



## Molecular characterization of methicillin-resistant *Staphylococcus aureus* from bovine mastitis and human contacts in Jordan

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### Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) infection has a significant impact on public health. Therefore, this study investigates the occurrence of MRSA isolated from cows with subclinical mastitis in Jordan during the period from January 2021 to December 2023. It is also to compare their genotypic and phenotypic characteristics with those of isolates from their associated human strains. To achieve this objective, milk samples were collected aseptically and cultured on mannitol salt agar and blood agar. The isolates were identified based on colony morphology, biochemical tests, and PCR confirmation targeting the *nuc* and *mecA* genes. In addition, molecular-based typing techniques (MLST and *spa* typing) have been applied and supplemented by antimicrobial susceptibility testing. A total of 310 bovine *S. aureus* isolates were identified, of which 52 (16.8%) were MRSA. Besides that, 49 and 33 MRSA strains were isolated from people related to and unrelated to these animals, respectively. Antimicrobial susceptibility testing revealed high resistance rates to  $\beta$ -lactams, macrolides, and tetracyclines. Nine distinct *spa* clusters and 21 different *spa* types were observed. The most prevalent *spa* type among the isolates was t386 (38%, n=17). For the first time, two novel *spa* types were discovered among the isolates examined in this study. The cluster pattern of *spa* isolates indicates a possible close relationship between human and animal isolates.

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### Introduction

Bovine mastitis is an inflammation of the mammary gland tissues due to trauma or bacterial infections (1). It is considered a source of economic losses in the dairy cattle industry, as the annual cost of mastitis is estimated to be \$147 per cow because of reduced milk production and culling rate (2). Up to 70% of total loss is caused by mammary tissue damage that leads to decreased milk production (3).

The degree of inflammation in mastitis determines whether it is clinical or subclinical. Clinical mastitis is

characterized by symptoms such as udder redness, swelling, fever, and flakes or clots in the milk (4). Subclinical mastitis is a common and economically important form of bovine mastitis characterized by the absence of visible signs of inflammation in the udder or abnormalities in milk appearance. However, it is associated with a significant increase in somatic cell count, as well as continuous bacterial shedding, allowing pathogens such as *S. aureus* to spread undetected in dairy herds (5). Many bacterial species have been identified as the etiological agent of mastitis, which can be contagious or environmental (6). Contagious pathogens

can be transmitted from cow to cow during milking and have the ability to colonize and multiply on the cow's skin of the teat and inside the udder (7). Environmental pathogens do not inhabit the udder skin or the teats; instead, they primarily exist in farm bedding. The infection can be reduced by lowering exposure of teat ends to environmental pathogens (8). Among bacterial pathogens, *S. aureus* is the main etiological agent of clinical and subclinical bovine mastitis worldwide (9-12). *S. aureus* can cause subclinical mastitis, resulting in a chronic infection for life (13). Chronically infected cattle serve as a reservoir for the rest of the flock, where levels of infection can be reduced by implementing proper udder hygiene and milking procedures (8).

The extensive use of antibiotics increases concern about antibiotic-resistant pathogens in the dairy industry and increases the chance of new antibiotic-resistant bacteria emerging (14). Multidrug resistance (MDR) is common among coagulase-positive and coagulase-negative *Staphylococcus* isolates (15). MDR reduces the effectiveness of mastitis treatments by presenting various bacterial resistance mechanisms, such as phagocytosis evasion, cell-mediated immunity, and the production of enzymes that inactivate most antimicrobial agents (16). Increased resistance in *S. aureus* isolates complicates mastitis treatment; the uncontrolled use of antibiotics in humans and animals has led to the emergence of resistant isolates (17), with particular concern about methicillin-resistant *S. aureus* (MRSA) (18), which is considered a global public health threat (19). MRSA strains were frequently associated with severe infections in humans and animals (20).

*S. aureus* is a major zoonotic pathogen that causes mastitis (21), which can be transmitted between humans and animals (22) and may result in amphixenotic infections (23). *S. aureus* inhabits the nasal cavity in approximately 30% of humans as a commensal organism. However, under certain circumstances, *S. aureus* can cause illness ranging from mild skin lesions to fatal infections (24).

To differentiate *S. aureus* strains among animals and humans, several typing methods can be used to classify organisms within a species using phenotyping and molecular typing (genotyping), where the molecular approach is known as the more appropriate one to study the bacterial population genetics (25). Several molecular typing methods have been developed to differentiate *S. aureus* isolates of the same species (26) to provide an insight into bacterial populations and confirm epidemiological linkage in outbreaks (27) where none of these methods is optimal for all types of epidemiological investigations (26). Various methods are used to type *S. aureus* isolates, including *spa* typing, multilocus sequence typing (MLST), staphylococcal cassette chromosome *mec* (SCC*mec*) typing, and pulse field gel electrophoresis (PFGE). *Spa* typing involves sequencing the variable X region of *S. aureus* surface protein A (*spa*) to identify genetic polymorphisms, whereas in MLST, seven housekeeping genes are sequenced. Sequence types (ST) are

assigned where identical strains are assigned to the same sequence type and closely related strains are assigned to the same clonal complex (24).

The aim of the present study was to determine the prevalence of MRSA in bovine mastitis cases along with humans who are closely related to animals and humans who aren't closely related to animals, as well as to detect the resistance profile, and *spa* types and to apply MLST to reveal shared similarities between all bacterial isolates.

## Materials and Methods

### Ethical statement

Ethical approval for this study was approved by the Animal Care and Use Committee (ACUC-JUST) of Jordan University of Science and Technology and the Institutional Review Board (IRB) of King Abdullah University Hospital prior to sampling.

### Sample collection

The milk samples were collected from 37 farms located in two districts in Jordan: Irbid and Zarqa (Dhlail region), which represent the northern regions of Jordan (Figure 1). 310 milk samples from subclinical mastitis cases were collected between April 2016 and November 2018. Both bacteriological and molecular studies were conducted at the Laboratory of Microbiology at the Faculty of Veterinary Medicine, Jordan University of Science and Technology.



Figure 1: Collection sites of the bovine milk samples used in this study. Map created with Google Earth Pro (Google LLC., Mountain View, CA, USA).

### Isolation and identification of *S. aureus*

In order to identify subclinical mastitis, milk samples were collected. The California Mastitis Test (CMT) was used to diagnose subclinical mastitis, in accordance with the guidelines of the National Mastitis Council (28). In brief, each mammary gland's teats were disinfected with an iodine solution and dried in 70% ethanol. A few foremilk strips were then discarded. A sample of approximately 10 mL of milk was collected in sterile 15 mL Falcon tubes, kept in an

icebox, and transported to the laboratory on the same day. Milk samples (10 $\mu$ L) were cultured in 5% sheep blood agar plates for 24 hours under aerobic conditions at 37°C. Suspected colonies showing hemolysis on blood agar were subcultured onto Mannitol Salt Agar (MSA) to confirm mannitol fermentation. The purified colonies were then examined by Gram staining, and Gram-positive cocci were further tested for catalase and coagulase activity. Presumptive *S. aureus* isolates were confirmed using PCR targeting the *nuc* gene.

### Genomic DNA extraction

Total genomic DNA was extracted from 4-5 colonies of each freshly sub-cultured *S. aureus* isolate grown on nutrient agar plates using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's protocol with some modifications to the pre-incubation step with 10 mg/ml lysozyme and 10  $\mu$ g/ml lysostaphin for 45 minutes. Extracted DNA was stored at -20 °C until further use.

All selected isolates were confirmed as *S. aureus* by the presence of the encoding thermonuclease gene (*nuc* gene) as well as the *mecA* gene conferring methicillin (29). We used the strain ATCC 29213 (American Type Culture Collection, Manassas, VA, USA) as a positive control. A PCR analysis was performed using a MyCycler thermal cycler (Bio-Rad, USA) under the following conditions: an initial 10-minute denaturation step at 94°C, followed by 30 cycles of denaturation at 94°C, annealing at 45°C, 2 minutes of extension at 72°C, and a final extension step for 5 minutes at 72°C. PCR products were evaluated by electrophoresis in 2% agarose gels stained with ethidium bromide.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility of *S. aureus* isolates to 13 antibiotics was tested using the disk diffusion method. The results were obtained in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (30) with a few minor modifications (31). The isolates were tested for resistance to thirteen different antimicrobial agents using antibiotic discs (Oxoid, England) included penicillin G, fusidic acid, oxacillin, erythromycin (15  $\mu$ g), clindamycin (10  $\mu$ g), ceftazidime (30  $\mu$ g), trimethoprim/sulfamethoxazole (25  $\mu$ g), doxycycline (30  $\mu$ g), tetracycline (30  $\mu$ g), chloramphenicol (30  $\mu$ g), ciprofloxacin (5  $\mu$ g), ceftazidime (30  $\mu$ g), and gentamicin (10  $\mu$ g).

### *Staphylococcus aureus Spa*-typing

*Spa* typing of the *S. aureus* protein A gene was performed as described by Mazi *et al.* (32) targeting the polymorphic X region after PCR amplification. BioNumerics 7.6 was used to assign the *spa* repeats and types. *Spa* codes were determined after data submission on the Ridom *Spa* Server website ([www.spaserver.ridom.de](http://www.spaserver.ridom.de)).

### Multilocus sequence typing

The conventional protocol and primers for performing MLST in *S. aureus* were performed as described by Enright *et al.* (33). The housekeeping genes were amplified by PCR and sequenced using Macrogen (Seoul, South Korea). As part of the MLST database [<https://pubmlst.org/>], sequences were assembled using BioNumerics software v7.6, previous and novel sequence types were determined, and data were submitted to it for analysis. The relatedness of the isolates was determined through housekeeping genes. In this manner, isolates could be grouped when at least five allele profiles were identical.

### Results

#### Distribution of MRSA among animal and human isolates

A total of 516 *S. aureus* isolates were recovered from bovine mastitis cases, humans with animal contact, and humans without animal contact. Most MRSA isolates were obtained from humans with animal contact (n=49, 46.2%), followed by humans without animal contact (n=33, 33%), and bovine mastitis (n=52, 16.8%) (Table 1). Figure 2 shows a representative gel of the PCR-amplified products for the *nuc* gene that confirms the identity of *S. aureus* and the *mecA* gene that confirms the presence of methicillin resistance.

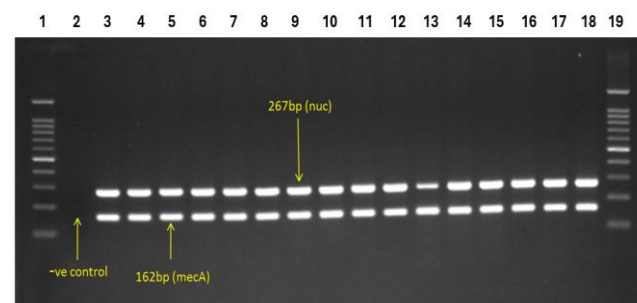


Figure 2: Gel of the PCR-amplified products for the *nuc* gene that confirms the identity of *S. aureus* and the *mecA* gene that confirms the presence of methicillin resistance.

#### Antimicrobial susceptibility testing of MRSA isolates

Antimicrobial resistance of MRSA isolates from bovine mastitis (n=24), humans with animal contact (n=48), and humans without animal contact (n=33) was tested against 13 antimicrobials (Table 2).

Among the 24 bovine isolates, the highest resistance was observed to penicillin G (100%, n=24), followed by oxacillin (79%, n=19), erythromycin (75%, n=18), ceftazidime (63%, n=15), and ceftazidime (63%, n=15). Resistance to tetracycline (58%, n=14), doxycycline (29%, n=7), fusidic acid (29%, n=7), clindamycin (17%, n=4), ciprofloxacin (8%, n=2), gentamicin (8%, n=2), trimethoprim/sulfamethoxazole (8%, n=2), and chloramphenicol (4%, n=1) was also detected (Figure 3).

Table 1. Prevalence of-methicillin-resistant *S. aureus* (MRSA) among bovines and humans in Jordan

Origin	No. of <i>S. aureus</i> isolates	Prevalence of MRSA (%; No. of isolate-positive samples/ No. of tested samples)
Bovine mastitis	310	16.8 (52/310)
Human (related to animals), nasal swap	106	46.2 (49/106)
Human (unrelated to animals), nasal swap	100	33 (33/100)

Table 2. Antimicrobial resistance among bovine mastitis, humans who are closely related to animals, and humans who aren't closely related to animals

Antimicrobial	No. of Mastitis (%)	No. of Related (%)	No. of Non-Related (%)	<i>p</i> -value
Ciprofloxacin	2 (8%)	2 (4%)	3 (9%)	0.637
Clindamycin	4 (17%)	9 (19%)	4 (12%)	0.727
Trimethoprim/ Sulfamethoxazole	2 (8%)	6 (13%)	0 (0.0%)	0.113
Chloramphenicol	1 (4%)	5 (10%)	4 (12%)	0.576
Doxycycline	7 (29%)	12 (25%)	4 (12%)	0.24
Gentamicin	2 (8%)	11 (23%)	3 (9%)	0.132
Erythromycin	18 (75%) <sup>a</sup>	15 (31%) <sup>b</sup>	10 (30%) <sup>b</sup>	0.001
Penicillin G	24 (100%)	48 (100%)	33 (100%)	NA
Cefazolin	15 (63%)	32 (67%)	28 (85%)	0.112
Cefoxitin	15 (63%)	32 (67%)	28 (85%)	0.112
Oxacillin	19 (79%)	40 (83%)	28 (85%)	0.848
Fusidic acid	7 (29%)	15 (31%)	5 (15%)	0.241
Tetracycline	14 (58%)	22 (46%)	16 (48%)	0.6

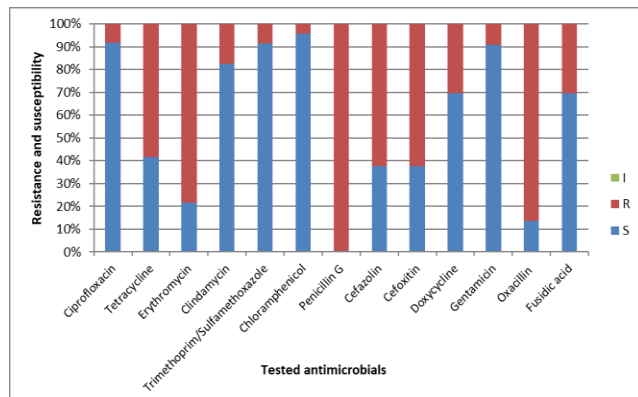


Figure 3: Resistance and susceptibility to a variety of antibiotics among bovine MRSA isolates. I: Intermediate, R: Resistant, and S: Susceptible.

Among the 48 isolates from humans with animal contact, resistance was highest to penicillin G (100%, n=48), followed by oxacillin (83%, n=40), cefazolin (67%, n=32), cefoxitin (67%, n=32), tetracycline (46%, n=22), erythromycin (31%, n=15), fusidic acid (31%, n=15), doxycycline (25%, n=12), gentamicin (23%, n=11),

clindamycin (19%, n=9), trimethoprim/sulfamethoxazole (13%, n=6), chloramphenicol (10%, n=5), and ciprofloxacin (4%, n=2) (Figure 4).

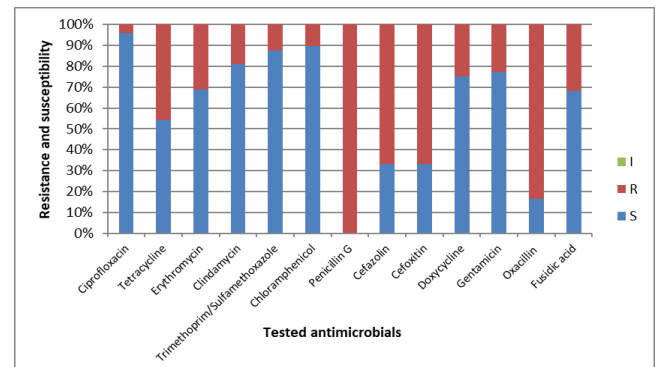


Figure 4: Resistance and susceptibility to a variety of antibiotics among humans who are closely related to animals. I: Intermediate, R: Resistant, and S: Susceptible.

Among the 33 isolates from humans without animal contact, resistance was highest to penicillin G (100%, n=33), followed by cefazolin (85%, n=28), cefoxitin (85%, n=28),

and oxacillin (85%, n=28). Resistance was also observed to tetracycline (48%, n=16), erythromycin (30%, n=10), fusidic acid (15%, n=5), clindamycin (12%, n=4), chloramphenicol (12%, n=4), doxycycline (12%, n=4), ciprofloxacin (9%, n=3), and gentamicin (9%, n=3), while no resistance was detected to trimethoprim/sulfamethoxazole (Figure 5).

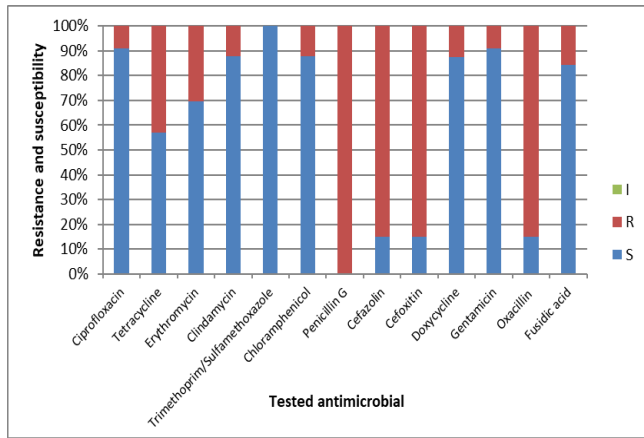


Figure 5: Resistance and susceptibility to a variety of antibiotics among humans who aren't closely related to animals. I: Intermediate, R: Resistant, and S: Susceptible.

**Molecular typing**

The isolates were assigned to nine distinct *spa* clusters and 44 different *spa* types (Table 3). We identified 19 different *spa* types among bovine isolates, including t386 (57%, n=11), t688 (5%, n=1), t223 (5%, n=1), t519 (5%, n=1), t690 (5%, n=1), t084 (5%, n=1), t1339 (5%, n=1), t17158 (5%, n=1), and novel (5%, n=1) (Table 4). Although some MRSA originated from humans who are closely related to animals, we identified 13 different *spa* types, where the most predominant *spa* type was t386 (23%, n=3), followed by t688 (15%, n=2), t021 (15%, n=2), t304 (15%, n=2), t019 (7%, n=1), t535 (7%, n=1), t852 (7%, n=1) and t223 (7%, n=1) (Table 4). Among MRSA isolates from humans without animal contact, 12 different *spa* types were identified, including t386 (25%, n=3), t688 (16%, n=2), t223 (8%, n=1), t14552 (8%, n=1), t1548 (8%, n=1), t318 (8%, n=1), t346 (8%, n=1), t6076 (8%, n=1), and novel (8%, n=1) (Table 4). Figure 6 shows the minimum spanning tree based on *spa* typing for *S. aureus* strains isolated from bovine mastitis, humans who are closely related to animals, and humans who aren't closely related to animals.

By the MLST method, a total of 23 sequences were grouped into four clonal complexes (CCs) named CC1, CC5, CC22, and undefined CC (Table 5). On the other hand, out of seven different sequences, four novel sequence types were identified as ST 7439 (n=1), ST 7440 (n=1), ST 7441 (n=1), and ST 7442 (n=1) (Table 4 and Figures 6 and 7), while the most common sequence types were ST 1 (57%, n=13), followed by ST 5 (22%, n=5), and ST 50 (n=1). Figure 7

shows the minimum spanning tree of MLST data of *S. aureus* strains isolated from bovine mastitis, humans who are closely related to animals, and humans who aren't closely related to animals to illustrate the genetic relationships among the strains and to provide deeper insights into their evolutionary divergence.

Table 3: Numbers and the *spa* cluster of *S. aureus* isolates

Clusters	Mastitis (n=19)	Related persons (n=12)	Non related persons (n=13)	Total
Cluster 1	2	0	0	2
Cluster 2	11	7	6	24
Cluster 3	1	3	2	6
Cluster 4	2	0	0	2
Cluster 5	0	1	3	4
Cluster 6	1	1	0	2
Cluster 7	1	0	0	1
Cluster 8	1	0	0	1
Cluster 9	0	0	2	2

Table 4: The genotypes of *S. aureus* isolates are determined by the molecular typing of protein A (*spa* type)

SPA Type	Mastitis (n= 19)	Non-Related (n= 13)	Related (n= 12)
t386	11	3	3
t688	1	2	2
t223	1	1	1
t021	0	2	0
t304	0	2	0
t519	1	0	0
t690	1	0	0
t084	1	0	0
t1339	1	0	0
t17158	1	0	0
Novel	1	0	1
t019	0	1	0
t535	0	1	0
t852	0	1	0
t14552	0	0	1
t1548	0	0	1
t318	0	0	1
t346	0	0	1
t6076	0	0	1

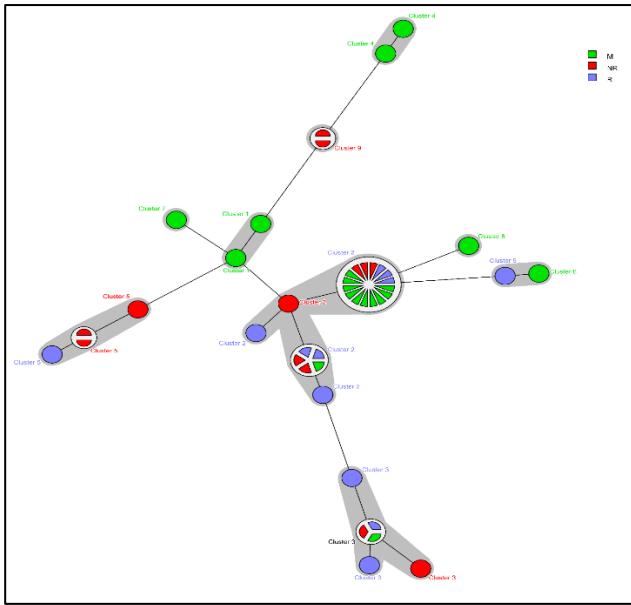


Figure 6: Minimum spanning tree for *S. aureus* strains isolated from bovine mastitis (green), humans closely related to animals (blue), and humans who aren't closely related to animals (red). The analysis was based on *spa* typing results and elaborated on with BioNumerics 7.6 (Applied Maths, Sint-Martens-Latem, Belgium). The size of the circles and the number of sections correspond to the number of isolates associated with each *spa* type.

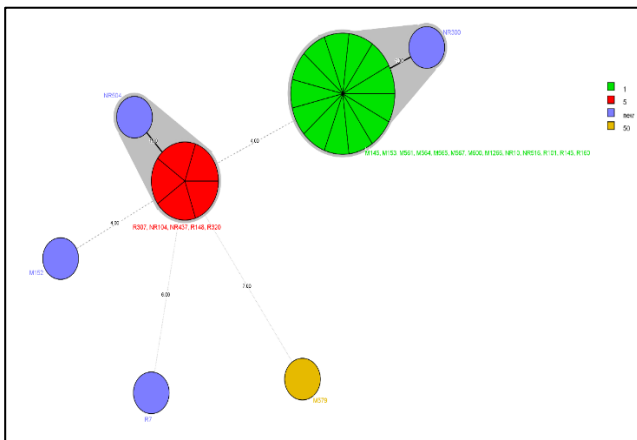


Figure 7: Minimum spanning tree of MLST analysis of *S. aureus* strains isolated from bovine mastitis (green), humans closely related to animals (blue), and humans who are not closely related to animals (red). The analysis was based on MLST typing results and was developed using BioNumerics 7.6 (Applied Maths, Sint-Martens-Latem, Belgium). The number of isolates of each type was indicated by the size of the circles and the number of sections.

Table 5: Isolate sources, numbers and the detected Sequence Types among each group

Clonal Complex	Sequence Type	<i>Spa</i> Type	Frequency
CC1	ST1	t386	8 M; 2 R; 3 NR
	ST7440*	t386	1 NR
CC5	ST5	t688	2 R; 1 NR
	ST5	t535	1 NR
	ST7441*	t1548	1 R
CC22	ST7441*	t688	1 NR
	ST7442*	Novel	1 R
Unknown	ST50	t386	1 M
	ST7439*	t688	1 M

### Discussion

In this study, 52 MRSA isolates (16%) were identified from mastitis cases. Previous studies in Jordan have shown varying prevalence rates of MRSA in bovine samples. Ismail (34) reported a prevalence of 27% of MRSA from clinical mastitis cases, while Obaidat *et al.* (35) found a 20% prevalence of MRSA in samples from bulk tank. Related studies conducted in different regions have reported prevalence rates ranging from 3.33% in Argentina (36) to 28.57% in Egypt (37) and 7.86% in India (38). Our recent study on *S. aureus* isolated from subclinical mastitis in sheep in northern Jordan found that 12.5% of those isolates were MRSA. These isolates were highly genetically similar between farms and did not cross-transfer to animal handlers. (39). Nonetheless, the presence of MRSA in milk heightens the risk of human infection (40) and MRSA has been a worldwide concern over the past two decades due to the increased levels of antimicrobial resistance (35). This highlights the importance of continued research and monitoring to address this global health issue.

In this study, 49 (46%) isolates were identified from individuals with farm-related exposures. To our knowledge, no previous studies have investigated farm workers in Jordan, highlighting the novelty of this research. Globally, MRSA prevalence rates have been reported to be 33.33% in Hungary, 23.9% in the Netherlands, and 4.54% in Korea, which shows varying prevalence rates across different regions.

Although human infections caused by livestock-associated MRSA (LA-MRSA) remain less common than those caused by HA- or CA-MRSA, LA-MRSA still presents risk factors (24), and cases of recurrent infections and even death have been reported and cases of recurrent infections and even death have been reported, since each MRSA isolate carries the potential to cause life-threatening disease (41,42).

This study identified 33 MRSA isolates (33%) from individuals who are not associated with farms and farm activities. Previous reports from Jordan have indicated that

the prevalence of MRSA among healthy individuals ranges from 7.5% to 19% (43-45). Other global reports indicated that MRSA prevalence rates have been reported in the Middle East (8.55%), the United States (23.78%), Poland (22.18%), Italy (16.34%), and China (18.07%) (46).

MRSA is a prevalent human pathogen, colonizing the nasal cavity of up to 33% of healthy individuals. Although nasal colonization is the primary site, extra-nasal colonization also occurs in the skin, throat, and digestive system (47). As a result, HA-MRSA carriers are five times more likely to develop an infection than non-carriers (48). Additionally, reverse zoonosis—where human MRSA is transmitted to cattle—is not uncommon, reinforcing the close interaction between human and animal health (49). In this study, all isolates demonstrated resistance towards penicillin, with the majority also exhibiting resistance to Oxacillin (79%), Erythromycin (75%), Cefazolin (63%), Cefoxitin (63%), and Tetracycline (58%). These resistance patterns align with findings reported by Lubna *et al.* (2023), where resistance levels were high against penicillin (100%), oxacillin (100%), and tetracycline (72.72%).

The resistance observed in subclinical mastitis cases towards penicillin can be attributed to the release of  $\beta$ -lactamase by *S. aureus*, which leads to the hydrolysis of  $\beta$ -lactam rings. Furthermore, it was noteworthy that 59.5% of the strains exhibited resistance to tetracycline, a broad-spectrum antibiotic class that, due to its widespread use, has contributed to the development of resistant strains (48).

Erythromycin resistance in this study was consistent with the findings reported by Obaidat *et al.* (35), although lower resistance rates were reported (50). This suggests that the misuse or overuse of antibiotics has likely played a significant role in the increased resistance observed towards erythromycin.

The most common and shared *spa* type was t386. A previous report from Jordan indicated that while some bovine mastitis isolates belonged to t386, most were of the novel *spa* type t17158 (51). In fact, t386 has been associated with clinical MRSA isolates from humans in Jordan (52), Iraq (53), Iran (54), and Palestine (55). The second most prevalent *spa* type was t688, MRSA t688 was the most prevalent in human hospitals in Kuwait (56), and in milk and milk products in Egypt (20) and Italy (57). In both reports, the t688 isolates were identified as ST5-CC5, a HA & CA-MRSA strain. However, in this study, the t688 MRSA isolate belonged to the novel ST7439, which could not be assigned to a Clonal complex, which suggests that a new MRSA strain is likely to emerge.

In the current study, the vast majority of MRSA isolates (from bovine, related humans and non-related humans) were ST1 and belonged to CC1. MRSA isolates belonging to CC1 were also reported in bovine in Brazil, China, Hungary Germany and the US of A (9), however, CC1 is mainly associated with human *S. aureus* infections. This finding suggests the host-switching ability of *S. aureus* (58), as also

reported by Richardson *et al.* (59), who demonstrated that host-switching events are facilitated by close human–animal contact and the industrialization of livestock production.

Six isolates in this study belonged to CC5 and were exclusive to humans either farm related or farm nonrelated, CC5 is one of the most spread Clone of MRSA worldwide (60, 61). A high prevalence of CC5 isolates was also reported in Libya (62), Kuwait (56), Kenya (63). ST5, which belongs to clonal complex 5 is highly associated with hospital-acquired infections (64). The least number of human isolates belonged to CC22, CC22 also associated with MRSA epidemics mainly ST22 (63), his study employed MLST to investigate the epidemiological status of MRSA in Jordan, Data analysis revealed four novel sequence types and two isolates that couldn't be assigned to clonal complexes. These findings highlight the importance and benefits of molecular-based typing and necessitate further investigation in Jordan.

## Conclusion

This study provides molecular evidence regarding the prevalence of MRSA among bovine mastitis cases and human contacts in Jordan. An analysis of antimicrobial resistance data and genotyping, including *SPA* and MLST analyses, revealed strain overlap between animal and human isolates, suggesting possible interspecies transmission. The results of these studies emphasize the need for an integrated antimicrobial stewardship and monitoring program to monitor, control, and prevent the spread of MRSA among humans and animals.

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## Conflict of interest

There is no conflict of interest.

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والمعزولة من أبقار مصابة بالتهاب الضرع تحت السريري في الأردن خلال الفترة من يناير ٢٠٢١ إلى ديسمبر ٢٠٢٣. كما تهدف إلى مقارنة خصائصها الجينية والظاهرية مع عزلات بشرية مرتبطة بها. ولتحقيق هذا الهدف، جُمعت عينات الحليب بطريقة معقمة وزرعت في أجار ملح المانيتول وأجار الدم. تم تحديد العزلات بناءً على مورفولوجيا المستعمرات والاختبارات الكيميائية الحيوية وتأكيد تفاعل البوليميراز المتسلسل PCR الذي يستهدف جيني nuc و mecA. بالإضافة إلى ذلك، استُخدمت تقنيات التصنيف الجزيئي MLST و التتميط ب sap، و استُكملت باختبارات الحساسية للمضادات الحيوية. تم تحديد ٣١٠ من عزلات المكورات العنقودية الذهبية البقرية، منها ٥٢ عزلة من المكورات العنقودية الذهبية المقاومة للميثيسيلين. كما عُزلت ٤٩ و ٣٣ عزلة من أشخاص مرتبطين بالحيوانات وغير مرتبطين بها، على التوالي. وكشفت اختبارات حساسية مضادات الميكروبات عن معدلات مقاومة عالية للبيتا لكتامز، والماكروليدات، والنتراسيكلين. و لوحظت تسع مجموعات مميزة من spa و ٢١ نمطا مختلفًا، وكان النمط t386 هو الأكثر شيوعًا (٣٨ %، العدد=١٧). كما تم الإبلاغ لأول مرة في هذه الدراسة عن وجود نوعين جديدين من spa. في الختام، يؤكد توزيع وتجمع العزلات المنمطة بجين spa في هذه الدراسة على الاحتمالية العالية لوجود علاقة وثيقة بين العزلات ذات الأصل البشري وتلك المعزولة من الحيوانات، مما يشير للإمكانية انتقال المكورات العنقودية الذهبية المقاومة للميثيسيلين بين الإنسان والحيوان.

## التوصيف الجزيئي للمكورات العنقودية الذهبية المقاومة للميثيسيلين من التهاب الضرع البقري و البشر المخالطين في الأردن

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### الخلاصة

تُعد العدوى بالمكورات العنقودية الذهبية المقاومة للميثيسيلين ذات تأثير كبير على الصحة العامة. لذلك، هدفت هذه الدراسة إلى التحقق من وجود عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين