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MOLECULAR DETECTION OF *ica* GENE AND SOME SURFACE PROTEINS IN BIOFILM PRODUCER OF METHICILLIN-RESISTANT AND METHICILLIN-SENSITIVE *STAPHYLOCOCCUS AUREUS*

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ABSTRACT : This study aimed to detect the connection between biofilm formation and the presence of surface proteins and intercellular adhesion gene (*ica*) in the isolates of methicillin-resistant (MRSA) and methicillin-susceptible *Staphylococcus aureus* (MSSA). Out of 212 samples, 67 *S. aureus* were isolated from different sources of cattle, MRSA represent 65.67% of isolates. Phenotypic biofilm formation was studied with microtiter plate assay. The biofilm producer constitutes of 59.1% from MRSA, comparing with 30.4% of MSSA isolates. The polymerase chain reaction was employed to detection *icaA, icaD, clfA, clfB*, *fnbA, fnbB*, *fib*, *cna, eno* and *ebps*, the frequency of thesegenes inMRSA isolateswere (11.54%, 88.46%, 100%, 46.2%, 100%, 0%, 73.1%, 38.5%, 100% and 0%) respectively, however, the frequency of the genes in MSSA isolates were (57.14%, 100%, 71.4%, 28.6%, 100%, 0%, 57.1%, 28.6%, 100% and 0%) respectively. The *ica A* and *ica D* genes were detected in majority of MSSA comparing with MRSA. The presence of the *clf A, clf B* and *fib* genes were higher in MRSA. In conclusion, the *ica A* and *ica D* may play important role in biofilm formation by MSSA, whereas, *clf A*, *clf B* and *fib* genes may play crucial role in biofilm formation by MRSA.

Key words : Biofilm formation, *S. aureus* methicillin resistant, MSCRAMMs, *ica*, *nuc* and *mec A*.

INTRODUCTION

Staphylococcus aureus is among the most common etiologic agents responsible for health-related infections and can produce numerous virulence factors including extracellular toxins and surface structures that promote colonization of the tissue, immune evasion and tissue destruction (Dunman *et al*, 2001).

Biofilms of staphylococci are common sources of infections (Arciola *et al*, 2015) begins from the microorganism's adhesion to the host's tissue. Adhesion is supported by virulence factors known as adhesives, grouped into a family known as Microbial Surface Components, which Recognize Adhesive Matrix Molecules (MSCRAMM) (Fischetti *et al*, 1998). It remains poorly understood that these factors are involved in pathogenesis. These MSCRAMMs are proteins bound on the bacterial surface and are capable of binding to one or more host's extracellular matrix and plasma components and are often covalently bounded to the cell wall's peptidoglycan and recognize collagen (collagen-binding protein), fibronectin A (fibronectin-binding protein), fibrinogen B (fibrinogen-binding protein), elastin (elastin-binding protein) and laminin (laminin-binding protein) (Foster and Hook, 1998).

The development of biofilm is mainly favored by Polysaccharide intercellular adhesion (PIA) as the most important component of the biofilm matrixconsisting of â-1,6-linked N-acetylglucosamine with partially de-acetylated residues. It surrounds the cells and protects them from immune defenses as well as antibiotic treatment (Gotz, 2002).

Exo-polysaccharides are essential components of the biofilm matrix, but recent studies have shown that bacterial surface-exposed proteins probably play a significant role in the developmentof biofilm production (McCarthy *et al*, 2015). The adhesion of cells during the formation of biofilm is possibly mediated via the *ica*locus, whereas, theremoval of the *ica* genes (icaADBC) removes the ability to produce polysaccharide intercellular adhesion (PIA) and to form a biofilm in vitro.On the other hand, surface proteins can also promote the accumulation of staphylococci in an *ica*-independent manner (special relevant for MRSA strains) (Cramton, 1999). Moreover, Fitzpatrick *al al* (2005) reported that, the biofilm formation

of *ica* ADBC operon deleted MRSA mutants was not affected whereas the formation of biofilm of *ica* ADBC operon deleted MSSA mutants was hindered.

The resistance of *S. aureus* to methicillin is associated with synthesis of a low affinity penicillin binding protein to change the drug –s site of action and is regulated by the *mec A* (Sawant *et al*, 2009). Biofilm formation is related with antimicrobial resistance. Also, McCarthy *et al* (2015) mentioned that the biofilm increases the level of resistance to beta-lactam antibiotics.

The aim of the presentstudy to investigate the presence of MSCRAMMs and *ica* genes among *S. aureus*, which were isolated from different sources in cattle including MRSA and MSSA in relation to biofilm production.

MATERIALS AND METHODS

Samples collection and bacterial isolates

A total of two hundred and twelve samples from cattle including; 42 mastitic milk samples detected by using California mastitis test (CMT) according to Galdhar *et al* (2005), 70 raw milk samples from local markets, 46 swabs from nares, 32 swabs from udder surface and 22 samples from coat of cattle were collected during the period from January to June 2018. All *S. aureus* strains were identified by using colony morphological characteristics, Gram–s staining, biochemical tests and *nuc* gene detection according to Wongboot *et al* (2013).

Phenotypic and genotypic detection of MRSA

All identified *S. aureus* isolates were tested for antimicrobial susceptibility to Cefoxitin to detect the phenotype of MRSA (NCCLS, 2013). Then molecular confirmation of MRSA isolates was performed by detection of *mecA* gene, primers sequences illustrated in Table 1. The thermal cycling protocol for PCR was comprised according to Bühlmann *et al* (2008), the amplified products were visualized by electrophoresis in a 1.5% agarose gels stained with ethidium bromide.

Table 1 : The primer sequences used to detection some genes in bacterial isolates.

Primers		Sequence	Size Products bp	Reference
clf A	**F**	5-ATT GGC GTG GCT TCA GTG CT-3	292	17
	R	5-CGT TTC TTC CGT AGT TGC ATT TG-3		
clf B	**F**	5-ACA TCA GTA ATA GTA GGG GGC AAC-3	205	16
	R	5-TTC GCA CTG TTT GTG TTT GCA C-3		
***fnb* A**	**F**	5 G-CAT AAA TTG GGA CA GCA TCA-3	127	17
	R	5-ATC AGC AGC TGA ATT CCC ATT-3		
fnb B	**F**	5-GTA ACA GCT AAT GGT CGA ATT GAT ACT-3	524	16
	R	5-CAA GTT CGA TAG GAG TAC TAT GTT C-3		
Eon	**F**	5-ACG TGC AGC AGC TGA CT-3	302	16
	R	5-CAA CAG CAT CTT CAG TAC CTT C-3		
Fib	**F**	5-CTA CAA CTA CAA TTG CCG TCA ACA G-3	404	17
	R	5-GCT CTT GTA AGA CCA TTT TCT TCA C-3		
Ebps	**F**	5-CAT CCA GAA CCA ATC GAA GAC-3	186	17
	R	5- CTT AAC AGT TAC ATC ATC ATG TTT ATC TTT G-3		
Can	**F**	5-GTC AAG CAG TTA TTA ACA CCA GAC-3	423	16
	R	5-AAT CAG TAA TTG CAC TTT GTC CAC TG-3		
nuc	**F**	5´- GCT TGC TAT GAT TGT GGT AGC C 3'	423	11
	R	5´- TCT CTA GCA AGT CCC TTT TCC A 3'		
mec A	**F**	5´- AAA ATC GAT GGT AAA GGT TGG C-3'	533	13
	R	5´- AGT TCT GGA GTA CCG GAT TTG C-3'		
ica A	**F**	5-CCT AAC TAA CGA AAG GTA G-3	1315	18
	R	5-AAG ATA TAG CGA TAA GTG C-3		
ica D	**F**	5-AAA CGT AAG AGA GGT GG-3	381	18
	R	5-GGC AAT ATG ATC AAG ATA C-3		

Phenotypic characterization of biofilm formation by micro-titer plate assay

The assay was done according to the method reported by Cucarella *et al* (2001). Briefly, each strain was grown on tryptic-soy broth (TSB), the culture was diluted 1:100 in TSB supplemented with 1% glucose, 200 µl of 1% inoculum were transferred to each well of microtiter plate (Coastr, China) and triplicate wells were used for each isolate. The negative control wells contained 200 µl of non-cultured (TSB supplemented with 1% glucose). After incubation at 37ºC for 24 h, each well was washed three times with 300µl of sterile phosphate buffered saline (PBS) pH 7.2, then fixed by methanol 150µl for 20 min. The plate was dried for overnight in an inverted position at room temperature; finally the adherent biofilm layer formed in each well was stained with 150µl of crystal violet 2% for 15 min. at room temperature. After that the micro-titer plate was washed three times with PBS and dried at room temperature, then150µl of 95% ethanol was added to each well and the wells were read during 30 min. The optical density OD was measured at 570 nm by using micro-titer plate reader (Biotek, USA). Optical density cut off was calculated according to Stepanovic *et al* (2000).

PCR assay for detection of *ica A*, *ica D* and MSCRAMMs genes

Genomic DNA of *S. aureus* isolates were extracted by using DNA kit (Geneaid, USA) according to manufacturer's protocol.

The primers were used to detect the genes encoding for *nuc, mec A*, *ica A*, *ica D*andeight of surface proteins mention in Table 1. Four surface proteins genes, *clf B, eno*, *cna* and *fnb B* were detected by using quadruplex PCR according to Tristan *et al* (2003), the genes *ebps*, *clfA* and *fib* detected by using triplex PCR according to Vancraeynest *et al* (2004), the genes *ica A* and *ica D* were detected by using duplex PCR (Vasudevan *et al*, 2003) and the gene *fnb A* was detected by using uniplex PCR according to Vancraeynest *et al* (2004).

Statistical analyses

The data were statistically treated with the software package IBM SPSS 22.

RESULTS

The suspected isolates of *S. aureus* resulted from conventional microbiological techniques were subjected to PCR to detect *nuc* gene. Out of 212 samples investigated, 104(49.05%) were had suspected isolates as *S. aureus* by conventional microbiological techniques, however by using *nuc* gene 67(31.60%) of isolates confirmed as *S. aureus.*

The results showed that, the high percentage of *S. aureus* were isolated from udder surface samples (43.75%), followed by subclinical mastitis (42.85%), whereas the lowest percentage was observed in the nasal swab (19.56%) (Table 2, Fig. 1). There is significant difference between the two methods for detection of *S. aureus* isolates.

Table 2 : *S. aureus* identified by using conventional microbiological techniques and molecular detection of *nuc* gene.

Samples	Total No. of tested sample	No. of *S. aureus* by conventional microbiological technique (%)	No. of *S. aureus* by *nuc* gene (%)
Subclinical mastitis	42	28 (66.66%)	18 (42.85%)
Raw milk from local markets	70	37 (52.85%)	21 (30 %)
Nasal swabs	46	15 (32.60%)	9 (19.56%)
Udder surface	32	15 (46.87%)	14 (43.75%)
Coat swabs	22	9(40.90%)	5 (22.72%)
Total	212	104(49.05%)	67 (31.60%)

Chi square = 75.79, df = 9, p<0.05

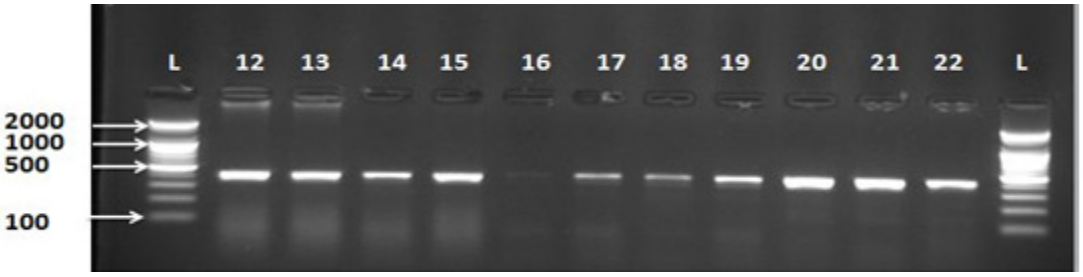

Fig. 1 : Electrophoresis of *nuc* gene amplification, the product size (423) bp, the mixture was run in 1.5% agarose gel, stained with ethidium bromide, L: Ladder.

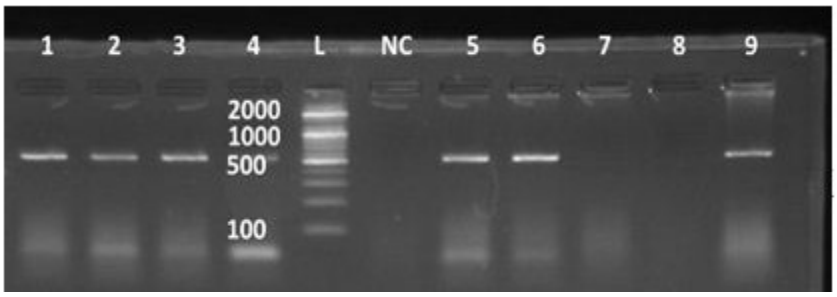

Fig. 2 : Electrophoresis of *mecA* gene amplification (the product size 533bp). The PCR product was run in 1.5% agarose and stained with Ethidium bromide. L: Ladder, NC: Negative control.

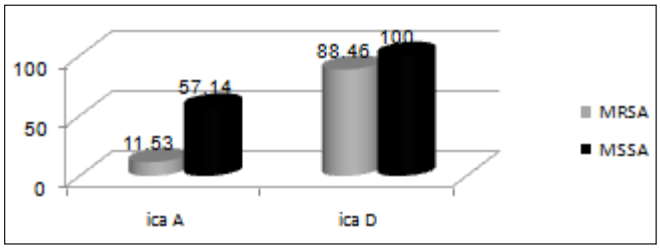

Fig. 3 : Distribution of *ica A* and *ica D* between MRSA and MSSA isolates. (Chi square of *ica D* 12.247, df=1, p< 0.05) (Chi square of *ica A* 46.11, df=1, p< 0.05).

among the MSSA isolates, 30.4% were able to produce biofilm, including 42.9% of the isolates were moderate and 57.1% were weak. However, there were significant differences (P<0.05) in the percentage of biofilm formation between MRSA and MSSA isolates (Table 4).

Among the strains isolated from nasal swabs was the highest percentage of biofilm producers (100%) and subclinical mastitis (83.3%). whileall strains (MRSA and MSSA) isolated from coat were unable to produce

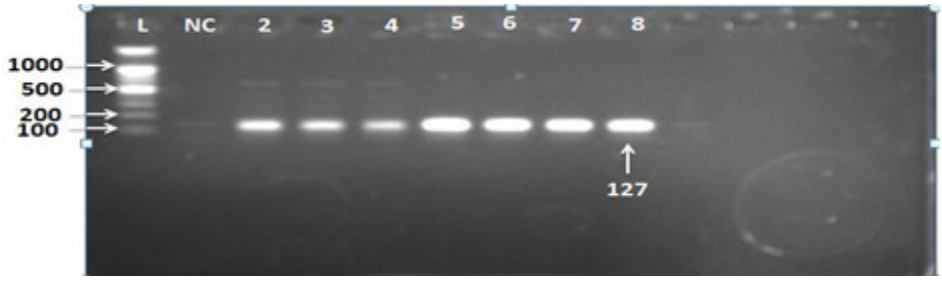

Fig. 4 : Electrophoresis of *fnb A* gene amplification by PCR. The pcr product was run in 1.5% agarose and stained with Ethidium bromide. L: Ladder, NC: Negative control.

Detection of MRSA using phenotypic and genotypicmethods

All confirmedisolates of *S. aureus* were tested for Cefoxitin susceptibility to detect the phenotype of MRSA. Table 3 showed that, the total resistance rate against Cefoxitin (65.67%). While, the results of the PCR used for detection the presence of *mec A*gene was 50.74% (Fig. 2). There is significant difference between the two methods for detection of MRSA.

The highest percentage 100% of MRSA were detected in *S. aureus* isolates from udder surface, followed by isolates from subclinical mastitis and nasal swabs with (66.66%) and the lowest percentage from coat isolates (40%) (Table 3).

Relationship between biofilm production and methicillin resistance

Among the MRSA isolates, 59.1% of the isolates were biofilm producer, regarding intensity of biofilm productions 42.3% of the isolates were found to be moderate and 57.7% were weak producer, whereas biofilm. Regarding the moderate and weak biofilm producers, no significant (p > 0.05) differences were found among MRSA and MSSA.

Relationship between methicillin resistance and *ica* genes

The results of PCR assay revealed that *ica D* gene was present in 91.04% isolates whereas *ica A* was present in 8.95% of *S. aureus* isolates. Among 26 MRSA strains studied 23 (88.46%) harbored the *icaD* gene and 3(11.54%) isolates were harbored *ica A*gene, however, the negative *icaD* strains were *icaA*-positive. Concerning MSSA strains 4/7(57.14%) were harbored *ica A* gene and 7(100%) harbored *icaD* gene. The differences between MRSA and MSSA for *ica A* and *ica D* were significant p< 0.05 (Fig. 3).

Molecular detection of surface protein genes (MSCRAMMs) by using PCR

Eight genes of surface protein were investigated in this study as follow, the gene *fnb A* (encoding fibronectin-binding protein A) detected by using uniplex PCR, the

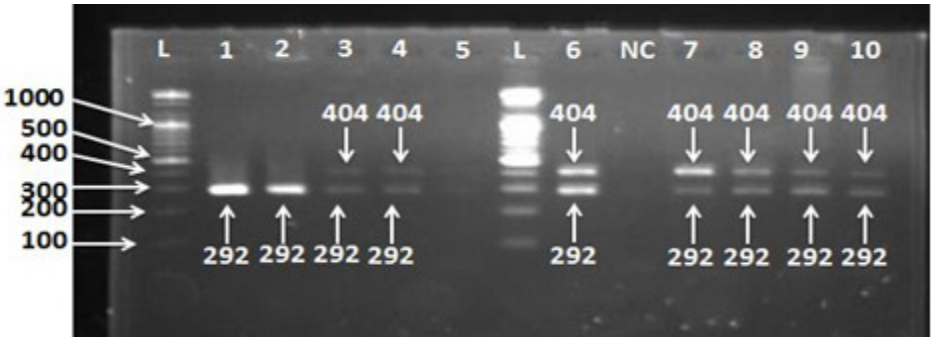

Fig. 5 : Electrophoresis of ebps, *clfA* and *fib* gene amplification by triplex PCR. The products of each gene were 186bp, 292bp and 404bp, respectively. The PCRproduct was run in 1.5% agarose and stained with Ethidium bromide. L: Ladder, NC: Negative control.

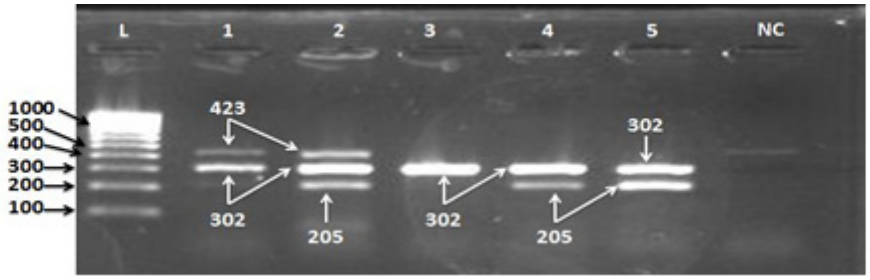

Fig. 6 : Electrophoresis of *clf B, cna*, *eno* and *fnb B* gene amplification by quadruplex PCR. The products of each gene were 205bp, 302bp, 423bp and 524bp, respectively. The pcr product was run in 1.5% agarose and stained with Ethidium bromide. L: Ladder, NC: Negative control.

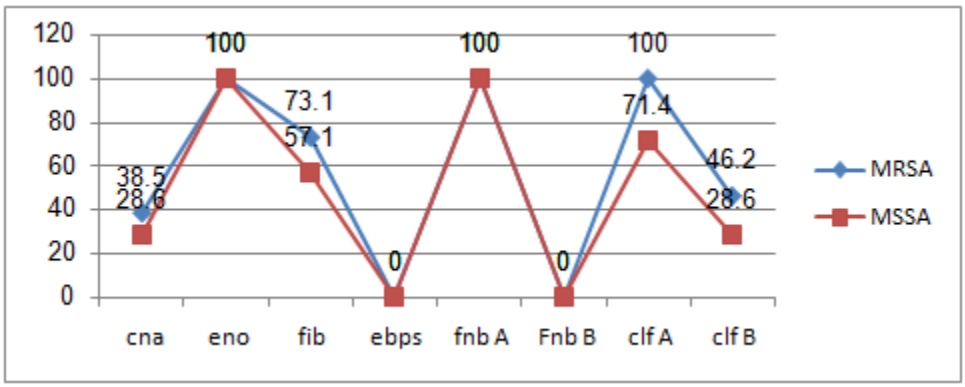

Fig. 7 : Distribution of MSCRAMMs between MRSA and MSSA isolates. (chi square of *cna* 0.138189, df=1, p> 0.05),(chi square of *fib* 5.634, df=1, p< 0.05) (chi square of *clf A* 33.372, df=1, p< 0.05) , (chi square of *clf B* 6.615, df=1, p< 0.05).

genes *ebps* (encoding elastin binding protein), *clfA* (encoding clumping factors A) and *fib* (encoding fibrinogen-binding protein) were detected by using triplex PCR and *clfB* (encoding clumping factors B), *eno* (encoding laminin-binding protein), *cna*(encoding collagen binding protein) and *fnb B*(encoding fibronectin-binding protein B) genes were detected by using quadruplex (Figs. 4, 5 and 6).

All biofilm producers *S. aureus* isolates included in this study were positive for *fnb A* and *eno* genes and negative for *fnbB* and *ebps*. The other MSCRAMM genes were heterogeneously detected as following: (93.9%, 69.7%, 42.4% and 36.4%) for genes (*clf A*, *fib*, *clf B* and *eno*) respectively (Table 5). All isolates from coat were unable to produce biofilm. The difference in genes *fnb A* and *eno* between isolates were not significant ($p > 0.05$).

Relationship between surface proteins (MSCRAMMs) and methicillin resistance

The present study reveals that most of surface proteins in MRSA were found to be higher than those of MSSA (Fig. 8). Generally, both MRSA and MSSA haven – t *ebps* and *fnb B*. All MRSA isolates have *fnb A*, *clf A* and *eno* in percent (100%), followed by (73.1%) for *fib* gene. The lowest rate was observed in *cna* (38.5%). However, in MSSA isolates, the highest rates were observed in *fnb A* and *eno* in percent (100%) followed by *clf A* and *fib* (71.4% and 57.1) respectively, the lowest rates were observed in both *cna* and *clf B* (28.6%).

Table 3 : Comparison of Phenotypic and Genotypic detection of MRSA isolates.

Samples	No. of *S. aureus* isolates	No. of resistant isolates to Cefoxitin / %	No. of isolates carried of *mec A*
Subclinical mastitis	18	12(66.66%)	11(61.11%)
Raw milk from local markets	21	10 (47.61%)	10(47.61%)
Nasal swabs	9	6 (66.66%)	4(44.44%)
Udder swabs	14	14(100%)	7(50%)
Coat swabs	5	2(40%)	2(40%)
Total	67	44(65.67%)	34(50.74%)

Chi square =122.416, df = 9, p<0.05.

Table 4 : Relationship between biofilm production and MRSA or MSSA.

Sample	Total no. of isolates	Susceptibility to Methicillin	No. of Isolates	Biofilm production			
				No. of None (%)	No. of biofilm producers (%)	No. of weak (%)	No of moderate (%)
Subclinical mastitis	18	MRSA	12	2 (16.7)	10(83.3)	6 (60)	4(40)
		MSSA	6	6 (75)	0(0)	0(0)	0(0)
Cow milk of market	21	MRSA	10	9(90)	1(10)	0(0)	1(100)
		MSSA	11	6 (54.5)	5(45.5)	3(60)	2(40)
Nasal swabs	9	MRSA	6	0(0)	6(100)	3(50)	3(50)
		MSSA	3	1(33.3)	2(66.7)	1(50)	1(50)
Udder swabs	14	MRSA	14	5(35.7)	9(64.3)	6(66.7)	3(33.3)
		MSSA	0	0(0)	0(0)	0(0)	0(0)
Coat swabs	5	MRSA	2	2(100)	0(0)	0(0)	0(0)
		MSSA	3	3(100)	0(0)	0(0)	0(0)
Total	67	MRSA	44	18(40.9)	26(59.1)	15(57.7)	11(42.3)
		MSSA	23	16(69.6)	7(30.4)	4(57.1)	3(42.9)

The differences between MRSA and MSSA in biofilm production were significant. Chi-square = 16.657, df = 1, p-value < 0.05

Table 5 : MSCRAMM gene distribution among biofilm producer *S. aureus* isolates.

Samples	No. of biofilm producer isolates	*cna* No.%	*eno* No.%	*fib* No.%	*ebps* No.%	*fnbA* No.%	*fnbB* No.%	*clf A* No.%	*clf B* No.%
Mastitic milk	10	00%	10100%	880%	00%	10100%	00%	10100%	330%
Raw milk from market	6	6100%	6100%	6100%	00%	6100%	00%	6100%	466.7
Udder surface	8	112.5%	8100%	337.5%	00%	8100%	00%	675%	225%
Nasal isolates	9	555.6%	9100%	666.7%	00%	9100%	00%	9100%	555.6%
Total	33	12(36.4)	33(100)	23(69.7)	0(0)	33(100)	0(0)	31(93.9)	14(42.4)
Chi square	-	253.78	-	100.28	-	-	-	80	48.887

Chi-square = 498.938, df =7, p-value < 0.05. The difference in genes (*eno* and *fnb A*) between isolates was not significant p> 0.05.

The statistical analysis of the genes *fib, clf A* and *clf B* revealed that differences were statistically significant, moreover, the percent of these genes were higher in MRSA (Fig. 7).

DISCUSSION

Staphylococcus aureus is an important pathogen-borne milk that causes a wide diversity of diseases in human and animals and is often associated with subclinical mastitis in dairy animals and can contaminate milk and other dairy products that act as vehicles for *S. aureus* infection (Felten *et al*, 2002). In the present study, the isolation rate of *S. aureus* from different sources of cows was 31.60%. This result is in line with many studies such as Khudaier *et al* (2013), Al-Iedani (2016) and Aboud (2019).

The detection rate of MRSA by using cefoxitin disc diffusion method was 65.67% compared with the results of the PCR used for detection of *mec A* gene in identified isolates was (50.74%). There was significant difference between the two methods; some isolates didn't carry the *mecA* gene by PCR assay while showing phenotypic resistance to cefoxitin. This result is in agreement with Fernandes *et al* (2005), who noted that the cefoxitin diffusion method is an alternative marker and potentially more sensitive for the detection of methicillin resistance.It also reported this finding by Cekovska *et al* (2005) and Davoodi *et al* (2012). The expression of phenotypic oxacillin resistance may be associated with changes in growth conditions, alterations in the production of PBP subtypes, or beta-lactamases overproduction (Moon *et al*, 2007). Also, this result is inagreement with Al-Iedani (2016).

Apropos the phenotypic biofilm formation, MSSA and MRSA isolates produced biofilm, the present study reveals that, 59.1% of MRSA isolates produced biofilm, whereas MSSA isolates 30.4%. There is significant difference ($P<0.05$) between MRSA and MSSA in biofilm production, this result is compatible with Fahimeh and Ebrahimzadeh (2016). While disagree with Ghasemian *et al* (2016) and Idbeis (2019) who showed no significant differences. Moreover, the formation of biofilm depends on many factors including environment, nutrient availability of and above all the presence and expression of biofilm-associated genes (Neopane *et al*, 2018).

The virulent factor that contributes to biofilm formation is PIA, which can be encoded by the *ica* ADBC operon (Cramton *et al*, 1999). *icaA* and *icaD* genes crucial factors for intercellular adhesion, it could be inferred that these genes are only important in the development of biofilms for the formation of a bacterial multilayer (Ghasemian *et al*, 2016). Therefore, both *icaA* and *icaD* genes were selected to study. The present results of PCR assay revealed that *S. aureus* isolates gave positive results for both *icaA* and *icaD* genes (8.95% and 91.04%), respectively. Similar results were obtained by several studies such as Mariusz *et al* (2007) and Da Costa *et al* (2015) and disagree with Vasudevan *et al* (2003). On other hand, this study reveals that most of MSSA isolates harbored *ica D* (100%) and *ica A* (57.14%) relative to MRSA isolates, which have *ica D* (88.46%) and i*ca A*(11.54%), this result may boost that MRSA depends on surface protein in biofilm production more than PIA in contrast to MSSA.N-acetylglucosaminyl transferase encodes by *icaA* gene, the enzyme involved in the synthesis of UDP-acetylglucosamine oligomers (Arciola *et al*, 2001). In addition, *icaD* has been reported to play a critical role in maximizing N-acetylglucosaminyl transferase expression, leading to the capsular polysaccharide's phenotypic expression (Gerke *et al*, 1998). Co-expression of *icaA* and *icaD* increases the activity of N-acetylglucosaminyl transferase and slime production (Arciola *et al*, 2001).

The present study, illustrated the prevalence of eight genes of surface proteins involved in biofilm formation including *fnbA* (100%), *eno* (100%), *clfA* (93.9%), *fnbB* (0%), *fib* (69.7%), *clfB* (42.4%), *cna* (36.4%) and *ebps* (0%). In this study all the *S. aureus* isolates from different samples were harbored *fnbA* and *eno* genes. These results are in agreement with De bentzmann *et al* (2004), Kumar *et al* (2011), Al -Ani and Al- Meani (2018). The fibronectin can bind to epithelial and endothelial surfaces, a part of blood clots, fibrin, collagen, fibronectin and fibrinogen (Pyor•al•a and Taponem, 2009; Ikawaty *et al*, 2010; Reinoso *et al*, 2008). Enolase has been identified as a 52 kDa laminin surface receptor and can thus, play a critical role in the pathogenesis of *S. aureus* by allowing its adhesion to extracellular matrix containing laminin-(Carneiro *et al*, 2004).

The differences in frequencies of *clfA* and *clf B* were statistically significant. In this study, *clfA* and *clf B* were detected in 93.9% and 42.4% respectively in all isolates; Clumping factors play a critical role in attachment and colonization of *S. aureus* in body surfaces, such as skin surface and epithelium (Klein *et al*, 2012), results of *clf A* in line with De bentzmann *et al* (2004), however, result of *clf B* disagree with De bentzmann *et al* (2004) and Klein *et al* (2012), who reported the ratios 95% and 91.8%, respectively.

The percentage of *fib* gene was 69.7%, these result in line with Singh *et al* (2011) and Eveline *et al* (2015), who reported that, the occurrence of *fib* in *S. aureus* were with frequencies of 67.2% and 68.4%, respectively. The fibrinogen-binding protein is an essential virulence factor in infections caused by *S. aureus* as it not only binds to fibrinogen, but can also interact with platelets aggregation and host complement cascade. The incidence of *fib* gene was significantly higher in MRSA isolates compare to the MSSA isolates, this result in agreement with Mir *et al* (2019).

Collagen is the most abundant group of proteins in the body, andis the main component of connective tissue, and *cna* gene is considered an important virulence factor instaphylococcal infections (Carneiro *et al*, 2004). In the present study, the frequency of this gene in *S. aureus* was 36.4%, which is in agreement with the finding of Pyor•al•a and Taponem (2009). Carneiro *et al* (2004)

reported the occurrence of the *cna* gene in 22.4% of 85 isolates of *S. aureus* isolated from bovine mastitis, while lower percentage of this gene were reported by Arciola *et al* (2006) and Klein *et al* (2012).

For *fnbB*, all isolates are negative, this data is in accordance with Taneike *et al* (2006). Some strains of *S. aureus* possesses a single *fnb* gene, results of this study are inagreement with Greene *et al* (1996) and Schulze *et al* (2001) and disagree with Ghasemian *et al* (2016). Other study by Al-Ani and Al-Meani (2018) recorded that, the ratio of *fnb B* lower than *fnb A*, 25% and 100%, respectively.

The elastin-binding protein gene (*ebps*) was not detected in *S. aureus* samples,this result is in contrast with the data reported by Al-Ani and Al-Meani (2018), who reported that the gene *ebps* was detected in 100% of 76 *S. aureus* isolated from bovine mastitis.

In conclusion, *fnb A* and *eno* genes may have a more important role in the pathogenic mechanisms of *S. aureus* infection because were detected in all isolates compared with other genes. The genes *fib*, *clf A* and *clf B* may play crucial roles in biofilm formation in MRSA isolates. Also, the results may indicate that MRSA depends on surface protein in biofilm production more than PIA in contrast to MSSA.

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