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Original article

Genetic relatedness, antibiogram and virulence factors of *Staphylococcus aureus* isolated from bovine mastitis and related human contacts

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Abstract

The objectives of this study were to determine the genetic relatedness, antibiogram and virulence factors of *Staphylococcus aureus* (*S. aureus*) isolated from bovine mastitis, associated farm workers, dairy cow farm veterinarians (private veterinarians), veterinary students, and non-veterinary university students. A total of 84 *S. aureus* isolates (27 from mastitis, 11 from farm workers, 9 from private veterinarians, 22 from veterinary students, and 16 from non-veterinary university students) were used to determine antimicrobial sensitivity patterns using disk diffusion test, virulence factors using PCR technique and phylogenic analysis using pulsed field gel electrophoresis. All *S. aureus* isolates were resistant to 2 or more commonly used antibiotics. All isolates from mastitis, farm workers, and veterinary students carried the genes encoding coagulase and thermonuclease factors while isolates from non-veterinary university students carried the genes encoding coagulase, clumping, and thermonuclease factors. The mecA gene was detected in 22.2%, 81.8%, 100%, 95.5% and 100% of isolates from mastitis, farm workers, private veterinarians, veterinary students, and non-veterinary university students, respectively. In the phylogenic analysis, 10 (45.5%), 6 (66.7%) and 8 (72.7%) isolates from veterinary students, private veterinarians and farm workers, respectively were more than 80% similar to isolates from mastitis. Results of this study indicate that *S. aureus* isolates from mastitis milk and those from related dairy cow personnel and veterinarians share similar antimicrobial sensitivity patterns and virulence factors, therefore a common source of bacteria may exist. Furthermore, possible transmission of *S. aureus* between cows and cow-related personnel and vice versa could also exist.

Key words: intramammary infection, dairy cows, multi-drug resistant S. aureus, antimicrobial resistance

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Introduction

Staphylococcus aureus (*S. aureus*) is a common opportunistic pathogen of zoonotic and, increasingly, public health concerns (Tong et al. 2015, Reddy et al. 2017). In humans, *S. aureus* is responsible for a variety of hospital-associated infections, dermatitis, endocarditis, and septicemia (Santos et al. 2014, Aberg et al. 2018). The pathogenesis of this organism is based on its ability to interact and adhere to host cells (Johannessen et al. 2012). Many virulence factors are responsible for this pathogen-host interaction including adhesive matrix molecules (AMMs) which includes clumping factor (Clf) A, ClfB, and serine-aspartate repeat containing protein C (SdrC), SdrD and SdrE (Barbu et al. 2010, Foster et al. 2014, Askarian et al. 2016, Pietrocola et al. 2017).

Treatment of *S. aureus* infections in both humans and animals using commonly available antimicrobials has become a great challenge and frustration in many situations. The development of antimicrobial resistance (AMR) among strains of *S. aureus* has become a cause of major concern for human health providers and veterinary services around the world (Garcia-Alvarezet al. 2011, Ammons et al. 2014, Khairalla et al. 2017). Infections caused by multi-drug resistant *S. aureus* (MDRSA) strains are usually non responsive to treatment due to the presence of multiple virulence factors including bio-film formation, toxic shock syndrome toxin (tsst-1), and gene-mediated resistance. Some such genes are responsible for methicillin and vancomycin resistance (mecA, mecC, and vanA, respectively) (Garcia-Alvarezet al. 2011, Ammons et al. 2014, Khairalla et al. 2017).

In animals, *S. aureus* usually resides in the nostrils, mouth and perineum (Al-Tarazi et al. 2011, Graveland et al. 2011, Shen et al. 2013, Woolhouse et al. 2015, Agabou et al. 2017). The bacterium is considered the most common cause of contagious mastitis in dairy cows in Jordan (Alekish et al. 2013, Alekish 2015, Bani Ismail 2017). Transmission of the bacteria between animals and humans is usually by direct contact with contaminated facilities, equipment, and contaminated worker's hands (Jahan et al. 2015). There has been a serious and rising zoonotic threat of some *S. aureus* strains due to contact with infected dairy cows with mastitis (Woolhouse et al. 2015). In fact, several recently published articles have reported that people having daily contact with infected animals including cows may be infected by some strains of *S. aureus* of animal origin (Graveland et al. 2011). However, little information is available in the literature regarding the genetic closeness and shared virulence factors of *S. aureus* isolates from bovine mastitis with those strains isolated from people at risk in Jordan. Therefore the objectives of this study were to evaluate the genetic relatedness, antibiogram and virulence factors of *S. aureus* isolated from bovine mastitis, associated farm workers, private veterinarians, veterinary students, and non-veterinary university students.

Materials and Methods

Sample collection

All experiential procedures performed in this project were approved by the institutional Animal Care and Use Committee of the Jordan University of Science and Technology (JUST-ACUC). Written consents were obtained from farm owners, farm workers, veterinary students, and university students before nasal swabs were collected.

Before sample collection, cows were subjected to a complete physical examination including udder and milk examination. In addition, California Mastitis Test (CMT) was performed and samples were then collected from CMT positive quarters only. Approximately 10 ml of quarter milk samples were collected from selected cows using an aseptic technique as described previously (Al-Tarazi et al. 2011) and placed in an ice box and transported to the laboratory within 4 to 6 hours.

Sterile cotton inoculating swabs (The Science Company, USA) were used to collect nasal samples from both nostrils of associated farm workers and from farm-resident private veterinarians, veterinary students at the Faculty of Veterinary Medicine and unrelated university students at the Jordan University of Science and Technology. To have nasal swabs collected from farm workers and farm-resident private veterinarians, they must have been in close contact with adult cows on a daily basis. After collection, swabs were transferred to the laboratory within 2 to 3 hours after collection.

Bacterial culture and antimicrobial sensitivity test

Milk and swab samples were initially grown on agar plates containing 5% sheep's blood (Oxoid, UK) at 37°C for 24 to 48 hours. Loop full of grown bacteria were then transferred onto culture plates containing mannitol salt agar (Oxoid, UK) and incubated again at 37°C for 48 h. *S. aureus* was then positively identified based on colony morphology, Gram-staining, biochemical tests such as catalase and coagulase and DNase testing (Oxoid, UK) (Jahan et al. 2015; Bani Ismail 2017). Confirmation was achieved then using the Microbact Staph 12S system (Oxoid, UK) and polymerase chain reaction (PCR) targeting 16S rRNA using

Table 1. Primers and sequences used to detect virulence genes in *Staphylococcus aureus* isolated from bovine mastitis, farm workers, veterinarians, veterinary students and university students (N=84).

Primer	Gene	Product size (bp)	Sequence
16S rRNA	16S rRNA		F: GTAGGTGGCCAAGCGTTATCC R: CGCACATCAGCGTCAG
mecA	mecA	163	F: ACTGCTATCCACCCTCAAAC R: CTGGTGAAGTTGTAATCTGG
coa	Coagulase	627	F: ATAGAGATGCTGGTACAGG R: GCTTCCGATTGTTCGATGC
clfA	Clumping factor	1024	F: GGCTTCAGTGCTTGTAGG R: TTTTCAGGGTCAATATAAGC
entA	Enterotoxin A	216	F: AAAGTCCCGATCAATTTATGGCTA R: GTAATTAACCGAAGGTTCTGTAGA
entB	Enterotoxin B	164	F: GTATGGTGGTGTAACTGAGC R: CCAAATAGTGACGAGTTAGG
nuc	Thermonuclease	279	F: CGATTGATGGTGATACGGTT R: ACGCAAGCCTTGACGAACTAAAGC
hla	Hemolysin	930	F: GCCAATCCGTTATTAGAAAAT R: CCATAGACGTAGCAACGGAT
icaA	Intercellular adhesion	770	F: GATTATGTAATGTGCTTGGA R: ACTACTGCTGCGTTAATAAT
Tsst-1	Toxic shock syndrome toxin	326	F: ACCCCTGTTCCCTTATCATC R: TTTTCAGTATTTGTAACGCC

previously published primers (Jahan et al. 2015, Bani Ismail 2017).

Antimicrobial sensitivity tests were performed on all *S. aureus* isolates using 9 commercially available antibiotics using the Kirby-Bauer disk diffusion method as described previously (Alekish et al. 2013, Alekish 2015, Bani Ismail 2017). The antibiotics used in the study were amoxicillin (10 µg), procaine penicillin (10 µg), streptomycin (10 µg), oxytetracycline (30 µg), ampicillin (10µg), doxycycline (30 µg), gentamicin (10 µg), enrofloxacin (5 µg), and trimethoprim-sulfa (15 µg). Sensitivity results were classified according to the diameter of no growth surrounding the disk into sensitive intermediate, or resistant according to previously described methods (Alekish et al. 2013, Alekish 2015, Bani Ismail 2017).

DNA extraction and PCR

The DNA was extracted from all *S. aureus* isolates using commercially available extraction kit according to manufacturer instructions (Majorbio, China). The quality and quantity of isolated DNA was then checked using a spectrophotometer. Several virulence genes were detected using previously published primer sequences (Jarraud et al. 2002, Tristan et al. 2003, Delgado et al. 2011, Bani Ismail 2017). The primers were synthesized at Princes Haya Biotechnology Center (Jordan) (Table 1). The PCR reaction was performed in a total of 25 µL reaction volume which contained 0.6 µM primers, 50 ng DNA template, 12.5 µL master mix, and completed to 25 µL using sterile purified water. The PCR machine was set at 94°C for 4 min for initial denaturation, then at 94°C for 45 s for 30 cycles for final denaturation, 55°C for 30 s for annealing, 72°C for 45 s for extension, and 72°C for 8 min for final extension (Jarraud et al. 2002, Tristan et al. 2003, Delgado et al. 2011).

Pulsed field gel electrophoresis (PFGE)

All *S. aureus* isolates including NCTC 8325 reference strain were subjected to genotyping using PFGE according to previously published procedures (Delgado et al. 2011). Briefly, bacterial DNA was subjected to digestion using SmaI endonuclease enzyme (New England Biolabs, USA) at 37°C for 18 hours. The electrophoresis was then carried out using electrophoresis device (Bio-Rad, USA) for 23 hours at 14°C at 6V/cm with 5 to 50 second pulses with an angel of 120u and a linear ramping factor. A standard pattern was included in the gels to allow comparison of the digitally normalized PFGE profiles (Lambda Ladder PFG Marker, New England Biolabs, USA). Obtained gels were stained and images (containing 84 bacterial isolates patterns) were obtained and analyzed using computer software (PyElph 1.4, Informer Technologies, Inc., USA). The dice coefficient represented by unweighted pair group using the arithmetic averages (UPGMA) clustering method with 1% band position tolerance and 0.5% optimization settings was used to determine clusters (Aklilu et al. 2012). An 80% cut-off similarity

Table 2. Antibiotic sensitivity patterns of *Staphylococcus aureus* isolated from bovine mastitis, farm workers, veterinarians, veterinary students and university students.

Isolate source	N	Sensitivity (%)								
		Amoxicillin (10 µg)	Procaine penicillin (10 µg)	Streptomycin (10 µg)	Oxytetracycline (30 µg)	Ampicillin (10 µg)	Doxycycline (30 µg)	Gentamicin (10 µg)	Entrofloxacin (5 µg)	Trimethoprim-sulfa (15 µg)
Mastitis milk	27	2(7.4)^{a}	3(11)^{a}	0(0)^{a}	0(0)^{a}	4(14.8)^{a}	3(11)^{a}	7(26)*^{a}	24(89)*^{a}	3(11)^{a}
Farm workers	11	0(0)^{b}	0(0)^{b}	2(18)^{b}	4(36.4)^{b}	0(0)^{b}	6(54.5)*^{b}	8(73)*^{b}	9(82)*^{a}	6(54.5)*^{b}
Private veterinarians	9	0(0)^{b}	0(0)^{b}	1(11)^{b}	4(44.4)*^{b}	0(0)^{b}	9(100)*^{c}	9(100)*^{c}	9(100)*^{a}	9(100)*^{c}
Veterinary students	22	1(0.45)^{b}	0(0)^{b}	6(27)^{c}	19(86.4)*^{c}	5(22.7)^{c}	20(91)*^{c}	22(100)*^{c}	21(85.4)*^{a}	19(86)*^{c}
University students	16	0(0)^{b}	0(0)^{b}	0(0)^{a}	10(62.5)^{d}	12(75)*^{d}	13(81)*^{c}	16(100)*^{c}	16(100)*^{a}	16(100)*^{c}

* Indicate significant differences ($p \leq 0.05$) in antimicrobial sensitivity against different antimicrobial agents.
Different litters between values in the same column indicate significant differences at $p \leq 0.05$.

Table 3. Virulence factors detected in *Staphylococcus aureus* strains isolated from bovine mastitis, farm workers, veterinarians, veterinary students and university students.

Isolate source	N	Virulence genes (%)								
		mecA	coa	clf	entA	entB	nuc	hlg	icaA	tsse1
Mastitis milk	27	6(22.2)	27(100)	26(96.3)	4(14.8)	4(14.8)	27(100)	21(77.8)	21(77.8)	19(70.4)
Farm workers	11	9(81.8)*	11(100)	9(81.8)	9(81.8)*	9(81.8)	11(100)	7(63.6)	9(81.8)	7(63.6)
Veterinarians	9	9(100)*	9(100)	6(66.7)*	9(100)*	3(33.3)	9(100)	2(22.2)*	8(88.9)	3(33.3)*
Veterinary students	22	21(95.5)*	22(100)	18(81.8)	4(18)	4(18)	22(100)	18(81.2)	20(91)	14(63.6)
University students	16	19(100)*	16(100)	16(100)	6(37.5)*	6(37.5)	16(100)	13(81.3)	13(81.3)	10(62.5)

* $p \leq 0.05$ in the same column indicate significant difference compared to the value in mastitis milk.

and criterion of a difference of #6 bands were both used to define a cluster (Aklilu et al. 2012). Isolate information and a dendrogram were created to estimate the significant genetic variation/similarities between the bacterial isolates using 22 representative isolates.

Statistical analysis

Descriptive analysis including frequencies and percentages were analysed using Excel Microsoft software program. Statistical differences between groups in terms of antimicrobial sensitivity and virulence factor prevalence were evaluated using analysis of variance (ANOVA) test (IBM SPSS statistical package version 23, USA). Statistical difference was considered significant at $p \leq 0.05$.

Results

The antimicrobial sensitivity patterns of all *S. aureus* isolates are summarized in Table 2. All isolates from mastitis (27) were resistant to streptomycin and oxytetracycline while 89% were sensitive to entrofloxacin. All isolates (11) from farm workers were resistant to amoxicillin, procaine penicillin, and ampicillin while 82% were sensitive to entrofloxacin. All isolates (9) from veterinarians were resistant to amoxicillin, procaine penicillin, and ampicillin while 100% of the isolates were sensitive to doxycycline, gentamicin, entrofloxacin and trimethoprim-sulfa. All isolates (22) from veterinary students were resistant to procaine penicillin while more than 85% were sensitive to oxytetracycline, doxycycline, gentamicin, entrofloxacin and trimethoprim-sulfa. All isolates (16) from university students were resistant to amoxicillin, procaine penicillin and streptomycin while more than 75% of isolates were sensitive to ampicillin, doxycycline, gentamicin, entrofloxacin and trimethoprim-sulfa. There were significantly ($p \leq 0.05$) more *S. aureus* isolates from university students (87.5%), veterinary students (86.4%), and private veterinarians (77.8%) that were sensitive to at least 4 different antibiotic agents than isolates from both farm workers (63.6%) and mastitis milk (28.4%) (Table 2).

Results of different virulence factors detected by PCR in *S. aureus* isolates from mastitis, farm

Table 4. Genetic distribution of *Staphylococcus aureus* isolated from bovine mastitis, farm workers, veterinarians, veterinary students and university students (N=84).

Clusters	Groups	Sources	N	Sample identification
Cluster 1	A	Mastitis milk	12	Mas13, Mas14, Mas15, Mas10, Mas3, Mas4, Mas5, Mas6, Mas8, Mas9, Mas11, Mas26
		Veterinary students	1	Vst18
	B	Veterinarians	2	Vet5, Vet6
		Veterinarians	3	Vet1, Vet2, Vet4
	C	Veterinary students	2	Vst2, Vst6
		Veterinary students	2	Vst1, Vst7
	D	Veterinarians	1	Vet7
	E	Veterinary students	1	Vst17
		Veterinary students	1	Vst16
		Veterinary students	3	Vst13, Vst14, Vst15
	F	Farm workers	2	Cowmen4, Cowmen8
	G	Mastitis milk	5	Mas16, Mas17, Mas18, Mas19, Mas20
		Mastitis milk	9	Mas21, Mas1, Mas2, Mas7, Mas23, Mas29, Mas28, Mas27, Mas25
		Farm worker	6	Cowmen9, Cowmen10, Cowmen11, Cowmen1, Cowmen2, Cowmen3
Cluster 2	H	University students	16	C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12, C13, C14, C15, C16
		Farm workers	3	Cowmen5, Cowmen6, Cowmen7
		Veterinarians	3	Vet3, Vet8, Vet9
	I	Veterinary students	6	Vst4, Vst5, Vst19, Vst20, Vs21, Vst22
		Mastitis milk	1	Mas12
	J	Veterinary students	4	Vst3, Vst9, Vst8, Vst11
		Veterinary students	2	Vst10, Vst12

workers, private veterinarians, veterinary students, and non-veterinary university students are summarized in Table 3. All isolates from mastitis, farm workers and veterinary students carried the genes encoding coagulase and thermonuclease factors. All isolates from veterinarians carried the genes encoding coagulase, thermonuclease and enterotoxin A factors. All isolates from non-veterinary university students carried the genes encoding coagulase, clumping, and thermonuclease factors. The mecA gene was detected in 22.2%, 81.8%, 100%, 95.5% and 100% of isolates from mastitis, farm workers, private veterinarians, veterinary students, and non-veterinary university students, respectively. Other virulence factors were detected in variable percentages of isolates from all groups.

The phylogenic analysis of *S. aureus* isolates from different sources in this study showed significant genetic variation (Table 4, Fig. 1). The genetic analysis revealed that 10 (45.5%), 6 (66.7%) and 8 (72.7%) isolates from veterinary students, private veterinarians and farm workers, respectively were more than 80% similar to isolates from mastitis while all isolates from non-veterinary university students were grouped in cluster 2 with 69.7% similarity with isolates from mastitis.

Isolates were genetically distributed into two major clusters. Cluster 1 included 7 groups (A, B, C, D, E, F, G) while cluster 2 included 3 groups (H, I, J). Overall, the degree of similarity between isolates in cluster 1 and isolates in cluster 2 was 38.3%.

The majority of *S. aureus* isolates from mastitis were grouped in cluster 1 (N=26) and only 1 isolate was grouped in cluster 2. Isolates from mastitis were further grouped into 2 different groups in cluster 1 (A and G). The degree of similarity between isolates from mastitis in cluster 1 was 75.2% while the similarity between the isolates from mastitis in cluster 1 and cluster 2 was 65.3%.

Isolates from veterinary students were grouped into 3 groups (A, C, and E) in cluster 1 and in 2 groups in cluster 2 (I, J). The degree of similarity between *S. aureus* isolates from veterinary students in cluster 1 was more than 89% while the similarity between these isolates in cluster 1 and isolates in cluster 2 was less than 65%.

Isolates from veterinarians were grouped in 2 groups

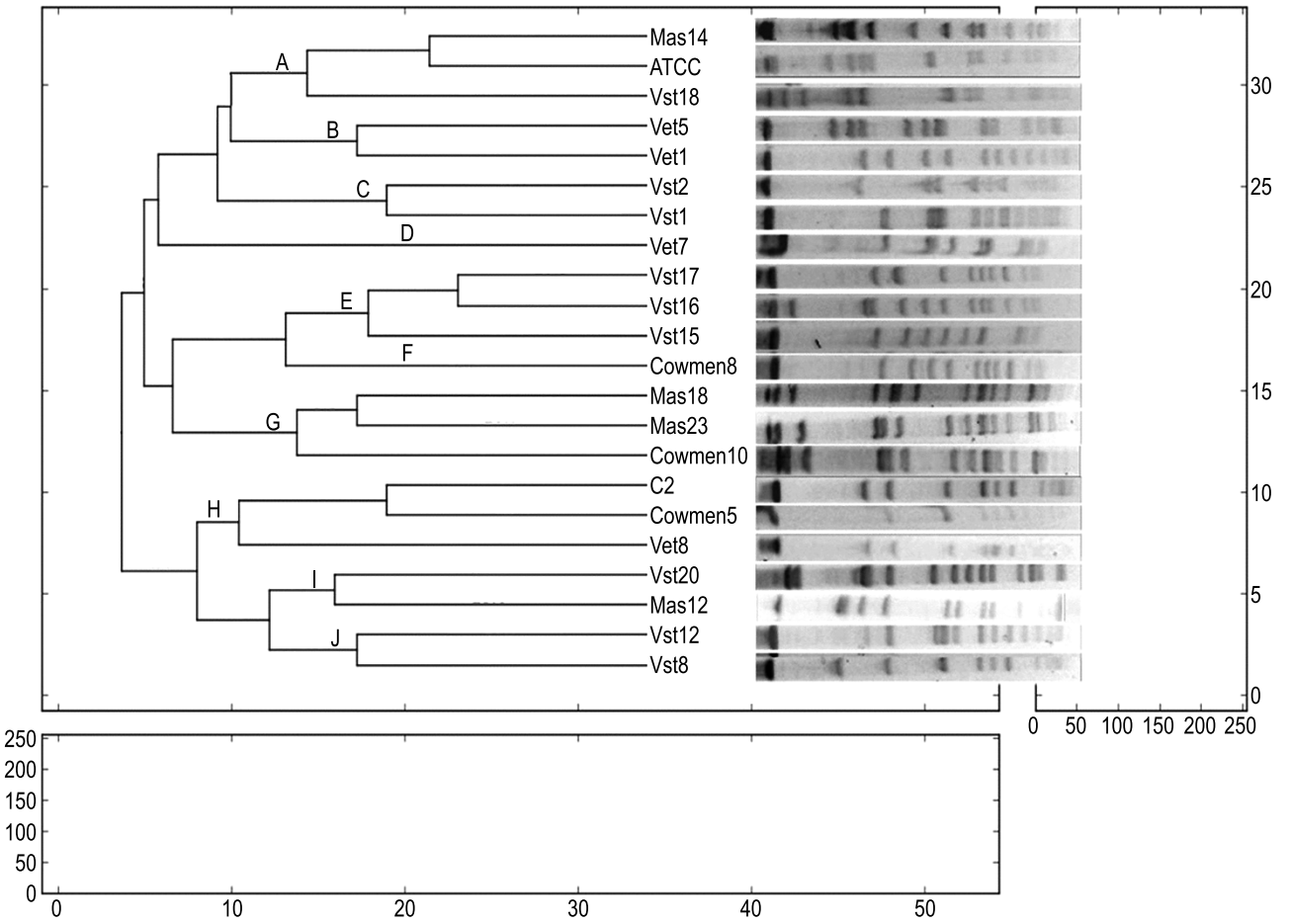

Fig. 1. Phylogenic analysis of *S. aureus* isolates from different sources showed considerable genetic variation. *Staphylococcus aureus* isolates were genetically distributed into two major clusters; cluster 1 included 7 groups (A-G) and cluster 2 included 3 groups (H - J).

(B, D) in cluster 1 and in 1 group in cluster 2 (H). The similarity between isolates from veterinarians in groups B and D was 95%. The similarity between isolates from veterinarians grouped in cluster 1 and cluster 2 was 89.3%.

Isolates from farm workers were grouped into 2 groups in cluster 1 (F, G) and in 1 group in cluster 2 (H). The similarity between isolates from farm workers grouped in cluster 1 was 91.8% while the similarity between isolates from farm workers grouped in cluster 1 and cluster 2 was 87.7%.

All isolates from non-veterinary university students were grouped in cluster 2 with 69.7% similarity with isolates from mastitis.

Discussion

Staphylococcus aureus is considered one of the most prevalent udder pathogens in dairy cows in Jordan and around the world (Alekish et al. 2013, Alekish 2015, Bani Ismail 2017). In the udder, *S. aureus* is invasive and the disease is characterized by chronic manifestations including fibrosis of glandular tissues with frequent flare ups of acute episodes (Alekish et al. 2013, Alekish 2015, Bani Ismail 2017). The condition is usually non-responsive to medical treatment despite multiple attempts using different categories of antimicrobials and therapeutic regimens. In fact, this has led to the appearance of multi-drug resistant strains of *S. aureus* (MDRSA) with serious veterinary, public health and economic implications (Alekish et al. 2013, Alekish 2015, Bani Ismail 2017).

In this study, the antimicrobial sensitivity test showed widespread resistance among all isolates of different sources against 2 or more commonly used antibiotics. These results are in congruity with previously published data (Jarraud et al. 2002, Tristan et al. 2003, Delgado et al. 2011, Persson et al. 2011, Alekish et al. 2013, Vishnupriya et al. 2014, Alekish 2015, Bani Ismail 2017, Obaidat et al. 2018). Multi-drug resistant S. aureus in cows' milk is considered a serious occupational health hazard affecting people who are most intimately associated with infected cows such as farm-associated workers, veterinarians and veterinary stu-

dents (Bani Ismail 2017, Obaidat et al. 2018). Indeed, severe clinical signs and sickness caused by infection of people with MRSA strains of animal origin have been reported around the world with high morbidity and longer hospitalization periods (Alekish 2015, Bani Ismail 2017, Obaidat et al. 2018).

Several genetic determinants such as the mecA, mecC and vanA genes are associated with the development of resistance to antimicrobial drugs among *S. aureus* strains (Bani Ismail 2017, Obaidat et al. 2018). In Jordan, the prevalence of mecA gene in methicillin-resistant *S. aureus* (MRSA) in mastitis milk has been reported previously at around 26.3% and 18.2% from culled dairy cows and acute mastitis cases, respectively (Bani Ismail 2017). In another study, the prevalence of mecA and mecC genes in MRSA obtained from cows, ewes, and goats' bulk tanks in Jordan was estimated at 26% (Obaidat et al. 2018). These results are in total agreement with the results reported here in this study regarding the presence of mecA gene in *S. aureus* isolates from mastitis.

Overzealous use of antimicrobials in livestock industries has been long blamed on the development and widespread occurrence of MRSA in humans and animals. In this study, however, detection of the mecA gene in high percentages of *S. aureus* isolates from farm workers, veterinarians, veterinary students, and non-veterinary university students (81.8%, 100%, 95.5% and 100%, respectively) compared to only in 22.2% of isolates from mastitis milk may indicate the opposite, at least among the targeted population in this study in Jordan. These figures indicate a serious medical concern in Jordan regarding the rapid and widespread diagnosis of MRSA in Jordan's population. Indeed, previous reports showed an alarming trend of MRSA spread in Jordan. Recent studies have indicated that 7.5-19% of individuals carry MRSA (Al-Zu'bi et al. 2004, Al-Bakri et al. 2013). Furthermore, a study that spanned over 3 years has reported a 56% MRSA in multiple medical centers in Jordan (Borg et al. 2007). In another study, 62% of *S. aureus* isolates from abscesses, skin wounds, skin infections, septicemia and nasal swabs were MRSA (Aqel et al. 2012). In Japan, MRSA strains isolated from bovine mastitis milk showed geno- and serotypes that were identical or similar to those of human MRSA isolates (Hata et al. 2010). These findings are also in agreement with those reported by Delgado et al. (2011) who found significantly higher resistance to penicillin among strains isolated from lactating women with mastitis in comparison to strains isolated from bovine mastitis. Delgado et al. (2011) explained these results by the presence of blaZ gene in human *S. aureus* strains (Delgado et al. 2011).

The pathogenesis of *S. aureus* is based on its ability to produce wide range of virulence factors and toxins *in vivo* (Barbu et al. 2010, Foster et al. 2014, Askarian et al. 2016, Pietrocola et al. 2017). In this study, the majority of *S. aureus* isolates from mastitis, farm workers, veterinarians and veterinary students consistently carried the genes encoding coagulase, thermonuclease, enterotoxin A, and clumping factors. Most of these factors are known to promote colonization, inflammation, invasion to deep tissues, evading effective immune system and survival inside the host leading to a serious alteration in cell functions, coagulation systems, and vascular integrity (Powers et al. 2014).

In one study, *S. aureus* strains causing bovine mastitis were found genetically similar to those strains that were isolated from extramammary sites including teat and udder skin, milking machine parts and milker's hands (Haveri et al. 2008). However, there was no obvious connection between various virulence genes and the origins of isolates which suggested a common source of the isolates (Haveri et al. 2008). In Jordan, this is the first study that investigated the genetic relatedness and molecular characteristics of S. aureus isolates from bovine mastitis compared to isolates obtained from associated farm workers, private veterinarians and veterinary students. In this investigation, PFGE was used successfully to distinguish between different S. aureus isolates. It is well recognized that PFGE typing is more discriminatory than phage typing (Zodaks et al. 2002). In one study, PFGE was successful in differentiating between *S. aureus* isolates from bovine teat skin and those isolates obtained from mastitis milk (Zodaks et al. 2002).

Despite a considerable genetic variation of *S. aureus* isolates from different sources, results of this study showed a great concern because 45.5%, 66.7% and 72.7% of isolates from veterinary students, private veterinarians and farm workers, respectively were more than 80% similar to isolates from mastitis. These 3 categories of people are closely associated with cows; and cross-species transmission of zoonotic pathogens such as *S. aureus* is likely. Therefore, appropriate precautionary measures must be implemented to prevent cross-species transmission of this important pathogen. Farmers and farm workers including veterinarians must be aware of the existence of zoonotic pathogens and must practice highest farm and personal hygiene standards to prevent transmission of such pathogens. Effective hand washing procedures, wearing disposable gloves, and isolating and treating of infected animals are indicated in affected farms.

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