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ORIGINAL ARTICLE

Methicillin resistance and clonal diversity of *Staphylococcus aureus* isolated from nasal samples of healthy horses in Iran

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Abstract

Purpose The aims of the current study were to investigate the frequency and genetic diversity of *Staphylococcus aureus* from healthy horses, including both methicillin-resistant (MRSA) and -susceptible *S. aureus* (MSSA).

Methods Three hundred-one nasal swabs were collected from healthy horses in three provinces, Iran. Sixty-one of the 301 tested samples contained *S. aureus* (20.3%), among which five were MRSA. Isolates were typed by *spa* PCR-RFLP and *agr* typing, followed by sequence-based *spa* typing and MLST on representative strains from each restriction pattern and SCC*mec* typing for MRSA strains. The presence of Panton-Valentine Leukocidin (PVL) encoding genes was also tested using PCR.

Results Eight distinct RFLP patterns (designated as N1-N8) were observed, with N2 (23/61; 37.7%) and N4 (18/61; 29.5%) the most common. On sequencing, N1-N8 patterns were found to be of clonal types ST15-t084, ST2151-t2484, ST291-t937, ST1-t127, and ST1-t1383, ST700-t11926, ST133-t1166, and ST1278-t12595, respectively. No PVL-positive *S. aureus* were detected. Five MRSA were identified as ST2151-t2484-SCC*mec*IVa (2 isolates), ST15-t084-SCC*mec*IVa, ST1-t1383-SCC*mec*IVa, and t12595-SCC*mec*IVa (one isolate each). Majority of *S. aureus* isolates were ascribed to *agr* types III ($n = 30$; 49.2%) and IV ($n = 28$; 45.9%), followed by types II ($n = 2$, 3.3%) and I ($n = 1$, 1.6%). The carriage of *S. aureus* was found to be associated with geographic locations.

Conclusions This study for the first time describes the circulation of diverse clones of MSSA and MRSA among the Iranian horse population. This may pose a public health risk, which supports the need for their epidemiological monitoring.

Keywords Horse · Methicillin resistant · Nasal · Clonal diversity · *S. aureus*

Introduction

Staphylococcus aureus is an important pathogen that can asymptomatically colonize the nares of diverse animals. Of particular concern is methicillin-resistant *S. aureus* (MRSA), which has become a major threat due to an increasing incidence in companion animals, including horses and animals raised for human consumption (Weese and van Duijkeren 2010). The emergence of methicillin-resistant strains of *S. aureus* is due to the acquisition of a staphylococcal cassette chromosome *mec* (SCC*mec*) element harboring the *mecA* or *mecC* gene (Liu et al. 2016). The populations of MRSA are classified on the basis of their origin as hospital associated (HA-MRSA), community associated (CA-MRSA), and live-stock associated (LA-MRSA) (Gopal and Divya 2017). Studies demonstrated that nasal mucosa of farm animals represents a potential reservoir of MRSA that, in turn, may serve as an important source for environmental con-tamination (Peterson et al. 2012). Colonization is also a substantial risk factor for autoinfection and colonized horses pose a risk to other animals and humans (Cohn and Middleton 2010; Axon et al. 2011; Agabou et al. 2017). In this regard, MRSA skin infections as a result of horse to human transmission have been reported in several studies (Weese et al. 2005, 2006).

Subtyping is a key point for epidemiologic investigation and subsequent design of public health control strategies. Many different molecular techniques have been extensively exploited for classifying *S. aureus* strains of which Staphylococcal Protein A (*spa*) typing and multilocus

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sequence typing (MLST) are considered to be well-established discriminatory methods (Enright et al. 2000; Koreen et al. 2004). These two methods can be used in conjunction with software-based clustering algorithms that group related isolates into clonal complexes (CCs). The algorithms used with *spa* typing and MLST to cluster *spa* types into *spa* CCs and MLST sequence types (STs) into MLST CCs are Based Upon Repeat Pattern (BURP) and Based Upon Related Sequence Types (BURSTs), respectively (O'Hara et al. 2016). Other techniques such as polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of the *spa* gene (Wichelhaus et al. 2001) and accessory gene regulator (*agr*) typing (Gilot et al. 2002) could also be used for epidemiological investigation of *S. aureus* strains. For MRSA, SCC*mec* typing is another indispensable method of typing which classifies SCC*mec* elements based on their structural organization and genetic content. To date, a total of 13 SCC*mec* types (I–XIII) have been reported, with SCC*mec* types I, II, or III predominant in HA-MRSA strains, whereas types IV or V were the most common in CA-MRSA strains (Lakhundi and Zhang 2018). In addition, Panton-Valentine Leukocidin (PVL) is a gene encoding a potent pore-forming cytotoxin that is strongly associated with CA-MRSA strains (Asghar 2014).

Although some studies have indicated that horses are colonized or infected by MRSA strains commonly related to CC8 (Weese and van Duijkeren 2010; Carfora et al. 2016), the genetic background of MRSA strains isolated from equids varies in different regions around the world (Carfora et al. 2016). In Iran, there is a long history in horse domestication and breeding and Iranian horse breeds can be classified into four main groups according to their origins and habitats as follows: Caspian breed in North alluvial plains, Turkmen breed in northeast fields, Kurd breed in west highlands, and Persian Arab breed (Asil) in central plateau (Moridi et al. 2013). However, the epidemiology of *S. aureus* in horses has not been well studied and it is also unclear how widely dispersed MRSA is in the horse population. Therefore, the aim of the present study was to investigate, for the first time, the frequency and genetic diversity of *S. aureus* isolates (either MSSA or MRSA) from healthy horses of various breeds housed in different geographic locations of Iran.

Materials and methods

Sample collection and bacterial identification

A total of 301 healthy horses were sampled from different Iranian provinces including Yazd (136 horses in five farms [A–E]) which is part of the Central Iranian Plateau where Iran's deserts are mainly located in, West Azerbaijan (63 horses in two farms [F and G] as well as a local rodeo) and East Azerbaijan (102 horses in four farm [H–K]) which are located in the northwest of the country. Horses were excluded if they had signs of upper respiratory tract infection or had antimicrobial administration in the preceding 14 days. Of the 301 horses swabbed, 195 were Iranian indigenous breeds including Persian Arab (Asil) ($n = 85$), Turkmen ($n = 47$), and Kurd ($n = 63$), the remaining horses were Thoroughbred ($n = 51$) and crossbred ($n = 55$) with a median age of 8 years (range 4 to 13 years old). Horses from West Azerbaijan province were raised near the northwest borders of the country on wide open spaces and used for work, while horses from Yazd and East Azerbaijan provinces housed in individual stalls in a stable and used for riding. On the other hand, farm in Yazd province characterized by confinement and intensive management and little direct contact between farms as well. Farms in the East and West Azerbaijan provinces had semi-intensive management system. Samples were obtained by inserting the same moistened sterile swab into both nostrils, and then rolling it against the nasal wall while removing. Collection of samples was approved by the Urmia University Animal Ethics Committee (UUAEC, authorization no. 1241). The swabs were enriched for 24 h at 37 °C in nutrient broth containing 6% NaCl and inoculated onto Mannitol Salt Agar (MSA) selective for *S. aureus* (Maddox et al. 2011). Incubation was carried out for 24 h at 37 °C. On evaluation of the growth on the selective medium, *S. aureus*-suspicious colonies were purified on blood agar plates containing 5% sheep blood for 24 h at 37 °C. Isolates were then subjected to conventional methods (Gram stain, catalase test, tube coagulase, DNase, and fermentation of mannitol) as well as PCR amplification of *S. aureus* specific-*nuc* gene (Brakstad et al. 1992) and frozen in brain heart infusion broth (BHI, Merck, Germany) with 15% glycerol at − 20 °C.

Detection of methicillin resistance

All *S. aureus* isolates were evaluated for methicillin resistance by disk diffusion method and endpoint PCR to detect the *mecA* gene, a determinant of methicillin resistance. Disk diffusion was performed on Mueller-Hinton agar plates (Oxoid) using 30-μg cefoxitin disk (Oxoid) (CLSI 2009). Isolates showing inhibition zone diameter ≤ 21 mm were classified as resistant. For the detection of *mecA*, we used the primer pair 5′-AAA ATC GAT GGT AAA GGT TGG C-3′ and 5′-AGT TCT GCA GTA CCG GAT TTG C-3′ as described previously (Murakami et al. 1991). A methicillin-susceptible *S. aureus* strain (ATCC 29213) and a MRSA strain (ATCC 33591) were used as control organisms for the disk diffusion and PCR assays.

Determination of accessory gene regulator (*agr*) type

The *agr* typing was carried out for determination of the *agr* groups (I–IV) using the *agr*- group-specific multiplex PCR by primers as described by Gilot et al. (2002), which involves a forward primer (pan-*agr*) common to all *agr* groups (Pan: 5′-ATG CAC ATG GTG CAC ATG C-3′) and four primers, each one specific to each *agr* group (agr1: 5′-GTC ACA AGT ACT ATA AGC TGC GAT-3′; agr2: 5′-TAT TAC TAA TTG AAA AGT GGC CAT AGC-3′; agr3: 5′-GTA ATG TAA TAG CTT GTA TAA TAA TAC CCA G-3′; and agr4: 5′-CGA TAA TGC CGT AAT ACC CG-3′). The PCR products of 441, 575, 323, and 659 bp represent the *agr* types I, II, III, and IV, respectively. *S. aureus agr* reference strains RN6390 (*agr* group 1), RN6923 (*agr* group 2), RN8462 (*agr* group 3), and A880740 (*agr* group 4) were used as controls.

RFLP analysis of the PCR-amplified *spa* gene

The primers SPA1 (5′-ATC TGG TGG CGT AAC ACC TG-3′) and SPA2 (5′-CGC TGC ACC TAA CGC TAA TG-3′) described by Wichelhaus et al. (2001) were used to amplify the polymorphic X region of *spa* gene. Amplification was carried out in Corbett thermocycler (Model CP2-003, Australia) through the following temperature program: 4 min of initial denaturation at 94 °C; 35 cycles consisting of 1 min at 94 °C, 1 min at 56 °C, and 3 min at 72 °C; and a final extension for 5 min at 72 °C. The presence of a PCR product was determined by electrophoresis in a 1.2% (*w*/*v*) agarose gels. Then, approximately 7–10 μL of PCR product (500 ng) was digested with 6 U of restriction endonuclease *Hae*II at 37 °C for 3 h. Ten microliters of digested PCR products was analyzed by electrophoresis in 1.2% agarose gel containing 0.5 μg/mL ethidium bromide at 100 V for 1 h and 20 min and visualized under UV light.

spa typing, BURP, and phylogenetic analysis

For this, *spa* gene fragment of the representative isolates from each RFLP patterns (N1-N8) was amplified using primer set 2 (spa-1113f: 5′-TAA AGA CGA TCC TTC GGT GAG C-3′ and spa-1514r: 5′-CAG CAG TAG TGC CGT TTG CTT-3′) under the following reaction conditions: (i) 5 min at 94 °C; (ii) 35 cycles of 45 s at 94 °C, 45 s at 60 °C, and 90 s at 72 °C; and (iii) 10 min at 72 °C (Johler et al. 2011). The resultant PCR products were separated in 1.5% agarose gel and the gel was photographed using ultraviolet transillumination. After gel purification (GeneJET Gel Extraction and DNA Cleanup Micro Kit; Thermo Scientific, Germany), the PCR products were sent for sequencing (SinaClon, Iran). The sequences were assigned to *spa* types using the Ridom SpaServer database (http://www.spaserver.ridom.de/) (Harmsen et al. 2003) via DNAGear (AL-Tam et al. 2012). The BURP (Based Upon Repeat Patterns) algorithm analysis of the *spa* types was performed in the Ridom StaphType software (Ridom GmbH, Germany) to cluster related *spa* types. Analysis was done with two default parameters for cluster designation: (1) "exclude *spa* types that are shorter than 5 repeats" and (2) "cluster *spa* types into the same group if cost distances are less than or equal to 4 repeats". A cluster consisting of two or more related *spa* types was regarded as a clonal complex. A singleton was defined as a *spa* type that was not grouped into a clonal complex (Mellmann et al. 2007). The *spa* sequences obtained in this study were aligned using ClustalW, and the phylogenetic analysis was performed employing the maximum likelihood (ML) method using MEGA 6.06 program. The confidence level of branching in the phylogenetic tree was evaluated with the bootstrap test based on 1000 resamplings.

Multilocus sequence typing and eBURST analysis

MLST was performed on representative *S. aureus* isolates from individual *spa* types as well as on MRSA isolates with the primers targeting seven distinct loci as described previously (Enright et al. 2000). After sequencing of the purified PCR products, allelic profile and sequence type (ST) of each isolate were assigned using the MLST website (http://www.mlst.net). The relatedness of identified STs against already deposited worldwide data was analyzed with the eBURST algorithm (version 3.0). Isolates that shared six of seven MLST loci belonged to the same CC.

SCC*mec* typing and type IV SCC*mec* subtyping

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of MRSA isolates was performed via multiplex PCR using the primers described previously (Boye et al. 2007). To distinguish between the different SCC*mec* IV subtypes, a further multiplex PCR was performed as previously described (Milheirico et al. 2007).

Detection of PVL genes

The detection of genes encoding the Panton-Valentine leukocidin (PVL, *lukS-lukF-PV*) was also carried out by PCR using the primers luk-PV-1 (5′-ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A-3′) and luk-PV-2 (5′-GCA TCA AST GTA TTG GAT AGC AAA AGC-3′) which amplify a 433 base pair fragment (Lina et al. 1999).

Statistical analysis

The association between *S. aureus* nasal carriage and geographical area was determined using Chi-Squared test. Statistical analysis was performed using SPSS Software version 22 (IBM SPSS Statistics for Windows, Armonk, NY,

USA: IBM Corp.). A P value < 0.05 was considered statistically significant.

Results

In all, 61 *S. aureus* (20.3%) strains were obtained from 301 horse nasal swab samples, of which 5 (8.2%) were found to be methicillin resistant (Table 1). As shown, *S. aureus* was isolated from 25 of 102 (24.5%) horses from East Azerbaijan, 26 of 63 (41.3%) horses from West Azerbaijan, and 10 of 136 (7.4%) horses from Yazd province. The prevalence of *S. aureus* colonization by breed was as follows: 29.1% (16/55) in crossbred, 19.1% (9/47) in Turkmen, 41.3% (26/63) in Kurd, 8.2% (7/85) in Arab, and 5.9% (3/51) in Thoroughbred.

By using the *agr* typing method, all retrieved isolates fell into one of four previously described *agr* groups, with types III ($n = 30$; 49.2%) and IV ($n = 28$; 45.9%) being predominant followed by types II ($n = 2$, 3.3%) and I ($n = 1$, 1.6%). Amplification of the polymorphic X region of the *spa* gene from the isolates yielded four differently sized amplicons of approximately 1360, 1310, 1260, and 1130 bp that were distributed in isolates at 44.3, 29.5, 16.4, and 9.8%, respectively. Restriction of the resulting amplicons with *Hae*II distinguished the isolates into eight genotypic patterns designated as N1-N8 (Fig. 1), of which patterns N2 (37.7%; $n = 23$) and N4 (29.5%; $n = 18$) were predominant in West and East Azerbaijan provinces, respectively (Table 1). As shown, the greatest diversity was found in isolates obtained from Arab breed with seven isolates assigned to five *spa*-RFLP patterns. Based on sequencing and determining *spa* repeat successions, the representative strains of each RFLP pattern (N1-N8) were assigned to *spa* type t084, t2484, t937, t127, t1383, t11926, t1166, and t12595, respectively. The *spa* gene sequence of the representative strain of each RFLP pattern (N1-N8) was submitted to the GenBank database and was assigned the following GenBank accession numbers: MF175192 (correspond to t084), MF175204 (t2484), MF175193 (t937), MF175203 (t127), MF175202 (t1383), MF175197 (t11926), MF175198 (t1166), and MF175200 (t12595).

Regarding the BURP analysis, the resulting *spa* types were clustered into one group (no founder) that consists of *spa* types t1383 and t127. The *spa* types t084, t937, t1116, t2484, t11926, and t12595 were not assigned to any BURP group and were identified as singletons (Table 2).

MLST resolved the representative *spa* types into seven different STs (Table 2). Using the stringent definition of a group, analysis with eBURST showed that the identified STs (with the exception of ST2151 that was shown to be unlinked 'singleton ST') can be divided into four major groups, out of a total of 83 groups dividing all of the currently available STs in the *S. aureus* MLST. Each of the detected STs associated with their clonal complexes represented by figures drawn by eBURST (Fig. 2).

The phylogenetic analysis of eight representative strains of each RFLP pattern along with five MRSA strains grouped them in two great clusters (I and II) (Fig. 3). The two predominant RFLP types N2 (correspond to ST2151-t2484) and N4 (correspond to ST1-t127) showed lower phylogenetic similarity and were placed in different clusters.

Five of 61 *S. aureus* isolates (8.2%) were found to be MRSA, which belonged to clonal types ST2151-t2484-SCC*mec*IVa ($n = 2$), ST15-t084-SCC*mec*IVa, ST1-t1383-SCC*mec*IVa, and ST1278-t12595-SCC*mec*IVa (one isolate each). Of these, three were isolated from Kurdish horses aged 7-year-old ($n = 2$) and 9-year-old ($n = 1$), one from Crossbred aged 7 years, and one from Persian Arab aged 8 years (Table 1). The *spa* sequence of a MRSA strains has been deposited in NCBI GenBank under accession numbers MF175194-MF175196, MF175199, and

Table 1 Prevalence and genotypic characteristics of nasal *S. aureus* isolates from healthy horses on the basis of breed and sampling region

Province	Breed	No. of horses sampled	No. of *S. aureus*-positive horses [%]	*spa*-RFLP pattern (*spa*-type)							
				N1 (t084)	N2 (t2484)	N3 (t937)	N4 (t127)	N5 (t1383)	N6 (t11926)	N7 (t1166)	N8 (t12595)
East Azerbaijan											
	Crossbred	55	16 [29.1%]	–	–	–	12	4[a]	–	–	–
	Turkmen	47	9 [19.1%]	–	3	–	6	–	–	–	–
West Azerbaijan											
	Kurd	63	26 [41.3%]	–	20[b]	3	–	–	–	–	3[a]
Yazd											
	Persian Arab	85	7 [8.2%]	2[a]	–	2	–	1	1	1	–
	Thoroughbred	51	3 [5.9%]	–	–	–	–	–	–	–	3
Total		301	61 [20.3%]	2	23	5	18	5	1	1	6

[a] One of these isolates was methicillin-resistant *S. aureus* (MRSA)

[b] Two of these isolates were methicillin-resistant *S. aureus* (MRSA)

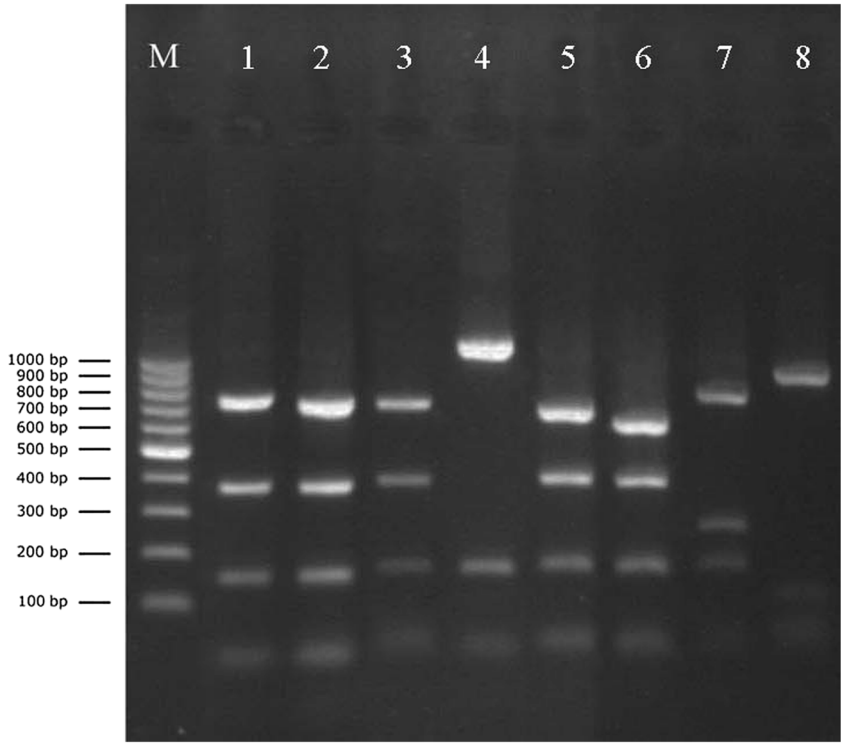

Fig. 1 Restriction polymorphism in the *spa* variable region of *S. aureus* nasal isolates recovered from healthy horses. Examples of the different restriction types obtained are shown: type N1 (lane 1), type N2 (lane 2), type N3 (lane 3), type N4 (lane 4), type N5 (lane 5), type N6 (lane 6), type N7 (lane 7), and type N8 (lane 8). Molecular weight marker (100-bp DNA ladder; Thermo Scientific) is shown in lane M

MF175201. Regarding Panton-Valentine leukocidin (PVL), the *lukS-lukF-PV* genes were not found in any of tested isolates.

Statistical analysis revealed that the rate of *S. aureus* nasal carriage ($P < 0.05$) varies among the studied geographical locations, with the high incidence in West Azerbaijan province (41.3%) and the lowest incidence in Yazd province (7.3%).

Discussion

The overall isolation rate of *S. aureus* from the nose of healthy horses was found to be 20.3% (61/301), with a significant difference across the studied regions. This rate is higher than those reported in healthy horses in Denmark (Islam et al. 2017) and Canada (Burton et al. 2008), but lower than the report in Malaysia (Zunita et al. 2008). Differences in environmental factors, equine husbandry practices and host characteristics could account for these discrepancies.

Our findings revealed that Iranian horses can act as a reservoir for diverse clones of methicillin-resistant and -susceptible *S. aureus*, which is consistent with the findings of Islam et al. (2017). However, the observed clonal types in this study were considerably different from that of other countries, with the exception of types ST133-t1166 and ST1-t127

Table 2 Overview of representative *spa* PCR-RFLP patterns and their corresponding *spa* types, *spa* clonal complex (*spa*-CC), MLST sequence types (MLST_ST), and MLST clonal complexes (MLST_CC)

RFLP pattern	*spa* type	*spa* repeat succession	*spa*-CC	MLST_ST	MLST_CC
N1	t084[a]	07-23-12-34-34-12-12-23-02-12-23	SGT	15	5
N2	t2484[a]	04-20-69-31-70-13-17-16-16	SGT	2151	SGT
N3	t937	08-16-34-24-34-34-17-17	SGT	291	398
N4	t127	07-23-21-16-34-33-13	Cluster 1 (NF)	1	5
N5	t1383[a]	07-23-21-33-13	Cluster 1 (NF)	1	5
N6	t11926	04-20-69-25-16	SGT	700	130
N7	t1166	03-16-21-17-23-13-17-17-17-23-24	SGT	133	133
N8	t12595[a]	04-17-34-17-32-23-24	SGT	1278	5

NF no founder, *SGT* singleton, *MLST* multilocus sequence typing, *ST* sequence type

[a] *spa* types containing MRSA

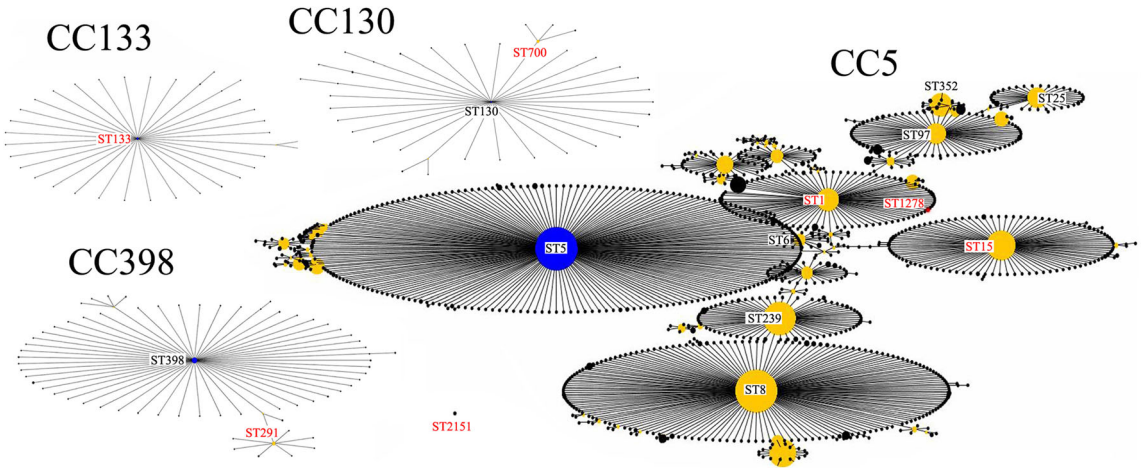

Fig. 2 Population snapshot of *S. aureus* lineages associated with their clonal complexes. All STs identified in this study are highlighted in red, together with their clonal complexes (CC). The primary and subgroup founders of the group are colored blue and yellow, respectively. Each dot represents a single ST, and the size of each dot is proportional to the number of isolates globally deposited

that have been described in MSSA of horses in Denmark (Islam et al. 2017). The later clonal type has also been isolated from horse MRSA infections in Austria (Cuny et al. 2008; Loncaric et al. 2014). These findings indicate that some specialized clones are world-wide in distribution while the others appear to be limited to a specific geographic area. Further studies are required to better understand the geographical distribution of *S. aureus* clones in different countries. The most common clonal types in East (ST1-t127) and West Azerbaijan (ST2151-t2484) provinces were found not to be closely related, indicating a possible difference in their evolutionary origin. ST1-t127 has been reported from various sources including milk samples (Basanisi et al. 2017), production chain of dairy products (Papadopoulos et al. 2018), seafood and the aquatic environment (Murugadas et al. 2017), small ruminants' meat (Sergelidis et al. 2015), as well as human specimens (Manara et al. 2018), while ST2151-t2484, as far as we know, has only been isolated from donkeys in Tunisia (Gharsa et al. 2012). *S. aureus* isolates belonging to clonal CC398 have emerged over the previous decade as a risk to livestock workers (Smith and Wardyn 2015). This study detected, for the first time, a double-locus variant (DLV) of the livestock associated *S. aureus* ST398 (ST291-t937) in five MSSA isolates for which MRSA ST291 has previously been

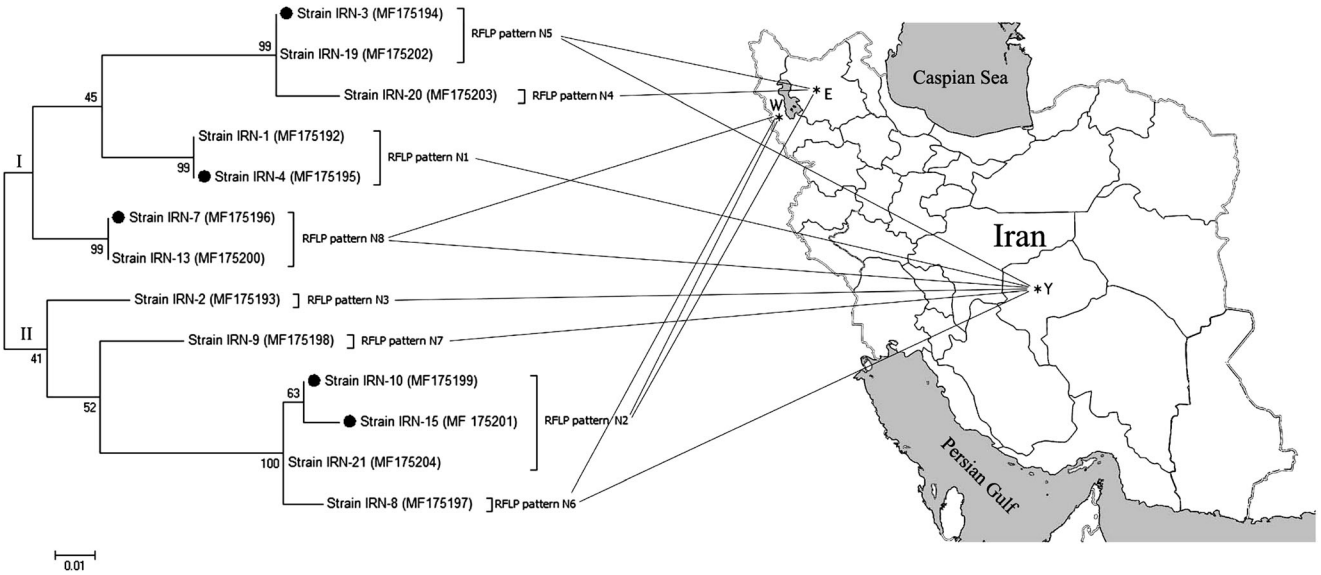

Fig. 3 Phylogenetic tree constructed based on nucleotide sequence of X region of the *spa* gene from eight strains of *S. aureus* (representatives of eight patterns obtained by RFLP) along with five MRSA strains isolated from the nares of horses, using the maximum likelihood method with bootstrap of 1000 replicates. Each strain is indicated by the *S. aureus* sample number followed by the GenBank accession number given in parenthesis. The MRSA isolates of the current study are indicated by round (●). E: East Azerbaijan province; W: West Azerbaijan province; Y: Yazd province

reported from human infections in Iran (Havaei et al. 2013; Ohadian Moghadam et al. 2015). This observation raises concerns about conversions of ST291-MSSA into ST291-MRSA in the community. Additionally, ST291-t937 MSSA has earlier been recognized in the nasal cavity of a dairy cow in Belgium (Vandendriessche et al. 2014) and a healthy human in India (Shambat et al. 2012). These findings indicate that nasal cavity of both humans and animals may constitute a colonization niche for this clone in some parts of the world. The finding of *spa* type t11926 for the first time in one MSSA horse nasal isolates is unexpected, as this has just been reported from Germany (https://spa.ridom.de/spa-t11926.shtml). Whole-genome sequencing (WGS) will be a useful tool to get insights into its epidemiology and phylogenetic relatedness. Despite the number of *S. aureus* from Persian Arab breed was limited, a wide variety of clonal types were identified. This may be accentuated by the genetic heterogeneity of this breed over time, as Persian Arab is one of the oldest breeds in the world and is distributed throughout the country for centuries and originated from a vast number of mares (Moridi et al. 2013).

The anterior nares are among the most widely studied MRSA colonization sites in animals and in humans (Karkaba et al. 2016). We detected MRSA in 1.7% (5/301) of the horses in the study population, which is somewhat similar to that reported in western Canada (Tokateloff et al. 2009), however, lower than those reported from horses in Denmark and Australia (Axon et al. 2011; Islam et al. 2017), but higher than the 0.53% found in horses in Belgium (Van den Eede et al. 2012). MRSA isolates were genoyped as ST15 (*spa* type t084), ST1 (t1383), ST2151 (t2484; $n = 2$), and ST1278 (t12595), and all of them contained SCC*mec* type IVa. *S. aureus* ST15 and ST1 are typical human associated clonal lineages (Cuny et al. 2010), pointing towards a possible human to horse transmission. It is also interesting to point out that the MRSA t084 strains have previously been recovered among isolates from nasal colonization of students (Japoni-Nejad et al. 2013) and also isolated from healthcare and community-acquired infections in humans in Iran (Fasihi et al. 2017), indicating a worrisome situation with the possibility of its zoonotic potential. As far as we know, the observed ST2151-MRSA in the current study has only been isolated from donkeys in Tunisia (Gharsa et al. 2012), while t12595-MRSA has just been reported from Pakistan (http://spa.ridom.de/spa-t12595.shtml). MRSA and MSSA isolates from our study were found to be genetically similar, which supports the hypothesis that some MSSA strains may be more receptive to the transfer of a mobile SCC*mec* element (Katayama et al. 2005). However, this finding is in contrast with the results of the previous study where MRSA and MSSA strains from horses were not clonally related and they presented different *spa* types (Islam et al. 2017). Three out of five MRSA isolates identified in this study were originated from Kurd breed, but the reasons behind this are unclear. However, stress due to transportation, cross-border dissemination, direct contact with humans and other animals during life in an open environment may be responsible factors for higher prevalence of MRSA carrier in this breed. To what extent these and other non-modifiable risk factors play a role in MRSA nasal colonization warrants further investigation. The *lukS-lukF-PV* genes were not found in any of the *S. aureus* isolates tested, which is in agreement with other studies (Tokateloff et al. 2009; Agabou et al. 2017; Islam et al. 2017).

Colonization is a multifactorial process that requires a variety of adaptive mechanisms, including nutrient acquisition, adherence to host tissues, and evasion of, or protection against, host defenses (Kiser et al. 1999). The role of *agr*-mediated inter-species and intra-strain interferences in nasal colonization has also been proposed (Lina et al. 2003; Barbagelata et al. 2011). In this study, isolates belonging to different clonal types often gave the *agr* types III ($n = 30$; 49.2%) and IV ($n = 27$; 44.3%), suggesting well adaptation of subset of *S. aureus* isolates to the nose of horses likely by *agr* regulatory system. More expanded study is, however, required to investigate this hypothesis.

In summary, this study, for the first time, revealed that Iranian horse population can be healthy carriers of diverse clones of *S. aureus* (either MSSA or MRSA). The results also provide evidence for the presence of human-related MRSA clones, emphasizing the importance of comprehensive epidemiological surveillance. In addition, the rate of *S. aureus* nasal carriage varied with geographic locations. Further studies are needed to determine the epidemiology and risk factors, such as cross-contamination via the environment and its reservoirs, for *S. aureus* nasal colonization of horses in Iran.

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Compliance with ethical standards

The study protocol was approved by the Urmia University Animal Ethics Committee (ethical clearance number 1241).

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

Research involving human participants and/or animals Not applicable.

Informed consent The authors confirm that this article's content has no animal or human participants in research.

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