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ORIGINAL ARTICLE

RAPD-PCR analysis of *Staphylococcus aureus* strains isolated from different sources

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Abstract Determination of genetic relationship among *Staphylococcus aureus* isolates is important for epidemiological surveillance and control of infections caused by this bacterium. The present study was conducted to determine the molecular diversity of *S. aureus* strains isolated from human, bovine, and food samples using random amplified polymorphic DNA (RAPD)-PCR method. A total of 208 *S. aureus* strains including 60 human (patients and healthy carriers), 83 bovine milk, and 65 food (pastry and cheese) isolates were assessed. The isolates were confirmed to be methicillin-resistance/susceptible *S. aureus* (MRSA or MSSA) using common biochemical tests and a species-specific PCR, and finally, they were genetically typed using RAPD-PCR method. Forty-seven RAPD profiles (13, 21, and 15 profiles for isolates from human, bovine, and food samples, respectively) were identified in the examined isolates. Based on these profiles and the constructed dendrogram, all of 208 *S. aureus* strains were divided into nine major clusters from A to I, at 80% similarity. Some clusters (A, B, C, E, and H) contained *S. aureus* strains isolated from only one source, while the others (D, F, G, and I) contained isolates from more than one source. The results revealed that although most of *S. aureus* strains of the same source were placed in certain clusters, there were also some strains of different sources which shared the same RAPD profiles indicating possible transmission of *S. aureus* strains (especially MRSA ones) among different hosts. Thus, effective hygiene measures should be considered to break transmission chains.

Keywords *Staphylococcus aureus* · Randomly amplified polymorphic DNA · Epidemiology

Introduction

Staphylococcus aureus, which is found on the skin and mucous membranes of mammals, surroundings, and food of animal origins, can potentially cause a wide range of infections in humans and animals (Adwan et al. 2006; Lowy 1998). In humans, *S. aureus* is one of the major bacterial pathogens that cause nosocomial infections and food poisoning cases (Wisplinghoff et al. 2004; Le Loir et al. 2003), while in animals, it is an important causative agent of mastitis which may result in milk reduction, thereby instigating huge economic losses to dairy industry (Beck et al. 1992).

Meanwhile, some strains of *S. aureus* possess *mecA* gene which confers resistance to methicillin and most of the commonly used antimicrobial agents including b-lactams and cephalosporins. These strains are called methicillin-resistant *Staphylococcus aureus* (MRSA) and they assumed to be more virulent than methicillin-susceptible *Staphylococcus aureus* (MSSA) strains (Cosgrove et al. 2003). The increasing rates of nosocomial and community-associated MRSA infections and their ability to transfer between humans, bovine, and food of animal origins have become a global menace (Juhász-Kaszanyitzky et al. 2007; Sakwinska et al. 2011; Nnachi et al. 2014).

As *S. aureus* is able to transmit from animals to humans and vice versa (Juhász-Kaszanyitzky et al. 2007; Roberson

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et al. 1994; Seguin et al. 1999) and it may also contaminate food products (lee 2003), knowing types and origins of *S. aureus* isolates, especially MRSA strains, is a crucial step for epidemiological surveillance and control of infections caused by this bacterium (Tambic et al. 1999). Moreover, typing of *S. aureus* strains may indicate possible differences in their characteristics and it is useful to determine circulation patterns of these bacteria among various hosts and sources (lee 2003). Several phenotypic and genotypic methods including biotyping, antibiotic susceptibility testing, multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), and PCR-based methods have been used for this purpose (Sabat et al. 2006; Rabello et al. 2007). Although PFGE and MLST are effective tools to type *S. aureus* strains, they are thought to be expensive, laborious, and technically demanding. However, PCR-based methods are simple, cheap, and useful techniques for epidemiological investigations (Morandi et al. 2010), and some of which like random amplified polymorphic DNA-PCR (RAPD-PCR) is an easy, fast, and economically affordable method which has been widely used for typing of *S. aureus* strains (Tenover et al. 1994; Neela et al. 2005). Here, RAPD-PCR was used to compare source of contamination and distribution of *S. aureus* strains isolated from human, bovine, and food samples and determine their genetic relationship.

Materials and methods

Bacterial isolates

A total of 60 human *S. aureus* strains were isolated from patients (n = 40, 12 from skin, 11 from tracheal discharges, 10 from urine, 4 from blood, and 4 from sputum) and healthy carriers (n = 20 from *nasal cavity*). All of the isolates were collected from individuals referred to Shahid Beheshti Hospital in Hamedan province of Iran. The isolates were confirmed to be *S. aureus* using common biochemical tests and a species-specific PCR. Moreover, 148 previously isolated *S. aureus* strains were also included in the study to extend the comparative analysis. These strains consisted of 83 strains isolated from bovine milk samples and 65 strains isolated from food samples (45 and 20 isolates from pastry and cheese samples, respectively) and had been identified in the same way.

Disk diffusion method to detect methicillin-resistant strains

Methicillin susceptibility among human isolates was tested by agar disk diffusion method using Mueller-Hinton agar (Merck, Germany), according to the guidelines of the National Committee for Clinical Laboratory Standards (Shanmugam et al. 2011). However, methicillin susceptibility of the other isolates had previously been performed using the same method.

Extraction of DNA samples

Bacterial DNA was extracted from an overnight culture of each isolate in tryptic soy broth (TSB). Briefly, 5 ml of the cultured medium was centrifuged at 8000 rpm for 3 min and the pellet was resuspended in TE buffer (10 mM Tris, 1 mM EDTA). After addition of SDS 10% and proteinase K, the mixture was incubated at 37 °C for 1 h. NaCl 5 M and CTAB/NaCl solutions were then added to the mixture followed by incubation at 65 °C for 10 min. Following addition of phenol/chloroform/isoamyl alcohol, the mixture was centrifuged at 14,000 rpm for 5 min and the supernatant was transferred to a new microtube and DNA was precipitated using isopropanol and ethanol 70%. Finally, the pellet was resuspended in 100 μl TE buffer. All DNA samples were frozen at −20 °C and used as DNA templates in PCR assays (Wilson 1987).

Duplex PCR

Extracted DNA samples from all of the biochemically characterized *S. aureus* isolates were examined by a duplex PCR assay targeting two genes, *femA* and *mecA*, to genetically detect *S. aureus* species and MRSA strains, simultaneously. The oligonucleotide primers were previously described by Mehrotra (Mehrotra et al. 2000) and their sequences are given in Table 1. The PCR reaction (25 μl) contained 3–5 μl of template DNA, 2.5 μl of ×10 PCR buffer, 0.75 μl of 50 mM $MgCl_2$, 0.5 μl of 10 mM dNTPs, 0.25 μl of 5 U/μl of Taq DNA polymerase, and 25 pmol of each of the primers. The PCR amplification was performed under the following conditions: initial denaturation at 94 °C for 5 min followed by denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min and extension at 72 °C for 1 min (35 cycles), and a final extension at 72 °C for 7 min (20). The PCR products were analyzed by electrophoresis on 2.5% agarose gel containing ethidium bromide (0.5 μg/ml).

Table 1 Primers used in PCR assays

Primer	Sequence 5′-3′	Product size (bp)
mecA-forward	ACTGCTATCCACCCTCAAAC	163
mecA-reverse	CTGGTGAAGTTGTAATCTGG	
femA-forward	AAAAAAGCACATAACAAGCG	132
femA-reverse	GATAAAGAAGAAACCAGCAG	
AP-7	GTGGATGCGA	200 to 1500

Random amplified polymorphic DNA-PCR

RAPD-PCR was performed using a previously designed primer AP-7 (Kurlenda et al. 2007) (Table 1(. The RAPD-PCR mixture (25 μl) included 12.5 μl of a commercial PCR Mastermix (Amplicon, Denmark), approximately 25 ng of DNA template and 20 pmol of the primer. The reaction was carried out under thermal conditions as follows: pre-denaturation at 95 °C for 6 min; 35 cycles of denaturation at 94 °C for 90 s, annealing at 35 °C for 60 s, and extension at 72 °C for 90 s with a final extension at 72 °C for 10 min. To check the repeatability, the experiment was repeated at least three times and a set of reproducible bands was defined as a profile (Morandi et al. 2010). Finally, the products were analyzed by electrophoresis as above. *S. aureus* ATCC 13566 and a sample contained no DNA were used as positive and negative controls in all PCR reaction.

Analysis of RAPD-PCR data

The patterns obtained from RAPD-PCR assays were analyzed using NTSYSpc software (version 2.1, USA). The presence or absence of each RAPD polymorphic band was scored 1 and 0, respectively. Cluster analysis and creation of dendrogram were performed by unweighted pair group method with arithmetic averages (UPGMA). The cluster cutoff line for detailed examination of genetic relationship among the isolates was set at 80% similarity and the genetic relationship between all of 208 isolates was portrayed by drawing of dendrogram (Idil and Bilkay 2014).

Results

A total of 208 *S. aureus* isolates were identified based on the biochemical tests. All of the isolates were also confirmed to be *S. aureus* species. Indeed, amplification of *femA* gene in the species-specific PCR produced a DNA band of the expected size (132 bp) from all of the extracted DNA samples (Fig. 1). Moreover, the results of this PCR indicated that 66.67% of human isolates (39 patient and 1 carrier isolates), 12.31% of food isolates (4 cheese and 4 pastry isolates), and 63.8% of bovine isolates were positive for *mecA* gene and showed the DNA band of about 163 bp in gel electrophoresis. On the other hand, the results of disk diffusion method were as the same as the results of the PCR assay to detect methicillin-resistant isolates.

RAPD-PCR was used to assess relative genetic relationship and compare the source of contamination among 208 *S. aureus* strains isolated from different origins. Polymorphic DNA bands were produced from all of the tested strains in RAPD-PCR using primer AP-7. As shown in Fig. 2a–c, 23 distinct amplicons ranged in size from 200 to 1500 bp were

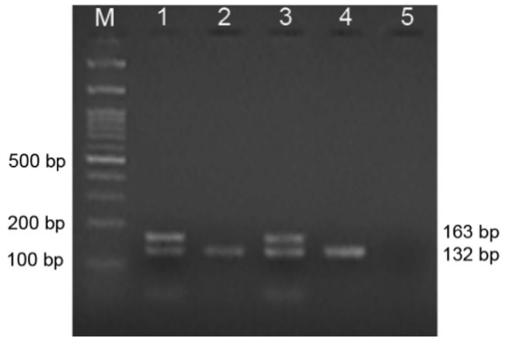

Fig. 1 Sample results of electrophoresis of PCR products. *M*: 100-bp DNA ladder; *lane 1*: *S. aureus* (MRSA, ATCC 33591) containing amplified DNA fragments from both *femA* and *mecA* genes (132 and 163 bp, respectively) as positive control; *lane 2*: *S. aureus* (MSSA, ATCC 25923) containing amplified DNA fragments from only *femA* gene (132 bp); *lane 3*: a tested isolate which was positive for both genes; *lane 4*: a *S. aureus* isolate which was negative for *mecA* gene; *lane 5*: negative control containing no template DNA

produced in RAPD-PCR, which formed 47 RAPD profiles. Thirteen RAPD profiles were characterized in human strains (patients and carriers), 3 of which (patient profiles 7, 8, and 9) were similar to carrier counterparts. However, 21 and 15 RAPD profiles were identified in *S. aureus* strains isolated from bovine and food samples, respectively. *S. aureus* isolates with the most common pattern (38 strains) were placed in a cluster (cluster D). This profile was observed in strain isolates from human (4 strains), bovine (28 strains), and food (11 strains) samples. However, constructed dendrogram indicated that some samples were not distinguishable by this primer (Fig. 3).

The generated dendrogram also showed that most of *S. aureus* strains isolated from different sources were placed in certain clusters. Based on the constructed dendrogram, all of the examined *S. aureus* strains were divided into nine major clusters (A–I), at 80% similarity. All of the isolates in clusters A, B, C, E, and H were isolated from the same source; however, strains in clusters D, F, G, and I had been isolated from different origins. In this case, 23 (60.5%), 11 (29%), and 4 (10.5%) strains in cluster D were bovine, food, and human isolates, respectively. Cluster F consisted of 13 strains including 1 (7.7%) bovine, 3 (23%) food, and 9 (69.2%) human isolates, and cluster G contained 4 (66.7%) food and 2 (33.3%) human isolates. Clusters A and B consisted of food isolates, while clusters C and H contained bovine isolates. Human isolates were grouped in cluster E.

Discussion

The use of rapid and accurate methods in typing of *S. aureus* isolates is essential to identify and control infections caused by this organism. There are many phenotypic and genotypic techniques to achieve this goal (Olive and Bean 1999). Genotypic

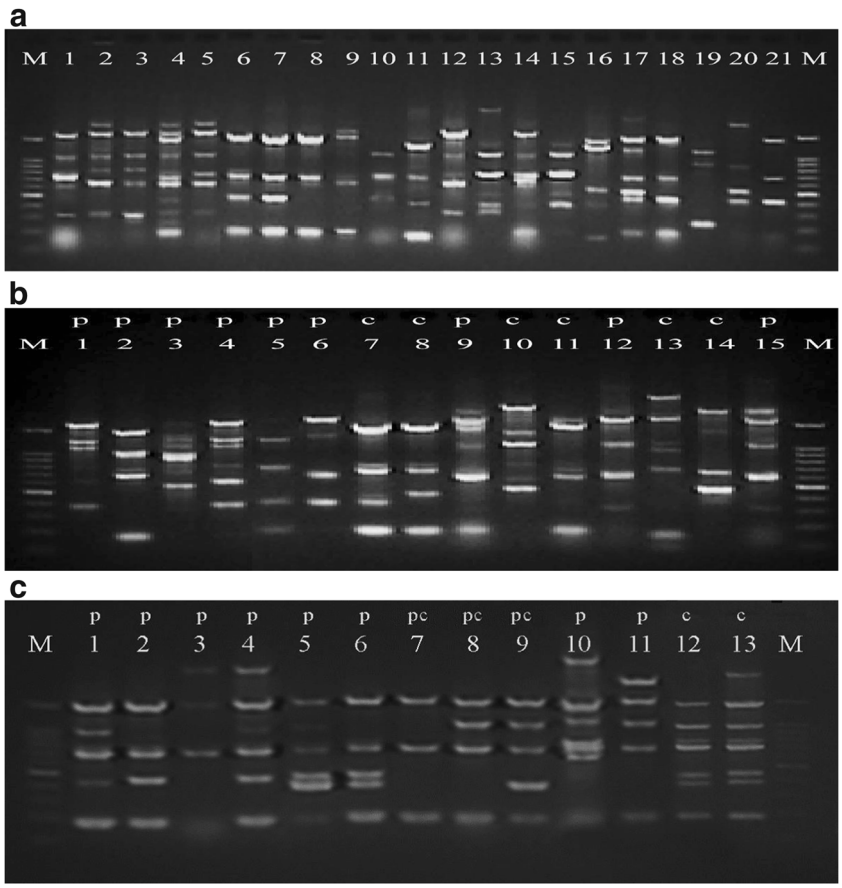

Fig. 2 **a** RAPD genetic profiles obtained from bovine isolates. *Lanes 1–21*: *S. aureus* isolates; *lane M*: 100-bp DNA ladder. **b** RAPD genetic profiles obtained from food isolates (cheese and pastry samples). *Lanes 1–15*: *S. aureus* isolated from cheese and pastry isolates; *lane M*: 100-bp DNA ladder (*p* pastry, *c* cheese). **c** RAPD genetic profiles obtained from human isolates. *Lanes 1–13*: *S. aureus* isolated from human (patients and carriers); *lane M*: 100-bp DNA ladder (*p* patient, *c* carrier, *pc* shared between patients and carriers)

methods are more accurate and faster than the phenotypic ones. RAPD-PCR is one of the known genotyping methods which has been widely used in various epidemiological investigations. Pereira et al. (2002) carried out a study to investigate genetic diversity and delineate geographical distribution among *S. aureus* strains from bovine and nosocomial origins. Five to nine groups were distinguished by RAPD-PCR and their results demonstrated genetic heterogeneity of the studied strains and large dissemination of some clones throughout different regions and hosts (Pereira et al. 2002). Reinoso et al. (2004) analyzed 80 *S. aureus* isolates from bovine and human hosts by RAPD-PCR to assess their genetic relationship. RAPD results showed that all of the isolates were clustered into 11 groups (A–K) (Reinoso et al. 2004). In another study carried out by Kurlenda et al. (2007), 234 isolates of *S. aureus* were typed to evaluate MRSA strains. All isolates were recovered from infection and carriage sites of patients referred to a hospital. RAPD-PCR analysis divided these 234 MRSA strains into 10 groups. However, they did not find any correlation between determined clones and hospital department or type of infection (Kurlenda et al. 2007). Prasad et al. (2015) performed a PCR-based fingerprinting for 56 *S. aureus* recovered from different regions using RAPD-PCR. All of 56 *S. aureus* isolates were typed into 15 clads (C1–C15). Their results also demonstrated genetic heterogeneity in the strains and dissemination of some clones through different regions (Prasad et al. 2015).

In the present study, RAPD-PCR was used to investigate the dissemination and clonal relatedness of *S. aureus* isolated from human, bovine, and food samples. The RAPD-PCR analysis revealed 47 distinct profiles among all of the studied isolates, including 21, 15, and 13 profiles for bovine, food, and human isolates, respectively, which indicated genetic heterogeneity among the isolates. On the other hand, based on the generated dendrogram, the isolates were grouped into nine clusters (A to I) at 80% similarity. Most of the *S. aureus* strains isolated from the same source were specifically placed in the same cluster suggesting that genetic differences among some *S. aureus* isolates were enough to be clustered separately. As shown in the dendrogram (Fig. 3), clusters A, B, C, and E only contained strains obtained from cheese, pastry, bovine, and human samples, respectively. However, there were also some strains from different origins which shared the same amplification profiles and grouped into the same cluster such as those placed in clusters D, F, and G. Among the defined clusters, cluster D had the highest degree of diversity and contained indistinguishable strains from almost all of the bovine, human, and food samples with the same electrophoretic pattern. This

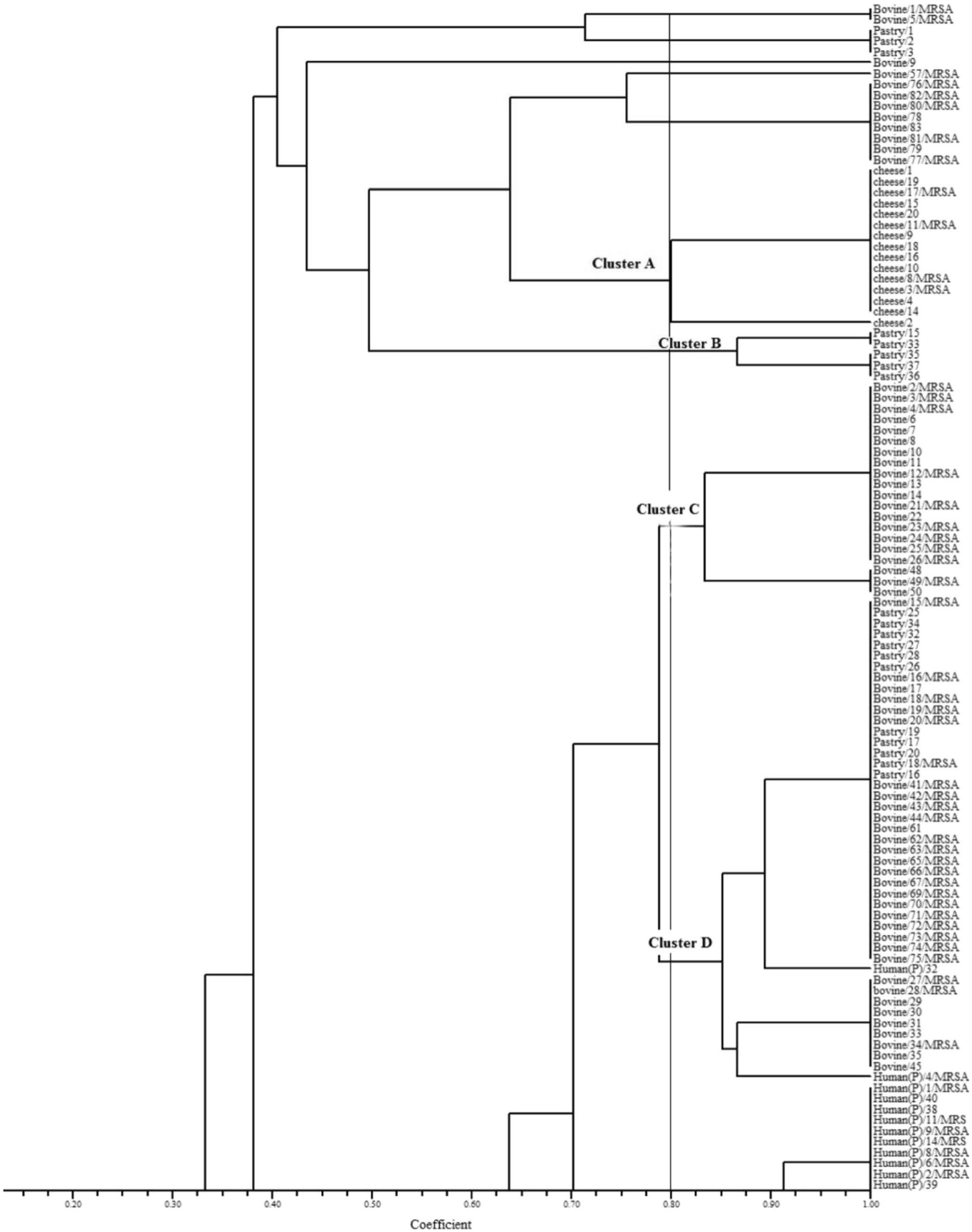

Fig. 3 Dendrogram of genetic relationship among 208 *S. aureus* strains isolated from human, bovine, and food samples. *P* patient, *C* carrier, *MRSA* methicillin-resistant *Staphylococcus aureus*

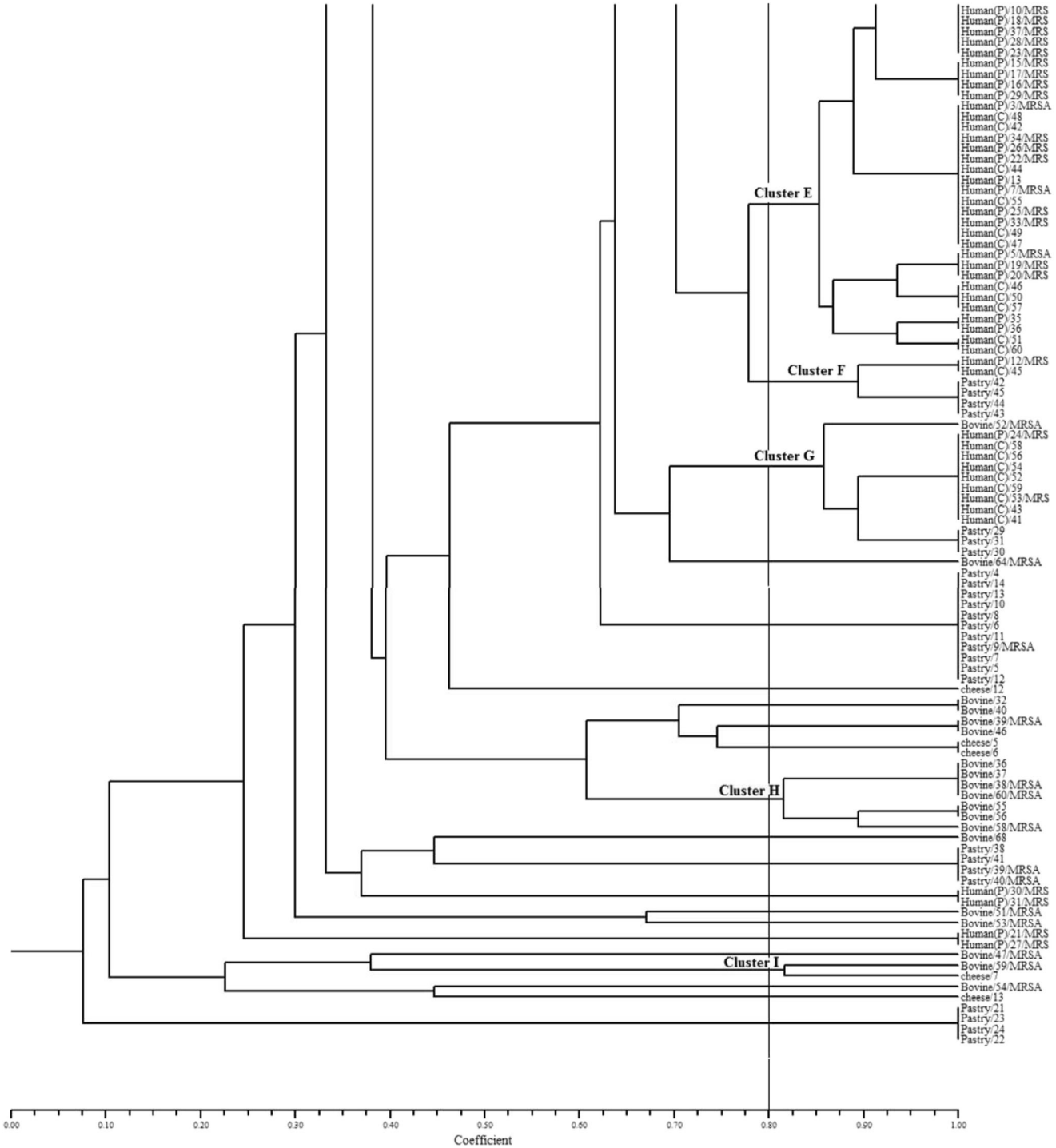

Fig. 3 continued.

may suggest circulation of these strains among different sources. These results are in agreement with the results obtained by lee (2003), who showed that *S. aureus* can be transmitted from food of animal origins to humans.

Besides, isolation of MRSA from food animals and the possibility of transmission to humans have raised worldwide concern (Nnachi et al. 2014). Juhász-Kaszanyitzky et al. (2007) reported direct transmission of MRSA between cows and humans (Juhász-Kaszanyitzky et al. 2007). However, Kapur et al. (1995) noted that transmission of *S. aureus* between cow and humans is not a frequent event (Kapur et al. 1995). In the present study, 26 MRSA isolates from human, bovine, and food origins shared the same RAPD profile and placed in cluster D, suggesting the possibility of transmission

of MRSA strains among these three sources. However, there was little profile similarity between human and bovine strains and only 4 out of 60 human isolates were characterized with the same RAPD profile and grouped with bovine strains. Indeed, as reported by Zadoks et al. (2000) which indicated that *S. aureus* strains were predominantly associated with a host species, a certain host specificity is usually seen between bovine and human isolates.

At 90% similarity, the generated dendrogram showed that *S. aureus* isolates from pastry samples (7 strains) were very closely related to strains from carriers (clusters F and G). This may also come from the fact that *S. aureus* may be present as normal flora at the skin surfaces and this can lead to contamination of pastries by workers handling them. However, *S. aureus* can also be transmitted from pastry to human. Moreover, according to the results of cluster analysis, some of the cheese isolates (15 strains, cluster A) had genetic relationship with some bovine strains suggesting that these strains may be originated from milks contaminated with bovine isolates, which in turn mirrors the effects of applied hygiene measures in dairy farms and chain of dairy production.

Meanwhile, 15 carrier isolates (75%) had a similar profile with isolates from patients and only 25% showed a different profile. This high similarity between carrier and patient strains may reflect the fact that carriers of *S. aureus* are at increased risk for infections with this organism. Wertheim et al. (2004) indicated that *S. aureus* nasal carriers are more susceptible to nosocomial infection than non-carrier individuals (Wertheim et al. 2004), and also emphasized the importance of nasal carriage of *S. aureus* in causing disease in human skin (Toshkova et al. 2001)

In the present study, *S. aureus* strains isolated from different origins have been successfully typed using RAPD-PCR and it has been confirmed that utilization of RAPD-PCR with the primer AP-7 is a suitable method for molecular typing, phylogenetic analysis, and detection of polymorphism in different isolates of *S. aureus*.

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Compliance with ethical standards The protocol of this study has been reviewed and approved by the Ethics Committee of the Hamadan University of Medical Sciences.

Conflict of interest The authors declare that they have no conflicts of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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