*spa*-RFLP pattern I) was also a dominant RFLP type among the human isolates, and *spa* type t16929 which belonged to an *S. aureus* strain isolated from a bovine sample (*spa*-RFLP pattern VIII) had the lowest frequency.

Conclusion

In the present study, *spa*-PCR–RFLP with *Hin*dIII and *Hin*fI restriction enzymes was successfully used to assess *S. aureus* isolated from human, bovine and food samples. The results of the RFLP analysis confirmed the possibility of transmission of pathogenic *S. aureus* strains between humans and foodstuffs. However, *S. aureus* isolates were predominantly associated with a host species in case of human and bovine samples. Given that *spa*-PCR–RFLP method is an available and cost-effective technique with high discriminatory power and repeatable results, the method can be considered as an appropriate procedure to genotypically type *S. aureus* isolates and to determine their clonal relationships.

Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

References

Abebe R, Hatiya H, Abera M, Megersa B, Asmare K (2016) Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. BMC Vet Res 12:270–281

Acosta A, Guelfer E, Ruiz A, Mota RA, Uffo O, Gomes-Filho MA, Barbosa SB (2014) Molecular subtyping of *Staphylococcus aureus* isolated from bovine mastitis in Pernambuco State, Brazil. Comp Clin Pathol 23:1037–1041

Adesida SAY, Likhoshvay W, Eisner AO, Coker OA, Abioye F, Ogunsola T (2006) Repeats in the 3'region of the protein A gene is unique in a strain of *Staphylococcus aureus* recovered from wound infections in Lagos, Nigeria. Afr J Biotechnol 16:1858–1863

Alni RH, Mohammadzadeh A, Mahmoodi P, Alikhani MY (2017) RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from different sources. Comp Clin Pathol 4:1–8

Dallal MM, Salehipour Z, Eshraghi S, Mehrabadi JF, Bakhtiari R (2010) Occurrence and molecular characterization of *Staphylococcus aureus* strains isolated from meat and dairy products by PCR–RFLP. Ann Microbiol 60:189–196

Frenay HM, Bunschoten AE, Schouls LM, Van Leeuwen WJ, Vandenbroucke-Grauls CM, Verhoef J, Mooi FR (1996) Molecular typing of methicillin-resistant *Staphylococcus aureus* on the basis of protein A gene polymorphism. Eur J Clin Microbiol Infect 15:60–64

Hallin M, Friedrich AW, Struelens MJ (2009) spa typing for epidemiological surveillance of *Staphylococcus aureus*. Mol Epidemiol Microorg Methods Protoc 551:189–202

Harmsen D, Claus H, Witte W, Rothgänger J, Claus H, Turnwald D, Vogel U (2003) Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for *spa* repeat determination and database management. J Clin Microbiol 41:5442–5448

Hunter PR, Gaston MA (1998) Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 26:2465–2466

Laplana LM, Cepero MP, Ruiz J, Zolezzi PC, Calvo MC, Erazo MC, Gómez-Lus R (2007) Molecular typing of *Staphylococcus aureus* clinical isolates by pulsed-field gel electrophoresis, staphylococcal cassette chromosome mec type determination and dissemination of antibiotic resistance genes. Int J Antimicrob Agents 30:505–513

Larsen J, Enright MC, Godoy D, Spratt BG, Larsen AR, Skov RL (2012) Multilocus sequence typing scheme for *Staphylococcus aureus*: revision of the gmk locus. J Clin Microbiol 5:2538–2539

Lee JH (2003) Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl Environ Microbiol 69:6489–6494

Mehndiratta PL, Bhalla P, Ahmed A, Sharma YD (2009) Molecular typing of methicillin-resistant *Staphylococcus aureus* strains by PCR–RFLP of SPA gene: a reference laboratory perspective. Indian J Med Microbiol 27:116–122

Oliver SP, Jayarao BM, Almeida RA (2005) Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications. Foodborne Pathog Dis 2:115–129

Omar NY, Ali HA, Harfoush RA, El Khayat EH (2014) Molecular typing of methicillin resistant *Staphylococcus aureus* clinical isolates on the basis of protein A and coagulase gene polymorphisms. Int J Microbiol 15:1–11

Persoons D, Van Hoorebeke S, Hermans K, Butaye P, de Kruif A, Haesebrouck F, Dewulf J (2009) Methicillin-resistant *Staphylococcus aureus* in poultry. Emerg Infect Dis 15:452–453

Rohinishree YS, Negi PS (2011) Detection, identification and characterization of staphylococci in street vend foods. Food Sci Nutr 2:304–313

Sabat AJ, Budimir A, Nashev D, Sá-Leão R, Van Dijl JM, Laurent F, Grundmann H, Friedrich AW, ESCMID Study Group of Epidemiological Markers (ESGEM) (2013) Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill 18:20380

Shakeri F, Ghaemi EA (2014) New Spa types among MRSA and MSSA isolates in north of Iran. Adv Microbiol 4:899–995

Shakeri F, Shojai A, Golalipour M, Rahimi Alang S, Vaez H, Ghaemi EA (2010) Spa diversity among MRSA and MSSA Strains of *Staphylococcus aureus* in North of Iran. Int J Microbiol 31:1–5

Stranden A, Frei R, Widmer AF (2003) Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? J Clin Microbiol 41:3181–3186

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

Tang D, Ando S, Takasaki Y, Tadano J (2000) Mutational analyses of restriction endonuclease-*Hin*dIII mutant E86K with higher activity and altered specificity. Protein Eng 13:283–289

Tesfaye GY, Regassa FG, Kelay B (2010) Milk yield and associated economic losses in quarters with subclinical mastitis due to *Staphylococcus aureus* in Ethiopian crossbred dairy cows. Trop Anim Health Prod 42:925–931

Toshkova K, Annemüller C, Akineden Ö, Lämmler C (2001) The significance of nasal carriage of *Staphylococcus aureus* as risk factor for human skin infections. FEMS Microbiol Lett 202:17–24

Walther B, Wieler LH, Friedrich AW, Hanssen A, Kohn B, Brunnberg L, Lübke-Becker A (2008) Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. Vet Microbiol 127:171–178

Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA (2004) Risk and outcome of nosocomial *Staphylococcus aureus* bacteraemia in nasal carriers versus non-carriers. Lancet 27:703–705

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

Wilson K (1987) Preparation of genomic DNA from bacteria. Curr Protoc Mol Biol 2:2–4

Sato'o Y, Omoe K, Ono HK, Nakane A, Hu DL (2013) A novel comprehensive analysis method for *Staphylococcus aureus* pathogenicity islands. Microbiol Immunology 57:91–99

Zadoks R, Van Leeuwen W, Barkema H, Sampimon O, Verbrugh H, Schukken YH, Van Belkum A (2000) Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. J Clin Microbiol 38:1931–1939