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Short Paper

spa typing of methicillin-resistant *Staphylococcus aureus* isolated from dogs in Ilam Province, Iran

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Abstract

Background: *Staphylococcus aureus* is a significant Gram-positive bacterium commonly found in both human and animal hosts. Among the variants of *S. aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA) has garnered particular concern, as it exhibits resistance to β-lactam antibiotics, including methicillin, posing substantial treatment challenges. **Aims:** The present study investigated the genotypic patterns of *S. aureus* isolated from dogs in Ilam Province, Iran, in order to enhance the understanding of strain diversity and distribution within this population. **Methods:** A total number of 250 samples were collected from the nose, mouth, and rectum of dogs, leading to the identification of 81 isolates confirmed as *S. aureus*. **Results:** Polymerase Chain Reaction (PCR) analysis identified 9 of these isolates as methicillin-resistant *S. aureus* (MRSA), carrying the *mecA* gene. Further *spa* typing categorized the MRSA isolates into three distinct *spa* types: t127, t309, and t3024. The most common types were t127 and t3024, each present in 4 isolates (44.44%), with t309 identified in only 1 isolate (11.11%). **Conclusion:** Our findings highlight a significant genetic diversity of *S. aureus* among dogs, potentially influenced by environmental factors, dietary habits, and social interactions. Notably, the prevalence of the t3024 *spa* type suggests specific regional influences that may contribute to its spread. The observed patterns of genetic diversity underscore the need for further epidemiological studies to address public health challenges posed by *Staphylococcus* infections in both animals and humans. The present study contributes to valuable insights on the dynamics of *S. aureus*, facilitating the development of targeted strategies for infection prevention and control.

Key words: Dogs, Ilam Province, MRSA, *spa* typing, *Staphylococcus aureus*

Introduction

Staphylococcus aureus, a Gram-positive bacterium is a significant concern in both human and veterinary medicine due to its ability to colonize various hosts, including domestic animals such as dogs and cats (Petinaki and Spiliopoulou, 2015; Haag *et al*., 2019; Elnageh *et al*., 2020). This bacterium is well-known for its production of cytotoxic toxins, which can lead to a range of infections, from superficial skin lesions to severe systemic diseases (Tong *et al*., 2015; Stevens *et al*., 2017; Foster *et al*., 2020). In both clinical and community settings, *S. aureus* poses a major public health risk, particularly with the emergence of methicillin-resistant *S. aureus* (MRSA) strains (Gandolfi-Decristophoris *et al*., 2013; Lee *et al*., 2018; Weese and Prescott, 2021). Its adaptability and resistance to many antibiotics make *S. aureus* a particularly challenging pathogen, necessitating ongoing research into effective treatment options and prevention strategies (Darby *et al*., 2022).

The global rise of MRSA has become a formidable challenge, complicating the management of infections and limiting treatment options. The increasing prevalence of MRSA in companion animals, particularly dogs, raises concerns about the potential for these animals to act as reservoirs for human infections, thereby fostering a cycle of transmission between species (Momtaz *et al*., 2018; Bhat, 2021). Understanding the molecular epidemiology of MRSA is crucial for devising effective control strategies that address both human and animal health (Shore *et al*., 2014; Lakhundi and Zhang, 2018; Kasela *et al*., 2023).

In recent years, Ilam province, located in south-western Iran, has drawn attention due to its unique environmental and ecological characteristics that may influence microbial diversity, including the presence and distribution of pathogenic bacteria such as *S. aureus*. Dogs, as common companions in this region, may serve as a critical interface for the transmission of MRSA between domesticated animals and humans. Thus, investigating the genetic characteristics of MRSA in dog populations in Ilam Province is of utmost importance for understanding the dynamics of bacterial transmission and

the pathogenesis of *S. aureus* infections.

The *spa* typing, a genetic characterization method based on the polymorphic X region of the *spa* gene, which encodes protein A, was employed. This technique enables precise strain differentiation and assessment of clonal relationships among MRSA isolates, providing valuable insights into the genetic diversity and epidemiological patterns of this pathogen (Mohammed *et al*., 2021; Alhakeem *et al*., 2024).

The primary aims of our study were to determine the prevalence of MRSA in dogs from Ilam Province, and to analyze the genetic diversity of MRSA isolates through *spa* typing. By conducting a thorough investigation of MRSA, our study also aimed to enhance understanding of the transmission dynamics between humans and dogs, ultimately contributing to the development of effective strategies to control the spread of this resistant pathogen and mitigate its impact on public health and veterinary medicine.

Materials and Methods

Study design, location, and sample collection

This study was conducted at the Microbiology Laboratory and Central Laboratory of Ilam University in Ilam Province, Iran—a mountainous western region with a unique pastoral and border-influenced ecology. The aim was to assess *S. aureus* prevalence in dogs from municipal shelters, veterinary clinics, and related facilities between mid-December 2022 and September 2023. A total number of 250 samples were collected from the nasal, oral, and rectal sites of 85 dogs, originating from a mix of shelters, municipal facilities, and clinics in Ilam Province. Samples could not be obtained from the nose in three cases and from the oral site in two cases. Procedures adhered to sterile conditions to prevent contamination, with each sample immediately transported in tryptic soy broth (TSB) to preserve viability.

Bacterial isolation and identification

Upon arrival at the laboratory, each sample was cultured on selective media, including Blood Agar, Mannitol Salt Agar, Baird-Parker Agar, and DNase Agar. Plates were incubated at 37°C for 24 h. Identification of suspected *S. aureus* colonies was performed through Gram-staining and a series of biochemical tests, including catalase, coagulase, and DNase tests. Gram-positive cocci that exhibited suitable biochemical reactions were further analyzed for confirmation.

Antibiotic susceptibility testing

The Kirby-Bauer disk diffusion method was used to determine resistance profiles on Mueller-Hinton agar with a bacterial suspension of 0.5 McFarland turbidity standards, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2022).

DNA extraction and PCR analysis

DNA was extracted from phenotypically identified S. aureus isolates using the boiling method, as previously described (Tavarideh *et al*., 2022). The resulting DNA was quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and adjusted to a concentration of 50 ng/µL. The *nuc* gene PCR verified *S. aureus* identity (Brakstad *et al*., 1992). All *nuc*-positive isolates were screened for *mecA* via established PCR (Havaei *et al*., 2013). All the methicillin-resistant isolates (*mecA* positive) were subjected to amplification of the *spa* gene (Kahl *et al*., 2005). The list of PCR primers used in the study is shown in Table 1.

spa typing

Spa amplicons were purified with a PCR Purification Kit (Favorgen, Taiwan; Cat. No. FAGCK001-2) per manufacturer guidelines, eluted in 25 µL nuclease-free water, and sequenced via the Sanger method (Macrogen, South Korea). The resulting sequences were analyzed for *spa* typing using the Ridom *Spa*Server online tool, which classified the sequences based on established *spa* type designations.

All experimental procedures were approved by the Animal Ethics Committee of Ilam University, Ilam, Iran (Approval No. IR.ILAM.REC.1403.031).

Results

Phenotypic and molecular identification

Initial phenotypic identification yielded 82 isolates presumed to be *S. aureus*. Of these, the rate of infection with *S. aureus* was related to the nose (43.9%, 36 isolates), mouth (37.8%, 31 isolates), and rectum (18.3%, 15 isolates), respectively (Table 2). Subsequent PCR confirmation targeting the *nuc* gene validated 81 of these isolates as *S. aureus* (data not shown).

Table 1: List of oligonucleotide primers used in this study

Target gene	(5´-3´ primer sequence)	Product size (bp)	Reference
nuc	GCG ATT GAT GGT GAT ACG GTT AGC CAA GCC TTG ACG AAC TAA AGC	279	Brakstad *et al*. (1992)
mecA	AAA ATC GAT GGT AAA GGT TGG C AGT TCT GCA GTA CCG GAT TTG C	533	Havaei *et al*. (2013)
spa	TAA AGA CGA TCC TTC GGT GAG C CAG CAG TAG TGC CGT TTG CTT	Variable	Kahl *et al*. (2005)

Table 2: Frequency of Staphylococcal isolates based on sample sources

Isolate source	Number of isolates	Frequency (%)
Mouth	31	37.8
Nose	36	43.9
Rectum	15	18.3

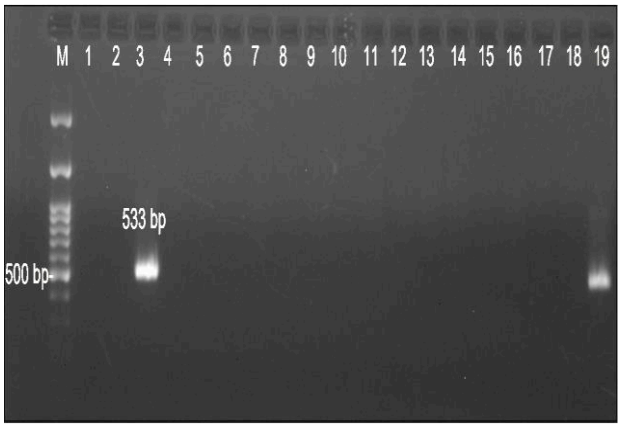

Fig. 1: A representative selection of the PCR products obtained for the *mecA* gene. Lane M: Molecular size marker 100 bp ladder. Lane 1: -ve control, Lane 2: *S. aureus* (ATCC 25293), Lane 3: *S. aureus* (ATCC 33591^{T}), and Lanes 4-18: Isolates codes 1-15, and Lane 19: Isolate code 16 (N112)

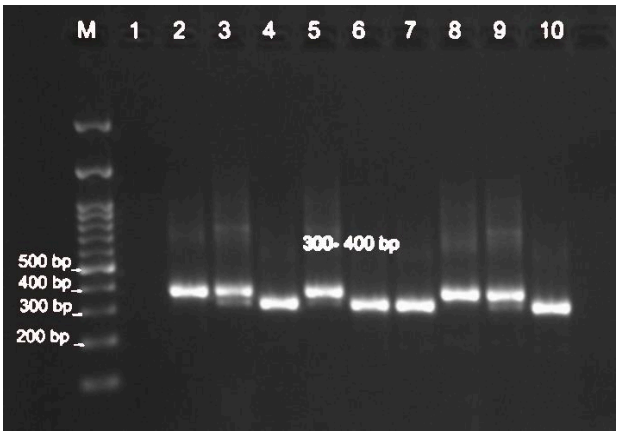

Fig. 2: PCR products of the *spa* gene for the methicillin-resistant isolates (*mecA* gene positive). Lane M: Molecular size marker 100 bp ladder. Lane 1: -ve control, Lane 2: Isolate code 16, Lane 3: Isolate code 17, Lane 4: Isolate code 25, Lane 5: Isolate code 31, Lane 6: Isolate code 34/1, Lane 7: Isolate code 40, Lane 8: Isolate code 50, Lane 9: Isolate code 66, and Lane 10: Isolate code 68

Antibiotic susceptibility test

The susceptibility of *S. aureus* isolates to the following antibiotics was determined: oxacillin, penicillin, vancomycin, cefoxitin, ceftriaxone, gentamicin, tetracycline, ampicillin, erythromycin, imipenem, clindamycin, streptomycin, doxycycline, and ciprofloxacin. Resistance rates were found to be (13.6%, 11 isolates), (79%, 64 isolates), (0%, any isolate), (21%, 17 isolates), (30.86%, 25 isolates), (51.85%, 42 isolates), (48.15%, 39 isolates), (80.25%, 65 isolates), (45.7%, 37 isolates), (1.24%, 1 isolate), (9.88%, 8 isolates), (11.12%, 9 isolates), and (37.1%, 30 isolates), respectively.

MRSA characterization

Among the 81 confirmed *S. aureus* isolates, 9 were identified as MRSA through PCR amplification of the *mecA* gene (Fig. 1). The unidentified species of the genus *Staphylococcus* (isolate code 72) lacked this gene.

spa typing

The *spa* typing analysis of the 9 MRSA isolates revealed three distinct *spa* types: t127, t309, and t3024. The most prevalent *spa* types were t127 and t3024, each found in 4 isolates (44.44%), while t309 was identified in only 1 isolate (11.11%) (Fig. 2 and Table 3).

Discussion

Our findings shed light on the molecular epidemiology of MRSA in dogs from Ilam province, Iran, revealing a concerning prevalence and a noteworthy genetic diversity among isolates. With a detection rate of 11.11% for MRSA in canine *S. aureus* isolates, the present study aligns with emerging evidence about the role of companion animals as reservoirs for antibiotic-resistant pathogens (Feuer *et al*., 2024). The implications for both animal and public health are significant, particularly given the zoonotic potential of certain strains (Algammal *et al*., 2020; Leonard and Markey, 2021).

The phenotypic antibiotic susceptibility testing of the 81 *S. aureus* isolates in this study revealed resistance to oxacillin in 13.6% (11 isolates) and to cefoxitin in 21% (17 isolates), key phenotypic markers of methicillin resistance that aligned closely with genotypic confirmation, where PCR amplification of the *mecA* gene identified 9 isolates as true MRSA, yielding a strong

Table 3: Results of *spa* typing obtained for the MRSA isolated from dogs in this study

No.	Isolate code	Repeat units	Repeat succession	*spa* type
1	16	9	26:23:05:17:25:17:25:16:28	t309
2	17	9	07:23:12:34:34:34:12:12:23	t3024
3	25	7	07:23:21:16:34:33:13	t127
4	31	9	07:23:12:34:34:34:12:12:23	t3024
5	34/1	7	07:23:21:16:34:33:13	t127
6	40	7	07:23:21:16:34:33:13	t127
7	50	9	07:23:12:34:34:34:12:12:23	t3024
8	66	9	07:23:12:34:34:34:12:12:23	t3024
9	68	7	07:23:21:16:34:33:13	t127

correlation between phenotypic resistance and the *mecA* determinant in 81.8% of oxacillin-resistant cases. This concordance highlights the reliability of *mecA* as the primary molecular driver of resistance, with minor discrepancies (e.g., two phenotypically resistant isolates lacking *mecA*) likely due to alternative mechanisms such as PBP modifications or efflux systems, or interpretive thresholds, and the absence of *mecA* in the unidentified Staphylococcus isolate (code 72), affirming genotypic specificity. Comparatively, a cross-sectional study from Punjab, Pakistan, involving 1000 clinical specimens reported a higher MRSA prevalence of 61.8% among 102 *S. aureus* isolates, with *mec* genes detected in 96.8% (61/63) of MRSA, including a notable 57.1% harboring both *mecA* and *mecC* alongside lone *mecA* (31.8%) and *mecC* (7.9%) variants, underscoring *mecC'*s emerging role in β-lactam resistance evolution and the need for dual-gene screening in high-burden settings where phenotypic resistance to agents like penicillin (near 100%) contrasted with retained susceptibility to vancomycin (98.4%) (Idrees *et al*., 2023). Similarly, an Iraqi outpatient study of 290 specimens isolated 65 *S. aureus* (22.4%), of which 95.4% (62) were phenotypically MRSA with 96.8% *mecA* positivity, mirroring the high genotypic-phenotypic alignment but revealing rare exceptions (e.g., two *mecA*-negative MRSA and one *mecA*-positive MSSA) akin to this cohort, alongside 7.7% vancomycin resistance and diverse *spa* types (e.g., dominant t304 at 30.3%) that suggest clonal dissemination (Mohammed *et al*., 2021). Collectively, these findings across regional studies reinforce a robust yet regionally variable correlation between phenotypic MRSA traits and *mecA*-mediated mechanisms, advocating integrated molecular surveillance to track discrepancies and guide empirical therapy amid rising multidrug resistance.

The identification of multiple *spa* types—specifically t127, t3024, and t309—further underscores the complexity and dynamism of MRSA epidemiology in this context (Goudarzi *et al*., 2016; Asadollahi *et al*., 2018; Di Ruscio *et al*., 2018).

The biofilm formation associated with *spa* type t127 has significant implications for treatment strategies. Biofilms provide a protective environment for bacteria, making them more resistant to antimicrobial agents (Asadpour, 2018; Rajkumar and Mohiddin, 2022).

The predominance of *spa* type t127, which is associated with enhanced biofilm formation, raises alarms about the persistence of MRSA within both veterinary and human populations (Omidi *et al*., 2020). Biofilms can confer significant survival advantages to bacteria, allowing them to endure in various environments and making management strategies more difficult (Ballah *et al*., 2024).

spa type t309 presents an intriguing intersection of human and canine MRSA epidemiology, suggesting a potential horizontal gene transfer mechanism whereby previously susceptible strains have acquired methicillin resistance; thus, its presence among MRSA isolates in this canine population suggests a possible evolution of methicillin resistance in a strain previously susceptible to β-lactam antibiotics (Liang *et al*., 2023). This indicates an evolving landscape in antibiotic resistance that underscores the necessity of ongoing surveillance and research.

The emergence of *spa* type t3024, previously unreported finding in the region, invites further investigation into local environmental factors that may contribute to the distribution of MRSA. Given its high frequency, t3024 could indicate the emergence of a new strain or local adaptation in response to specific selective pressures, such as environmental or social factors associated with canine populations. Its equal presence alongside t127 indicates that new strains may thrive in response to the unique local ecology, potentially influenced by human-animal interactions. Its equal presence alongside t127 indicates that new strains may thrive in response to the unique local ecology, potentially influenced by local animal husbandry practices and human-animal interactions. Understanding these ecological drivers is crucial for developing effective control measures (Baig *et al*., 2020; Abdulah and Al-Hejjaj, 2022).

Overall, this study highlights the importance of integrating public health, veterinary medicine, and environmental perspectives to address the challenges posed by antibiotic resistance. Public awareness among pet owners and veterinary professionals regarding the potential transmission of MRSA is essential (Khairullah *et al*., 2023). Implementing robust hygiene practices and responsible antibiotic use in both spheres will be pivotal in mitigating the spread of these pathogens.

In conclusion, our study provides essential insights into the molecular epidemiology of MRSA among dogs in Ilam Province, revealing significant prevalence rates and genetic diversity among isolates. The identification of *spa* types t127, t3024, and t309 emphasizes the urgent need for increased surveillance and preventive measures targeting the human-animal interface. Due to the zoonotic potential of these strains, public health initiatives should prioritize education and awareness to reduce the risk of transmission.

The findings also serve as a call to action for further research, including elucidation of the molecular mechanisms behind antibiotic resistance, exploration of the virulence factors associated with emerging strains, and long-term monitoring to track changes in prevalence and diversity. Through collaborative efforts across disciplines, we can tackle the ongoing challenges posed by MRSA and protect both animal and human health effectively.

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Conflict of interest

The authors declare no conflicts of interest.

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Use of generative AI and AI-assisted technologies statement

No generative AI tools were used in drafting, analyzing, or revising this manuscript. Grammarly (via university license) assisted solely with language polishing.

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