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ORIGINAL ARTICLE

Molecular profiling of methicillin-resistant *Staphylococcus aureus* isolated from healthy pet dogs and their owners in western Iran

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ABSTRACT

Background and Objectives: Growing apprehension surrounds methicillin-resistant *Staphylococcus aureus* (MRSA) strains. The objective of this investigation was to molecularly profile MRSA strains recovered from asymptomatic companion dogs and their human caretakers in Kermanshah, located in western Iran, marking the inaugural research of its kind within the country.

Materials and Methods: During a six-month period, specimens were obtained from the oral cavities and nasal passages of 200 clinically normal pet dogs, as well as their owners. MRSA isolates were identified, DNA was extracted, and characterized via staphylococcal cassette chromosome mec (*SCCmec*), accessory gene regulator (*agr*), and staphylococcal protein A (*spa*) typing, plus detection of *mecA* and Panton-Valentine leukocidin (*PVL*) genes.

Results: Eighteen MRSA isolates were identified (12 from dogs, six from owners). All carried *mecA*. Most had *SCCmec* type III. Human isolates included one *agr* II and two *agr* IV; no dog isolates were *agr*-positive. Isolates belonged to four *spa* types: t690 (dogs) and t325, t037, t030 (owners). *PVL* genes were absent. Isolates from dogs and owners showed type diversity.

Conclusion: This study reveals MRSA epidemiology in healthy dogs and owners in Iran, with low colonization rates. Continuous monitoring is essential to track MRSA circulation, as colonization raises infection risk.

Keywords: Genotyping; Drug resistance; Methicillin-resistant *Staphylococcus aureus*

INTRODUCTION

Members of the *Staphylococcus* genus routinely inhabit the skin and mucous membranes of people and animals. About 20 to 30 percent of healthy individuals persistently harbor *S. aureus* in the front part of the nose, which can serve as a major source of subsequent infections (1). In dogs, colonization occurs at multiple sites, including the skin, oral cavity, ears, and perineal region. Carriage rates are modulated by host genetics, hormonal influences, age, and the innate antimicrobial properties of nasal secretions (2).

This bacterium can cause many different diseases, including skin and soft-tissue infections, mastitis, bacteremia, infection of the heart valves, osteomyelitis, pneumonia, and foodborne disease (3). In recent years, the pathogen has accumulated resistance mechanisms against multiple antibiotic groups, and

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MRSA infections carry a substantially greater risk of mortality compared with infections by methicillin-sensitive *S. aureus.* The *mecA* gene underlies most of this resistance by producing PBP2a, an altered penicillin-binding protein with low β-lactam affinity, and it is borne on the mobile *SCCmec* cassette. To date, researchers have described thirteen *SCCmec* variants (types I through XIII), but clinical isolates are still most commonly represented by types I to V (4).

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first reported in 1961 and initially confined to nosocomial settings, predominantly affecting elderly or immunocompromised patients. Epidemiologically, MRSA is divided into three principal groups: strains tied to healthcare environments (HA-MRSA), those found in the general community (CA-MRSA), and variants associated with animals and agricultural settings (LA-MRSA). Public health surveillance currently treats healthcare-associated, community-associated, and livestock-associated MRSA as the primary categories for monitoring and studying transmission patterns (5). Each epidemiological category shows a characteristic *SCCmec* signature: community strains are most often associated with types IV and V, hospital strains typically carry types I–III, and livestock-associated isolates are frequently linked to *SCCmec* IVa and V (6).

Community-associated MRSA (CA-MRSA) isolates often carry the *lukS/F-PV* genes, which code for Panton-Valentine leukocidin—a two-part cytolysin that induces lysis of white blood cells and leads to necrotic damage in tissues. Detection of *PVL* is closely associated with life-threatening necrotizing lung infections and repeated abscesses or cellulitis in skin and subcutaneous layers (7). In *S. aureus,* the production of various pathogenic determinants is regulated by the *agr* locus, a density-dependent signaling network that senses population levels. Researchers have identified four *agr* alleles (types I through IV), making *agr* classification a valuable tool for tracing outbreaks. Additionally, *agr* function may influence resistance to antimicrobials in certain scenarios (8).

MRSA serves as a highly infectious agent, with lateral gene transfer playing a key role in maintaining and propagating the bacterium. Several approaches for molecular strain identification have been established to follow the dissemination of MRSA lineages. The *spa* locus produces a cell-surface antigen that contributes to the virulence mechanisms of *S. aureus.* Within this gene, the hypervariable X domain comprises differing quantities of 24-nucleotide motifs, facilitating detailed strain differentiation. Utilizing sequencing of just one locus, *spa* genotyping delivers resolution on par with multilocus sequence typing, yet it proves more efficient in terms of time and resources (9).

Human–animal contact creates opportunities for bidirectional transmission of resistant pathogens. Dogs, the most popular companion animals in Iranian households, represent a significant potential reservoir for zoonotic MRSA, particularly during the COVID-19 pandemic. Notably, pet dog ownership saw a substantial rise amid the COVID-19 outbreak (10). Therefore, monitoring colonization in clinically normal dogs and their owners is crucial for grasping the spread of antibiotic-resistant strains within communities. Given MRSA's significant public health implications, the present investigation aimed to identify the *mecA* and *PVL* genes, *SCCmec* variants I through V, *agr* groups I through IV, and *spa* genotypes in MRSA strains recovered from healthy pet dogs and their owners in Kermanshah, located in western Iran. Kermanshah was chosen for this study because it is located on the Iran-Iraq border, a region potentially vulnerable to cross-border transmission of antimicrobial-resistant pathogens like MRSA, given the documented "catastrophic" rise in antibiotic resistance in Iraq due to decades of conflict. Additionally, previous studies have reported a high prevalence of MRSA isolates in Kermanshah Province, making it a critical area for surveillance of community-associated strains in pets and humans (11). As the largest urban center in western Iran, Kermanshah has experienced increasing pet ownership, which may further heighten the risk of zoonotic transmission.

Most research conducted in Iran has concentrated on the epidemiological aspects and molecular profiling of MRSA among human populations. To our knowledge, no prior publications exist in Iran regarding the genetic analysis of MRSA strains obtained from asymptomatic companion dogs and their human guardians. Dogs were chosen as the target species because they are the most common companion animal in Iranian households, exhibit the closest physical contact with owners, and have been repeatedly identified worldwide as important reservoirs. Dogs are also vectors for bidirectional transmission of human-associated MRSA strains, unlike cats and other less-interactive pets (12, 13).

MATERIALS AND METHODS

Ethical statement. The study received ethical clearance from the Ethics Committee of Sanandaj Branch, Islamic Azad University (approval code IR.IAU.SDJ.REC.1399.055). All pet owners provided informed consent prior to sampling. Participants younger than 15 years were not enrolled.

Study population and sampling method. Sampling took place at veterinary clinics in Kermanshah city from January to June 2022, which is the capital of Kermanshah Province and serves as the largest urban and referral center in western Iran.

We collected specimens from apparently healthy companion dogs presenting for routine care or immunization, along with samples from their healthy owners; owners were people who kept one or more dogs and were not involved in livestock farming. Dog owners who consented to enrollment were required to sign written informed consent forms. All participants completed a detailed questionnaire that collected the following data; for owners – age, sex, any healthcare facility exposure in the previous 12 months, smoking status, and alcohol use disorder; for dogs – age, sex, and any veterinary healthcare facility exposure in the previous 12 months. Dogs and owners with a history of antimicrobial therapy within the preceding six months were excluded from the study.

Cotton swabs moistened in saline solution were employed to take cheek, gum, and nostril samples from 200 healthy dogs. The owners were also sampled by sterile swabs through the nostrils. Swabs were inserted about 2/3 cm deep, rotated in both nostrils and touched with the nasal septum (14). Samples were inoculated into TSB (Q-Lab, Canada) with 6% NaCl and incubated at 37°C for one day.

Isolation and identification of MRSA. The enriched TSB samples were vortexed, and a loopful was plated onto mannitol salt agar (Merck, Germany). The plates were incubated at 35-37°C for one to two days. Mannitol fermenting colonies were subcultured to 5% sheep blood agar and incubated under the same conditions. Morphology and hemolytic activity of colonies were evaluated, and Gram reaction and the presence of catalase, coagulase, and DNase were tested to recognize targeted isolates (15). To further confirm the identification of *S. aureus*, a molecular test was performed using primers for the reference *nuc* gene (16). PCR run parameters: 94°C for 5 min (initial), 30 cycles of 94°C/1 min, 61°C/1 min, 72°C/1 min, and final extension at 72°C for 5 min. Control: *S. aureus* ATCC 25923.

For phenotypic MRSA confirmation, isolates at 0.5 McFarland were plated on Mueller-Hinton agar and tested with a 30 µg cefoxitin disk. Plates were incubated at 35°C for 16-18 h, after which inhibition zones were measured and results categorized according to the 2024 CLSI standards (17). An inhibition zone of 21 mm or smaller (≤ 21 mm) was considered as resistance to methicillin. All MRSA isolates were frozen at -70°C in TSB containing 20% (v/v) glycerol for downstream analyses.

Genomic DNA extraction. DNA from MRSA strains was obtained with a SinaClon Gram-positive kit per the kit manual, and used as a PCR template. Quantification was performed by measuring absorbance at 260 nm; purity was checked by the 260/280 absorbance ratio, with 1.7–2.0 considered pure. Extracts were stored in the refrigerator.

Detection of *mecA* and *SCCmec* types in MRSA isolates. All PCR reactions in the present study were performed in an Eppendorf (Germany) thermal cycler. Included in every PCR run were a sterile deionized water negative control and a positive control (either a reference ATCC strain or a laboratory stock isolate) as specified in (18). The *mecA* assay used a 25 µL reaction mix: master mix (SinaClon; 1 U Taq, 1X buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP), the specified forward and reverse primers (Table 1), and DNA template, according to the early-mentioned thermal cycles (19).

***agr* typing.** *agr* group assignment (I–IV) employed multiplex PCR with published primers (20). Amplicon size overlap necessitated paired amplification of *agr* I and II and of *agr* III and IV (Table 1), and PCR conditions matched those previously described. Positive (*S. aureus* ATCC 25923 or in-house MRSA strains) and negative (nuclease-free water) controls were included in each run.

Detection of *PVL* genes. The presence of genes encoding the S and F subunits of Panton-Valentine leukocidin (*lukS*-PV and *lukF*-PV) was investigated in all isolates by PCR assay. Amplification was performed using the primer pair described by (21). This

Table 1. Primer sequences, annealing temperature settings, and expected amplicon sizes

Target gene	Primer sequence (5′-3′)	Product (bp)	Annealing temperature (°C)	Reference
mecA	GTGAAGATATACCAAGTGATT/ ATGCGCTATAGATTGAAAGGAT	147	52	(19)
SCCmec I	GCTTTAAAGAGTGTCGTTACAGG/ GTTCTCTCATAGTATGACGTCC	613	56	
SCCmec II	CGTTGAAGATGATGAAGCG/ CGAAATCAATGGTTAATGGACC	398		
SCCmec III	CCATATTGTGTACGATGCG/ CCTTAGTTGTCGTAACAGATCG	280	55	
SCCmec IV	GCCTTATTCGAAGAAACCG/ CTACTCTTCTGAAAAGCGTCG	776		
SCCmec V	GAACATTGTTACTTAAATGAGC/ TGAAAGTTGTACCCTTGACACC	325		
agr I	ATGCACATGGTGCACATGC/ GTCACAAGTACTATAAGCTGCGAT	440	55	(20)
agr II	ATGCACATGGTGCACATGC/ GTATTACTAATTGAAAAGTGCCA-TAGC	572		
agr III	ATGCACATGGTGCACATGC/ CTGTT-GAAAAAGTCAACTAAAAGCTC	406		
agr IV	ATGCACATGGTGCACATGC/ CGATAATGCCGTAATACCCG	588		

primer set specifically generates a 433-bp product covering the *lukS*/F-PV locus. PCR was conducted according to the original cycling conditions reported in the same reference (22). Every run incorporated positive controls (*S. aureus* ATCC 25923 or in-house confirmed MRSA isolates) and negative controls (nuclease-free water).

Forward (*luk*-PV-1): 5′-ATCATTAGGTAAAATGTCTGGACATGATCCA-3′

Reverse (*luk*-PV-2): 5′-GCATCAASTGTATTGGATAGCAAAAGC-3′ (S = G/C).

Separation of PCR products. The amplified fragments from PCR were loaded onto agarose gels at a concentration of 1.5% (w/v) in 0.5X Tris-Borate-EDTA (TBE) buffer, with separation achieved through electrophoresis. Fragment lengths were evaluated using a 100 bp Plus DNA ladder supplied by SinaClon. After loading, the gels underwent staining with Safe Stain (SinaClon) and were visualized using a UV transilluminator.

***spa* typing.** The variable X region within the *spa* gene was targeted for PCR amplification employing the primer pair described by (23). Amplification proceeded in reaction volumes of 25 µL.

Forward: 5′-AGACGATCCTTCGGTGAGC-3′

Reverse: 5′-GCTTTTGCAATGTCATTTACTG-3′.

Data analysis. Statistical analyses were conducted employing SPSS version 16 (SPSS Inc., Chicago, IL, USA). Categorical data are reported as counts and percentages. For *spa* typing, strains were classified based on the protocols outlined on the Ridom SpaServer platform. Nucleotide sequences were uploaded to this database for type identification. The system generates a consensus sequence, automatically identifies *spa* repeat units, and designates types. Strains sharing identical *spa* allele patterns are grouped into the same category.

RESULTS

Isolation of MRSA. During the study period, from 200 healthy dogs and their healthy owners, 18 MRSA isolates were obtained from 12 dogs (n=12 isolates, 6%) and six owners (n=6 isolates, 3%). Six dogs and

their owners were both colonized with MRSA. The *mecA* gene was found in all MRSA isolates.

Table 2 shows the characteristics of dogs harboring MRSA. The prevalence of MRSA in dogs less than 1 year old was higher than in other age groups. Of the 12 dogs, 7 were less than 1 year, one dog was between 1 and 3 years old, two dogs were between 3 and 5 years old, and two dogs were more than 5 years old. Moreover, six dogs had a history of exposure to healthcare facilities in the preceding 12 months. The diversity in dog breeds observed in this study (Table 2) suggests that MRSA colonization may not be breed-specific, but further studies are needed to confirm this.

Table 3 shows the characteristics of MRSA-positive owners. All MRSA-positive owners had a history of exposure to healthcare facilities, three were smokers, and three had alcohol use disorder. The age range was from 25 to 43 years. Six owners and dogs were both colonized with MRSA (cases 1-4, 7, and 9). Cases 5, 6, 8, 10-12 were from MRSA-negative owners, although their dogs harbored MRSA.

To further illustrate the epidemiological connections between dog and owner isolates, a paired comparison of the six households where both were MRSA-positive is presented in Table 4. This table highlights the diversity in *SCCmec* types, *agr* groups, and *spa* types, with no identical clones shared between pairs.

Distribution of *SCCmec* types. Out of 18 MRSA isolates, 15 isolates (83.3%) were classified into *SCCmec* types: 10 isolates were from dogs and five isolates were from dog owners. Most isolates carried type III followed by type IV; one isolate carried type I, two isolates type II, eight isolates type III, three isolates type IV, and one isolate carried type V (Fig. 1).

Typing of *agr* and detection of *PVL* genes. Out of 18 isolates, only three isolates (16.7%) carried *agr*: one isolate had *agr* type II, and two isolates carried *agr* type IV. *agr* I and *agr* III were not found. All *agr*-positive strains were isolated from owners, and no dog isolates were *agr*-positive. The *PVL* genes were not found in any isolates.

Table 2. Attributes of canines harboring MRSA strains.

Case	Breed	Age	Sex	Exposure to health care facilities in the preceding 12 months
1	Pug	6 months	Male	Positive
2	Chihuahua	9 months	Male	Positive
3	Malinois	8 months	Female	Positive
4	Doberman	4 years	Male	Positive
5	Great Dane	5 months	Male	Positive
6	Terrier	4 years	Male	Negative
7	Shih Tzu mix	3 months	Female	Negative
8	Terrier	8 years	Female	Negative
9	Shih Tzu-Terrier	7 years	Female	Negative
10	Poodle	7 months	Female	Positive
11	Siberian husky	2 years	Female	Negative
12	German shep-herd	6 months	Male	Negative

Table 3. Features of canine owners carrying methicillin-resistant *S. aureus* strains

Case	Age (years)	Sex	Exposure to healthcare facilities	Smoking	Alcohol use disorder
1	31	Male	Positive	Negative	Positive
2	27	Female	Positive	Positive	Positive
3	27	Female	Positive	Negative	Negative
4	43	Male	Positive	Positive	Positive
7	25	Female	Positive	Negative	Negative
9	33	Male	Positive	Positive	Negative

Table 4. Matched genetic profiling of MRSA strains obtained from canines and their corresponding owners (n=6 pairs)

Pair ID	Dog isolate	Owner isolate	*SCCmec* match	*agr* match	*spa* match	Overall clonal identity
1	*SCCmec* III / *agr*-negative / *spa* t690	*SCCmec* III / *agr* II / *spa* t030	Yes	No	No	Different
2	*SCCmec* III / *agr*-negative / *spa* t690	*SCCmec* III / *agr* IV / *spa* t037	Yes	No	No	Different
3	*SCCmec* III / *agr*-negative / *spa* t690	*SCCmec* III / *agr*-negative / *spa* t325	Yes	Yes	No	Different
4	*SCCmec* IV / *agr*-negative / *spa* t690	*SCCmec* IV / *agr* IV / *spa* t030	Yes	No	No	Different
5	*SCCmec* III / *agr*-negative / *spa* t690	*SCCmec* III / *agr*-negative / *spa* t037	Yes	Yes	No	Different
6	*SCCmec* V / *agr*-negative / *spa* t690	Untypeable / *agr*-negative / *spa* t325	No	Yes	No	Different

This Table pairs the 6 owner MRSA isolates with corresponding dog isolates (from the 12 dog isolates, select-ing paired cases as per the study). It highlights epidemiological diversity, directly addressing the reviewer's concern without needing a visual diagram. All data derived from study results: dogs mostly t690/*spa*-negative *agr*; owners varied. *PVL* was absent in all.

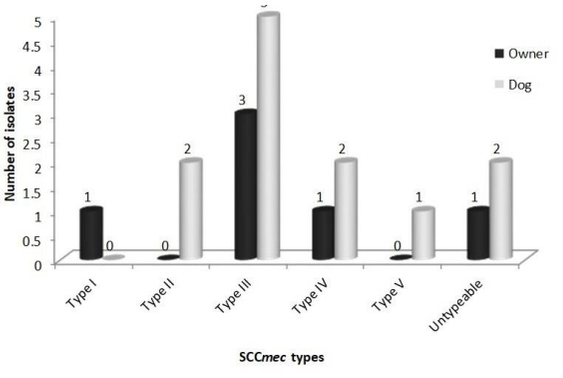

Fig. 1. Breakdown of staphylococcal cassette chromosome mec (*SCCmec*) variants in MRSA strains recovered from canines and owners.

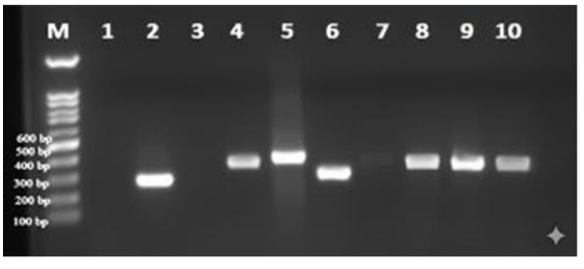

Fig. 2. Gel image of the *spa* gene. M: 100 bp plus DNA marker; wells 1-10: MRSA isolated from healthy dogs and their owners. Wells 1 and 3: *spa*-negative isolates; wells 2, 4-10: *spa*-positive isolates. Fragments ranging in size from approximately 300 to 500 bp are seen.

***spa* typing.** In the amplification of the *spa* gene, fragments ranging in size from approximately 300 to 500 bp were obtained (Fig. 2). Out of 18 isolates, 13 isolates (72.2%) harbored the *spa* gene, of which nine were isolated from dogs and four isolates were from owners. In the dog group, out of nine isolates harboring the *spa*, three isolates had *spa* t690, while six isolates could not be typed. In the dog owner group, out of four *spa*-positive isolates, three isolates had *spa* types t325, t037, and t030, while one isolate was untypeable.

Comparison of MRSA isolates grouped by dogs and their owners. Table 5 shows the comparison of isolates from dogs and owners. As shown, diversity in types was found between the strain isolated from the dog and that isolated from the owner in each case.

In the dogs, three isolates belonged to *spa* t690 (*spa* t690-*SCCmec*V- *agr* non-typeable (NT), *spa* t690-*SCCmec*II, IV-*agr*NT, and *spa* t690-*SCCmec*NT-*agr*NT). Two isolates were *spa* negative-*SCCmec*III-*agr*NT, one isolate was *spa*NT-*SCCmec*III, IV- *agr*NT, one isolate *spa* NT-*SCCmec*II, III- *agr*NT, and one isolate was *spa*NT-*SCCmec*III-*agr*NT. Four isolates could not be typed with any typing method.

In the owners, isolates belonged to *spa* t037-*SCCmec*III- *agr*NT, *spa* t325-*SCCmec*NT- *agr*IV, *spa* t030-*SCCmec*I, III- *agr*II, *spa* negative-*SCCmec*III-*agr*NT, and *spa* NT-*SCCmec*IV-*agr*IV. One isolate could not be typed by any method.

DISCUSSION

Recently, recognition has grown that dogs may act as reservoirs for antimicrobial-resistant organisms, a concern intensified by the marked rise in pet-owning households during the COVID-19 pandemic (22). This study involved genotyping MRSA strains recovered from healthy companion dogs and the people

Table 5. Evaluation of methicillin-resistant and methicillin-susceptible *S. aureus* strains organized by canines and owners.

Cases	Host	Isolate	*mecA*	*SCCmec* type	*agr* type	*spa* type	*PVL* genes
1	Dog	MRSA	Pos.	V	NT	t690	Neg.
	Human	MRSA	Pos.	III	NT	t037	Neg.
2	Dog	MRSA	Pos.	NT	NT	NT	Neg.
	Human	MRSA	Pos.	NT	IV	t325	Neg.
3	Dog	MRSA	Pos.	II, IV	NT	t690	Neg.
	Human	MRSA	Pos.	I, III	II	t030	Neg.
4	Dog	MRSA	Pos.	III	NT	NT	Neg.
	Human	MRSA	Pos.	NT	NT	Neg.	Neg.
5*	Dog	MRSA	Pos.	NT	NT	NT	Neg.
	Human	MSSA	Neg.	ND	ND	ND	Neg.
6*	Dog	MRSA	Pos.	III, IV	NT	NT	Neg.
	Human	MSSA	Neg.	ND	ND	ND	Neg.
7	Dog	MRSA	Pos.	NT	NT	NT	Neg.
	Human	MRSA	Pos.	III	NT	Neg.	Neg.
8*	Dog	MRSA	Pos.	II, III	NT	NT	Neg.
	Human	MSSA	Neg.	ND	ND	ND	Neg.
9	Dog	MRSA	Pos.	NT	NT	t690	Neg.
	Human	MRSA	Pos.	IV	IV	NT	Neg.
10*	Dog	MRSA	Pos.	III	NT	Neg.	Neg.
	Human	MSSA	Neg.	ND	ND	ND	Neg.
11*	Dog	MRSA	Pos.	III	NT	Neg.	Neg.
	Human	MSSA	Neg.	ND	ND	ND	Neg.
12*	Dog	MRSA	Pos.	NT	NT	Neg.	Neg.
	Human	MSSA	Neg.	ND	ND	ND	Neg.

*: Dogs harbored MRSA, but their owners carried MSSA.

NT: unable to type; ND: undetermined; Pos.: affirmative; Neg.: absent

who care for them. During 6 months, 12 *S. aureus* isolates (6%) from dogs and six isolates (3%) from their owners showed methicillin resistance. Studies typically report low rates of MRSA carriage in both healthy companion animals and their owners (24). In a study in Germany on nasal colonization of 112 dogs and 179 humans, MRSA isolates were detected in 2 (1.1%) of the humans but in none of the dogs (25). In the USA, of 499 human subjects, 2.8% carried MRSA, while among 113 dogs, none were MRSA-positive. Among 112 owner–dog pairs sampled in Trinidad, no MRSA isolates were recovered from the animals or the human participants (26). Multiple factors—including a person's age and sex, behaviors such as smoking, exposure to medical facilities, genetic and environmental conditions, hormonal state, non-targeted antibiotic use, compromised immunity, and the innate antimicrobial activity of nasal mucus—can strongly determine nasal colonization by *S. aureus* (27). The MRSA carriage rates were higher among dogs than among their owners in the current study. The higher rate of methicillin resistance seen in isolates from dogs could reflect exposure to antibiotic residues present in animal food.

Demonstrating the relationship between isolates and determining the types of isolates may be useful in preventing the spread of MRSA isolates. Previous studies indicate that *SCCmec* types I–III are commonly linked to healthcare-associated MRSA, while types IV and V predominate in community-associated strains (28). Type III was the most common genotype among MRSA isolates from both dogs and their owners in our study, a variant typically associated with hospital-acquired MRSA. Although the data suggest a hospital origin for these isolates, the evidence is indirect, and further investigations are needed to substantiate this link. However, the association with hospital origins remains speculative and

requires additional epidemiological data, such as patient history or genomic sequencing, for validation. An earlier Iranian screening of nasal swabs from 50 veterinary staff and 49 healthy dogs recovered four MRSA isolates—two from veterinarians and two from dogs—all carrying *SCCmec* type II (29). In contrast, a large South Korean survey recovered only six canine and 27 human isolates, predominantly *SCCmec* type IV with two type III (30). Similarly, a Brazilian study of 241 dogs and 208 owners detected just three MRSA (one canine, two human), all *SCCmec* type II (31).The presence of untypeable *SCCmec* elements in our collection suggests possible novel variants or structural rearrangements (32).

Only three (16.7%) isolates were *agr*-typeable (one *agr* II, two *agr* IV), all from human carriers. This is consistent with earlier observations where the majority of canine MRSA isolates were *agr*-untypeable (33). Although *agr*-defective strains typically exhibit reduced virulence, they often demonstrate enhanced colonization persistence and reduced susceptibility to certain antibiotics, potentially favoring long-term carriage (34). None of the isolates harboured *lukS/F-PV* genes. *PVL* is more commonly detected in clinical isolates from severe or recurrent infections than in colonization strains and shows a strong association with *agr* group III, which was absent in our collection (35). Among the 13 *spa*-typeable isolates, t690 predominated in dogs, while t325, t037, and t030 were found in owners. Previous Iranian reports described t186 and t10897 in canine isolates and t1816 in veterinary personnel (36). The observed diversity between paired dog–owner isolates suggests no direct recent transmission within households, though more discriminatory methods (e.g., WGS) would be required for definitive conclusions.

In the *spa* typing, out of 12 dog isolates, nine had the *spa* gene: in three isolates, t690 was found, and in six isolates *spa* types could not be determined. In MRSA isolated from the owners, *spa* t325, t030, and t037 were found. Limited studies were carried out on the *spa* types of MRSA in healthy dogs and their owners. The types are affected by several factors, including the sample source and geographical location. In Iran, MRSA recovered from veterinarians and healthy dogs comprised three *spa* types: t186 and t10897 from dogs and t1816 from two veterinarians (36). In the Spanish cohort of 67 healthy people and 66 healthy animals, only one MRSA (t5173-*SCCmec*IVa) was detected, while 27 owner-derived isolates were methicillin-susceptible *S. aureus* (37). *spa* typing provides an efficient method for investigating phylogenetic and clonal relationships between isolates and can be practical for monitoring the prevalence of the bacterium. MRSA isolates belonging to type t037-*SCCmec* III and type t690-*SCCmec* IV were previously reported in Iran (38) and Malaysia (39), respectively. MRSA t325-*agr*IV, t030-*SCCmec*III-*agr*II, and t690-*SCCmec*IV are reported in the present study. Diversity in types was found in our study between the strain isolated from the dog and that isolated from the owner in each case. To confirm transmission of MRSA between pets and humans, molecular methods such as WGS or MLST are necessary. According to Weese (2010), when the epidemiology of MRSA alters in a given species, similar alterations may appear in other species (40). Whether, in both species (dogs and humans), new resistant lineages with the capacity to spread between dogs and humans will emerge should be considered.

Limitations. Because the study included a limited number of participants from one city, the findings may not represent the broader population; however, Kermanshah's status as the largest city in western Iran and a regional referral hub increases the local relevance of the results. Our data cannot distinguish between transient carriage, real colonization, or mere contamination, and MRSA in the current study may have been transmitted from the environment. Only long-term studies can determine the real burden of dogs as sources of MRSA. Another limitation is the exclusion of children (<15 years) from the owner cohort. Children often have intense physical contact with pets and could represent an additional transmission route. However, ethical requirements for dual parental consent and child assent made their inclusion logistically impractical in the veterinary clinic setting. Previous studies indicated that adult household members are the primary source of MRSA transmission to dogs; nevertheless, future studies specifically targeting families with young children would be valuable to clarify their role.

CONCLUSION

These results outline MRSA circulation among healthy pet dogs and their owners in Iran, revealing low carriage rates (6% of dogs, 3% of owners),

a predominance of *SCCmec* type III, no detection of *PVL* genes, and notable molecular heterogeneity between canine and human isolates. We believe this work is the first in Iran to molecularly profile MRSA from healthy companion dogs and their owners, and it underscores that the full extent of MRSA in animals and its implications for human and environmental health remain poorly defined, emphasizing its relevance to both veterinary and medical fields.

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