Iranian Journal of Microbiology
Volume 13 Number 1 (February 2021) 65-73

ORIGINAL ARTICLE

Bovine subclinical mastitis-associated methicillin-resistant *Staphylococcus aureus*, selective genotyping and antimicrobial susceptibility profile of the isolates in Kurdistan province of Iran

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Received: July 2020**, Accepted:** January 2021

ABSTRACT

Background and Objectives: *Staphylococcus aureus* is frequently involved in bovine subclinical mastitis worldwide. Besides, the methicillin-resistant *S. aureus* (MRSA) carrier state of animals is a matter of worrisome. This study aimed to evaluate the frequency of MRSA, discriminatory geno-analysis and antibiotic resistance scheme of the strains isolated from bovine subclinical mastitis in Kurdistan province of Iran.
Materials and Methods: A total of 283 samples were collected and analyzed for *S. aureus* phenotypically and molecularly. SCC*mec* and *coa* types, and *pvl* gene were evaluated using polymerase chain reaction (PCR). Finally, the restriction fragment length polymorphism (RFLP) patterns of *coa* types and the antimicrobial susceptibility profile of the isolates were assessed.
Results: Among the 95 isolates of *S. aureus*, 11 (11.57%) strains were recognized as MRSA. Six, one, and four SCC*mec* types represented for IVa, IVc, and V were determined, respectively, among which an individual IVa and V determinant harboured *pvl* gene. Restriction digestion products of 490 bp, 680 bp, and 730 bp of *coa* bands were generated. Tobramycin, mupirocin, fusidic acid, clindamycin, and chloramphenicol were the most effective drugs against the MRSA isolates.
Conclusion: The detrimental involvement of *S. aureus* in bovine subclinical mastitis is proved herein. Besides, the contribution of MRSA and potential contamination of milk and dairy products with the bacterium may impose a serious public health risk. This demands serious and long-lasting efforts to control the infection. The results may be effective in the implementation of accurate controlling strategies.

Keywords: Mastitis; Bovine; Methicillin-resistant *Staphylococcus aureus*; Genetic profile; Antibacterial drugs resistance

INTRODUCTION

Staphylococcus aureus, a notorious etiological agent of bovine intramammary infection, leads to both clinical and subclinical mastitis in dairy cattle. The importance of the disease, particularly in the subclinical form, is due to not only vast economical loss in animal husbandry but also the potential zoonotic risk imposed by the dissemination of the infection through close contact and/or the food chain (1). Although methicillin-resistant *S. aureus* (MRSA) was originally introduced as a hospital-acquired (HA-MRSA) infection in human (2), the emergence of community- (CA-MRSA) or livestock-acquired MRSA (LA-MRSA) is noteworthy, recently (3). This is a matter of serious concern due to the complications of the therapeutic trials in both the public and

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the veterinary sectors (4). Carrying *mec*A gene, the bacterium may encode a modified low-affinity penicillin-binding protein, conferring resistance to methicillin and most other beta-lactams. Staphylococcal cassette chromosome *mec* (SCC*mec*) types are designated through the embedment of *mec*A-harboring mobile genetic elements in the bacterial chromosome (2). So far, because of the diverse variations in genetic content and structural organization, SCC*mec* elements are classified into 12 types, some of which are further subdivided into subtypes (5).

Panton-Valentine Leukocidin (PVL), a virulence feature of MRSA encoded by *pvl* gene, is a cytotoxin incriminated in tissue necrosis and leucocyte demolition (5). This attributes to skin and necrotizing pulmonary infections in human, in addition to its role in bacterial persistence and spread within the host and the environment (6).

Considering the variability in 3′ end of coagulase gene in *S. aureus*, distinct clones of the bacterium constitute (7). Epizootiological studies have revealed the attribution of particular clones in bovine mastitis in a single farm, some of which have pandemic distribution (1, 8, 9). Hence, exploiting this property through the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) typing of the *coa* gene might be prerequisite in epidemiological insight and applying countermeasures to control the outbreaks within and among herds (8).

Antibiotic therapy in *S. aureus*-originated mastitis is a controversial issue as it is poorly effective (3). It is even more complicated in the cases of MRSA since the cross-resistance of isolates to almost most beta-lactams used in the protocol of mastitis remedy (4). Likewise, some MRSA isolates are multi-drug resistant against various antimicrobial classes. This highlights the significance of surveillance efforts in controlling antimicrobial resistance (AMR) and prescription of the appropriate antimicrobial agent in both veterinary and public operations (10, 11).

Despite the lack of most common virulence traits in LA-MRSA in comparison with CA- or HA- MRSA, it has involved in human infections (4). Based on the scanty of knowledge regarding the frequency of mastitis-involved MRSA and therefore the role of animals as a reservoir of MRSA and their virulence content in the West of Iran, the present research aimed to elucidate the emergence of LA-MRSA and the prevalence of *pvl* gene and SCC*mec* types, *coa* gene diversity, and antimicrobial susceptibility profile of the isolates recovered from bovine subclinical mastitis in Kurdistan province of Iran.

MATERIALS AND METHODS

Sampling. In a cross-sectional study from June to December 2019, 283 subclinical bovine mastitis milk samples, identified in California Mastitis Test (CMT) (12) were collected from all over Kurdistan province, in the West of Iran. An equal amount of approximately 5 mL milk was collected from each teat of an individual cow following disinfection of the udder, blended in a sterile glass bottle and transported to the laboratory within maximum 3 hours. The samples were chilled until delivery. Table 1 represents sampling details regarding season, location, and herd numbers. The study was approved by the Ethics Committee of Sanandaj Islamic Azad University.

***S. aureus* isolation.** Initial cultivation of 50-100 µL from each milk sample was performed onto Columbia agar (CA, Merck, Germany) containing 7% defibrinated sheep blood. An individual presumptive colony of *S. aureus* (round and convex with an approximate of 1-4 mm in diameter) from each plate was subcultured on blood agar (BA, Merck, Germany). Further, the isolates were phenotypically recognized as *S. aureus* based on Gram staining and biochemical reactions including catalase, coagulase, and DNase tests, in addition to a yellow appearance on mannitol salt agar (MSA, Merck, Germany).

DNA extraction and molecular assessments. DNA of *S. aureus* isolates was extracted from overnight culture of the isolates in tryptic soya broth (TSB, Merck, Germany) using Gram-positive bacterial DNA extraction kit (CinnaGen, Iran). Molecular identification of the isolates as *S. aureus* was accomplished in a species-specific PCR reaction by the protocol introduced elsewhere (13).

In the next step, the isolates were phenotypically categorized as either MRSA or methicillin-sensitive *S. aureus* (MSSA) using cefoxitin disk (Patanteb, Iran) diffusion test (14), followed by *mec*A gene amplification in MRSA isolates (15).

A multiplex PCR reaction was applied to assess the frequency of SCC*mec* types as introduced by Ghaznavi-Rad et al. (16). The incidence of *pvl* gene was analysed based on the primer pair and thermal condition

Table 1. The frequency of sampling and MRSA isolates in relation to city, herd number, season, and the studied genes in the present study.

City	Farm number	No. of samples		No. of *S. aureus* isolates		No. of MRSA isolates		SCC*mec* type		*pvl*		*coa* type (bp)	
		winter	spring	winter	spring	winter	spring	winter	spring	winter	spring	winter	spring
A	A1	5	5	3	1	-	-	-	-	-	-	-	-
	A2	3	5	2	2	-	-	-	-	-	-	-	-
	A3	5	4	2	1	-	1	-	V	-	-	-	680
	A4	3	5	2	2	-	-	-	-	-	-	-	-
	A5	5	5	3	1	1*	-	V	-	-	-	680	-
	A6	5	4	1	1	-	-	-	-	-	-	-	-
B	B1	5	3	2	1	-	-	-	-	-	-	-	-
	B2	5	5	2	1	-	-	-	-	-	-	-	-
	B3	4	5	1	2	-	1	-	IVa	-	-	-	730
	B4	4	5	1	1	-	-	-	-	-	-	-	-
C	C1	5	5	3	1	1	1	IVa	IVa	-	1	730	730
	C2	5	5	1	2	-	-	-	-	-	-	-	-
	C3	5	4	2	3	-	-	-	-	-	-	-	-
	C4	5	5	1	1	-	-	-	-	-	-	-	-
	C5	3	4	1	2	-	1	-	IVa	-	-	-	730
D	D1	5	4	0	1	-	-	-	-	-	-	-	-
	D2	3	5	1	1	-	-	-	-	-	-	-	-
	D3	4	4	2	1	-	-	-	-	-	-	-	-
	D4	4	5	1	1	-	-	-	-	-	-	-	-
	D5	5	5	2	3	-	-	-	-	-	-	-	-
E	E1	3	4	1	0	-	-	-	-	-	-	-	-
	E2	3	5	1	2	-	-	-	-	-	-	-	-
	E3	4	4	1	1	1	1	IVc	IVa	-	-	490	730
	E4	5	4	2	1	-	-	-	-	-	-	-	-
F	F1	5	5	3	1	-	-	-	-	-	-	-	-
	F2	4	5	1	1	-	-	-	-	-	-	-	-
	F3	5	3	2	0	-	-	-	-	-	-	-	-
	F4	5	4	2	2	-	-	-	-	-	-	-	-
G	G1	5	5	2	2	1*	-	V	-	-	-	680	-
	G2	3	4	0	1	-	-	-	-	-	-	-	-
	G3	5	4	2	2	2	-	IVa V	-	1 -	-	730 680	-
	G4	3	4	2	2	-	-	-	-	-	-	-	-

*It was not confirmed as MRAS by PCR.

proposed by Lina et al. (17).

Finally, genotyping of *coa* gene was carried out using *Alu*I endonuclease (Fast digest, CinnaGen, Iran), digested PCR product amplified based on the procedure presented by Hookey et al. (18).

In all steps, the amplicons were compared to prototypes used as the positive control. All PCR products were electrophoresed on 1.2% agarose gel.

Antimicrobial susceptibility testing. The antimicrobial susceptibility patterns of the MRSA strains were determined using the Kirby-Bauer method according to CLSI guidelines (14). The used antibiotic disks (Patanteb, Iran) included amoxicillin (20 µg), chloramphenicol (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), erythromycin (15 µg), fusidic acid (10 µg), gentamicin (10 µg), mupirocin (200 µg), penicil-

lin (10 U), rifampin (5 µg), tetracyclin (30 µg), trimethoprim-sulphamethoxazole (1.25/23.75 µg), and tobramycin (10 µg). Besides, vancomycin minimum inhibitory concentration (MIC) was determined by serial microdilution method. *S. aureus* ATCC 25923 was the quality control organism.

Statistical analyses. The statistical association of the frequency of *mec*A determinants with the variables including season, herd, and location were evaluated in SPSS software (version 21.0) using Fisher's exact test. *P* values of less than 0.05 indicated as statistically significant.

RESULTS

In general, 96 (34.16%) strains of *S. aureus* were isolated from the samples phenotypically, all of which were confirmed by generating the expected 275 bp product in *nuc*-based PCR reaction (Fig. 1). Thirteen out of 95 isolates (13.68%) were conventionally identified as MRSA, among which two isolates did not produce the predicted 310 bp *mec*A-specific PCR product (Fig. 2). These two isolates were omitted from further analyses in the study. Likewise, the distribution of SCC*mec* types among the molecularly recognized MRSA isolates were six, one, and four for IVa, IVc, and V, respectively (Fig. 3). Two isolates, each from an individual IVa and V SCC*mec* determinant, harboured *pvl* gene (Fig. 4).

Three different types, with the sizes of 490 bp, 680 bp, and 730 bp, were generated in the partial 3′ end region amplification of the *coa* gene among the isolates (Fig. 5). Also, three distinct restriction fragment length polymorphism (RFLP) patterns were perceived following the digestion of the amplicons with *Alu*I endonuclease, arbitrarily nominated

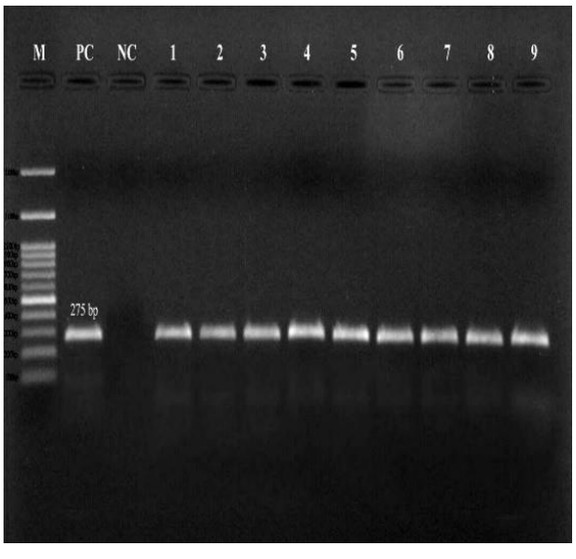

Fig. 1. Agarose gel electrophoresis of PCR products generated from *nuc* gene amplification in *S. aureus* isolates. M: 100 bp DNA Ladder (CinnaGen, Iran), PC: positive control (*S. aureus* ATCC 25923), NC: negative control, Lanes 1-9: field samples.

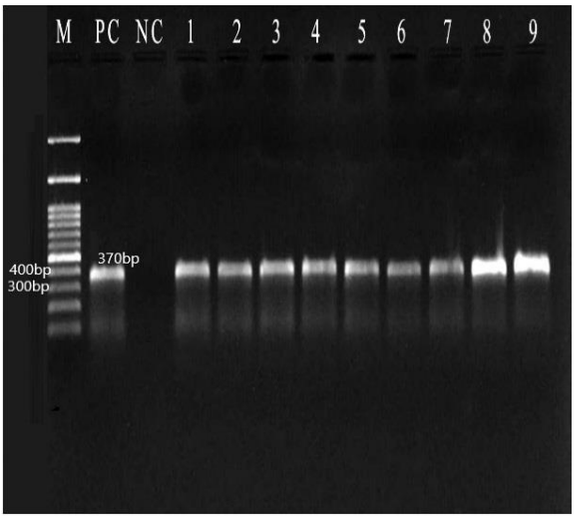

Fig. 2. Agarose gel electrophoresis of PCR products generated from *mec*A gene amplification in *S. aureus* isolates. M: 100 bp DNA Ladder (CinnaGen, Iran), PC: positive control (*S. aureus* ATCC 25923), NC: negative control, Lanes 1-9: field samples.

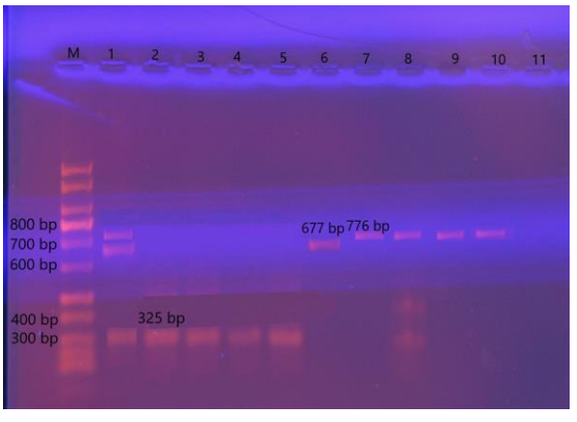

Fig. 3. Agarose gel electrophoresis of PCR products representing SCC*mec* variation among MRSA isolates. M: 100 bp DNA Ladder (CinnaGen, Iran), Lane 1: positive controls, Lanes 2-5: V SCC*mec* type, Lane 6: IVc SCC*mec* type, Lanes 7-10: IVa SCC*mec* type, Lane 11: negative control.

I to III. Each individual amplicon produced a single RFLP pattern including 190 + 270 bp (pattern I), 300 + 410 bp (pattern II), and 320 + 410 bp (pattern III)

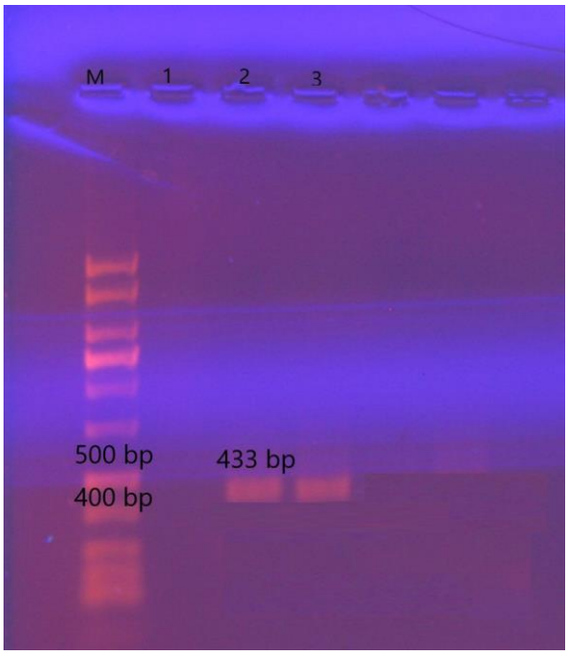

Fig. 4. Agarose gel electrophoresis of PCR products generated from *pvl* gene amplification from MRSA isolates. M: 100 bp DNA Ladder (CinnaGen, Iran), Lane 1: negative control, Lane 2: positive control (*S. aureus* ATCC 49775), Lane 3: field sample.

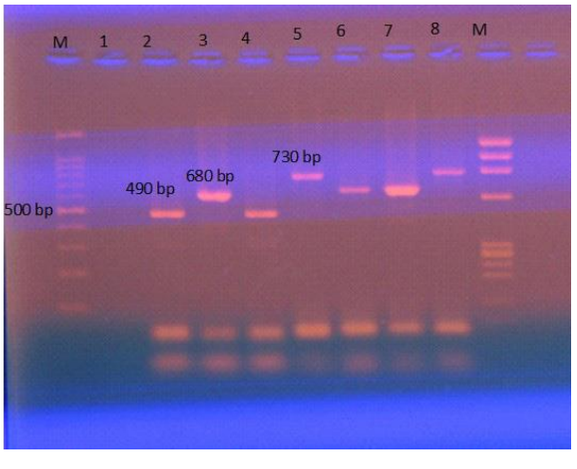

Fig. 5. Agarose gel electrophoresis of PCR products generated from *coa* gene amplification from MRSA isolates. M1: 100 bp DNA Ladder (CinnaGen, Iran), Lane 1: negative control, Lanes 2 and 4: 490 bp product, Lanes 3, 6, and 7: 680 bp product, Lanes 5 and 8: 730 bp product, M2: phiX174 DNA/BsuR1 (*Hae*III) marker, ready to use (Thermo Scientific™).

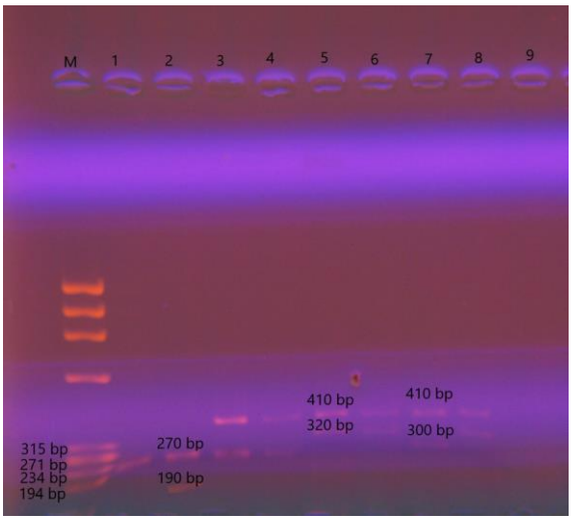

Fig. 6. RFLP patterns generated from *Alu*I endonuclease digestion of *coa* PCR types generated from MRSA isolates. M: phiX174 DNA/BsuR1 (*Hae*III) marker, ready to use (Thermo Scientific™), Lanes 1-2: genotype 1, Lanes 3, 4, 7, 8: genotype II, Lanes 5-6: genotype III.

fragments (Fig. 6). Pattern III was the predominant one (54.54%) among the MRSA isolates, represented in four cities and five herds. While pattern II was retrieved in two cities, and among two herds in each one. Pattern I was detected only in one herd of a city. The co-existence of isolates with patterns II and III was also detected in one herd. The characteristics of MRSA isolates are depicted in Table 1 in details.

Finally, antimicrobial sensitivity analyses of the MRSA isolates represented the most susceptibility to tobramycin, mupirocin, fusidic acid, clindamycin, and chloramphenicol. Sensitivity to gentamicin, rifampin, and vancomycin were in the next step. The frequency of antimicrobial resistance among MRSA isolates is indicated in Table 2 in details.

Besides, the results represented no statistical association between the frequency of MRSA with season (P = 0.25), location (P = 0.99), and herd numbers (P = 0.63).

DISCUSSION

The recent emergence and propagation of LA-MRSA has been considered a public health matter. Given the lack of any obvious clinical signs in subclinical mastitis and therefore delayed recognition of the infection, the isolates may frequently enter the human food chain through the milk (1). This is especially

Table 2. Antibiotic resistance profile of MRSA isolated from subclinical mastitis in the studied region.

MRSA isolate	Amoxicillin (A)	Chloramphenicol (Ch)	Clindamycin (Cl)	Ciprofloxacin (Ci)	Erythromycin (E)	Fusidic acid (F)	Gentamicin (G)	Mupirocin (M)	Penicillin (P)	Rifampin (R)	Tetracyclin (Te)	Trimethoprim-sulphamethoxazole (Ts)	Tobramycin (To)	Vancomycin (V)
1	R	S	S	S	R	S	S	S	R	S	S	S	S	I
2	R	R	S	R	R	S	R	S	R	S	I	S	S	S
3	R	S	S	S	I	I	S	S	R	S	I	I	S	S
4	R	S	S	S	S	S	S	S	R	S	S	S	S	S
5	R	I	I	R	I	S	R	I	R	I	R	S	S	S
6	R	S	S	I	S	S	S	I	R	R	I	R	S	I
7	R	S	S	S	S	S	S	S	R	S	S	S	I	S
8	R	S	S	S	S	S	S	S	R	S	S	I	S	S
9	R	S	S	S	S	S	S	S	R	S	S	S	S	I
10	R	S	I	I	R	I	I	S	R	S	I	R	S	S
11	R	S	S	S	S	S	S	S	R	R	S	S	S	S
Total: N (%)	R: 11 (100) I: 0 (0) S: 0 (0)	R: 1 (9.09) I: 1 (9.09) S: 9 (81.81)	R: 0 (0) I: 2 (18.18) S: 9 (81.81)	R: 2 (18.18) I: 2 (18.18) S: 7 (63.63)	R: 3 (27.27) I: 2 (18.18) S: 6 (54.54)	R: 0 (0) I: 2 (18.18) S: 9 (81.81)	R: 2 (18.18) I: 1 (9.09) S: 8 (72.72)	R: 0 (0) I: 2 (18.18) S: 9 (81.81)	R: 11 (100) I: 0 (0) S: 0 (0)	R: 2 (18.18) I: 1 (9.09) S: 8 (72.72)	R: 1 (9.09) I: 4 (36.36) S: 6 (54.54)	R: 2 (18.18) I: 2 (18.18) S: 7 (63.63)	R: 0 (0) I: 1 (9.09) S: 10 (90.90)	R: 0 (0) I: 3 (27.27) S: 8 (72.72)

R: resistant; I: intermediate; S: sensitive; N: number

more significant in districts, like ours, where unpasteurised milk and dairy products are frequently used. In the present survey, the total prevalence of *S. aureus* and MRSA was 34.16% and 3.88%, respectively. Lower frequency of mastitis-associated MRSA has been reported from Ahvaz, Iran (1.3%) (3) and Finland (1.6%) (19), in contrast with the higher frequency informed from Isfahan, Iran (20%) (20), Belgium (4.4% and 10%) (21, 22), Japan (39.74%) (23), India (13.1%) (24), Bangladesh (29%) (25), Thailand (8.33%) (26), and the Northwest of Iran (15.51%) (27). Despite these, the viable comparison is challenging due to the differences in sampling methodology (21). It is remarkable to mention that the precise prevalence of MRSA in subclinical mastitis has not been assessed in the present research as just a limited proportion of the farms enrolled. As this pathogen is considered a zoonotic threat, continuous monitoring of animal health status and improving the sanitary practice of milking, as well as effective disinfection of plants and training of staff are of great importance in controlling the infection (20).

A possible reason for positive results of MRSA detection in phenotypic method, which were not confirmed by molecular methods may be related to a potential truncation in the open reading frame, resulting in the poor expression of *mec*A gene (7). Besides, variation in the expression of resistance constitutes based on the growth condition (osmolarity of the medium, pH, etc.) (12). This is why the suspected MRSA isolates in the phenotypic method should also be confirmed at molecular level or in a broth microdilution-based MIC detection test (4). On the other hand, the identification of a novel *mec*A homolog, undetectable in routine PCR reactions, emphasizes the application of a selective MRSA-detection medium for MSSA (4).

Currently, SCC*mec* typing is applied as a beneficial tool in molecular epidemiological studies of MRSA. Diverse SCC*mec* types are proved to differ in toxin content and antimicrobial sensitivity. As a shred of evidence, harboring a smaller cassette in SCC*mec* types IV, V, and VII, in comparison with types I, II, and III, they are mostly resistant to beta-lactams. While the latters are commonly resistant to multiple classes of antibiotics (5, 20). The identification of types IV and V MRSA in the present study coincides with the results described elsewhere (19,

20-22). In contrast, Hata has reported the presence of SCC*mec* type II in mastitis associated MRSA outbreak in Japan (23).

Generally, *pvl*-harboring isolates are considered as CA-MRSA. Two of the isolates in the present research were recognized as PVL-positive. PVL-positive MRSA has also been recognized in mastitis milk samples in Bangladesh (4.1%) (25) and Thailand (96%) (26). In contrast, no isolates harboring *pvl* gene have been documented in MRSA isolated from bovine mastitis milk samples in Finland (19), Isfahan (Iran) (20), and Wallonia (southern part of Belgium) (22). Therefore, it is highly recommended to determine the clonal lineage of the isolates, using multi locus sequence typing (MLST), in upcoming studies to accurately evaluate if these isolates are belonging to a CA-MRSA or LA-MRSA genotype. The mountainous subtropical climate of Kurdistan province of Iran is assumed as a risk factor which may influence the higher prevalence of *pvl* gene in the region (28). Meanwhile, because of the possible transmission of the gene through transduction from MSSA to MRSA strains, screening of MSSA isolates for *pvl* is highly recommended (2).

The results showed that three different PCR products were yielded in the amplification of the 3′ end of the *coa* gene. Application of the same prime pair, the presence of different *coa* types in this study agrees with the results reported in some other surveys (1, 8, 9). Although the main reason of this phenomenon in not clear, it is attributed to the insertion or deletion mutations in the *coa* gene which may lead to the alterations in the size and probably antigenic properties of the coagulase enzyme. It is assumed that this variable part of the *coa* gene may play a fundamental role in antigenic variation and therefore the escape of the enzyme from the inhibitory effect of the anticoagulase factors in the body (8). Moreover, the existence of a single-banded *coa* PCR product complies with the results stated elsewhere (8, 9). In comparison, some studies have stated the production of double-bonded *coa* PCR amplicons (1, 29), as a consequence of the presence of different allelic forms of the gene (30). The predominant frequency of particular *coa* types may reflect their elevated compatibility to overcome host defense mechanisms like neutrophil activities than those with the rare genotypes (31). The *coa* types of 730 and 970 bp from Turkey (1), 970 bp from Isfahan and Chaharmahal va Bakhtiari provinces of Iran (9), 850 and 680 bp from Northwest of Iran (27), 759 bp from Brazil (29), and 730 bp in the present study have been demonstrated as the dominant types. Coevolution of the pathogen and its host, herd size, nutrition, management, differences in reservoirs, and the environment of each geographical area are the plausible explanations for this distribution (31). Hence, it is necessary to identify the epizootiology of the involved isolates before applying mastitis control measures. Given the specified targeting of the virulence factors, the presence of limited genotypes in a herd/region is beneficial in controlling the mastitis associated *S. aureus* (10). This is even more significant in the cases of MRSA-originated mastitis.

The genetic homogeneity and the prevalence of 730 bp *coa* type of MRSA in approximately all cities represent the possible dissemination of this strain from one region/herd to another through the introduction of heifers and cows among them, easy dispersion of the strain in close geographical regions, as well as the successful host adaption of this genotype. In contrast, the genetic heterogenicity was also observed as a particular genotype (490 bp) was found in a specific herd merely. This infrequent strain may not well adapted to the udder and therefore its spread is limited. Likewise, the coexistence of multiple genotypes in an individual herd was also detected. These findings are is in line with the reports by Dastmalchi Saei et al. (8).

*Alu*I endonuclease has been frequently used for RFLP analysis of the *coa* PCR products (1, 9, 30). Despite this, Dastmalchi Saei et al. (16) have documented the lack of any restriction site of this enzyme on the repeated region of the *coa* gene in *S. aureus* derived from bovine mastitis in Northwest of Iran. It is validated that the differences in point mutation of the variable regions in individual repeats, may fluctuate the existence of restriction sites for endonucleases among the isolates (10). Following the digestion of *coa* PCR products with *Alu*I, one single RFLP pattern was yielded in all isolates. Interestingly, the pattern in each *coa* type was the same, representing no heterogenicity in *Alu*I restriction site among the isolates. This is concordant with the results documented in another study (9). While some other researchers have indicated distinct genetic diversity in a particular *coa* type (1, 8, 29). Based on the potential public health hazard imposed by MRSA strains and the importance of *S. aureus*-originated mastitis in the dairy industry, these data might be useful in the establish-

ment of effective controlling strategies.

Regarding either the approximately poor respond of *S. aureus*-originated mastitis to chemotherapy or the potential threat for selection and dissemination of resistance genes, precise prescription of antibiotics, based on the results of the antibiogram test, is important for successful elimination of the infection (10). This is even more important in the cases of mastitis associated MRSA. The routine antimicrobial agents administrated for mastitis remedy are beta-lactams, aminoglycosides, lincosamides, and macrolides (32). On the other hand, some antibiotics which are common in the public health sector are also included in the study. The results revealed limited or no resistance to various antibiotic classes. The increasing frequency of resistance to beta-lactams in staphylococci has also been reported in some internal (10, 33), and external studies (21, 34, 35). The selection pressure provoked by the abuse/misuse of beta-lactams is one of the main reasons for this phenomenon, as cloxacillin is frequently prescribed in treatment regime of intramammary infections (IMI) in the region without antibiogram test. Despite our results, some studies have represented high rates of resistance to tetracycline and cephalosporins among MRSA (21, 22, 33, 34). High sensitivity to rifampin, lincosamides, macrolides, and aminoglycosides are in agreement with the results obtained elsewhere (10, 32, 34, 35). Besides, three (27.27%) of the MRSA isolates were multi-drug resistant (MDR). In addition, the fact concerning the horizontal transfer of most antibiotic-resistance genes in *S. aureus* isolated from mastitis udder has previously been reported (34). Fortunately, no resistance was observed against the exclusively prescribed medical antibiotics (fusidic acid, mupirocin, tobramycin, and vancomycin). In another study undertaken in the same district, the frequency of resistance in CA-MRSA to the latters was also limited (5). Generally, due to the importance of the matter in both the veterinary and the public health sector, careful and judicious administration of antimicrobial agents is a must.

CONCLUSION

Holistically, the involvement of MRSA, some isolates as *pvl* determinant, in subclinical bovine mastitis in Kurdistan province imposes the potential public health menace in the region. Acknowledging the *coa* types and antimicrobial patterns of the isolates, precise treatment decisions and management strategies should have been adopted to control the infection.

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