DETECTION OF INTRACELLULAR ADHESION GENE (icaA and icaD)AND BIOFILM FORMATION STAPHYLOCOCCUS AUREUS ISOLATES FROM MASTITIS MILK OF SHEEP AND GOAT

Hasan I. Idbeis ; Mohammed H. Khudor

Department of Microbiology, College of Veterinary Medicine ,University of Basrah, Basrah,Iraq

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Corresponding Author: mohamedh_79@yahoo.com

ABSTRACT

In the present study, a total of 150 mastitis milk samples were collected from sheep and goat (75 for each one) and were analyzed for the presence of *S.aureus*.. The obtained results indicate that this bacterium observed in 20% of these samples (21.33% from sheep and 18.66% from goat) .The study of antibiotic susceptibility test to 9 different antibiotics showed that *S. aureus* was 100% resistant to penicillin and 100% sensitive to vancomycin, gentamycin , clarithromycin and chloramphenicol . whereas for cefoxitin (alternative to methicillin) resistance was 47%.There were a variable sensitivity percentage for the rest of antibiotics: Tetracycline (70%), Ciprofloxacin (80%) , Clindamycin (83%). The biofilm-forming ability of *S. aureus* was evaluated via microtiter plates and the result revealed that, all the studied isolates were either moderate biofilm producer or weak biofilm producer while the non-biofilm producer and strong biofilm producer were not detected among the tested isolate.The relationship between biofilm formation and resistance to methicillin showed there as no significant differences (P>0.05) in the percentage of weak and moderate biofilm producers between MRSA and MSSA isolates. PCR analysis was applied to DNA extracted from *S.aureus* isolates from milk samples .The results of PCR assay revealed that all *S.aureus* isolates gave positive results for both icaA and icaD genes (100%) with Product size 151 and 211 bp, respectively.

Results of this study indicate that biofilm producing *S.aureus* have a major role player on the occurrence of mastitis .In addition, there was high prevalence of MRSA isolates (47%) in mastitic milk at the study area

INTRODUCTION

Mastitis means inflammation of the udder and is a common disease among dairy animals worldwide. It is often associated with bacterial intramammary infections (IMI),influence milk quality and yield negatively, therefore, mastitis is of major economic concern for the farmer (1,2).

Staphylococcus aureus is generally regarded as one of the major etiologic agents of mastitis in dairy animals (3,4,5). This pathogen has the potential to develop resistance to almost all the antimicrobial agents used for the management of the disease (3,5,6). *S. aureus* is also well known for its tolerance to a wide range of adverse circumstances. This tolerance is related to diverse genetic capabilities including the ability to form biofilms in the host, which contributes to the resistance of this microorganism against antibiotics (7,8).

S. aureus biofilms are considered major facilitators of different animal and human infections contributing 80% of all infections (9) .The major component of *S. aureus* biofilms is an exopolysaccharide, Polyβ-1, 6-linked N-acetylglucosamine (PNAG)(10). Four proteins including IcaA, IcaD, IcaB and IcaC encoded by the icaADBC operon are associated with the production of PNAG. IcaA and IcaD are the most important proteins for the production of PNAG(11). Carriage of the ica operon is a characteristic of most clinical *S. aureus* strains (12) Production of the extracellular polysaccharide in *S. aureus* is currently the best understood mechanism of biofilm development, this ica operon can be further differentiated to the icaA, icaD, icaB and icaC loci each responsible for relevant pathogenic and virulent factors involved in polysaccharide intercellular adhesin synthesis (13).

This study aimed to determine the isolation rate of *S. aureus* from sheep and goat mastitis cases, potential of these isolates to carriage ica operon and it is phenotypic evaluation of antibiotic susceptibility and biofilm formation.

MATERIALS AND METHODS

Samples collection

A total 150 milk sample were collected from clinical and subclinical mastitis of sheep and goat (75 for each one). The samples were collected after cleaning the udder by a piece of cloth then using cotton moistened by alcohol 70% and removing the first flowage of milk and collecting 10 ml in sterile tube, transported with ice box. The subclinical mastitis was confirmed with California mastitis test according to (14). From each sample, 1 ml of milk was pipetted into sterile microcentrifuge tubes and centrifuged at 5000 rpm for 5 min at room temperature. The supernatant was then discarded and the pellet was directly inoculated onto plated of mannitol salt agar(14).

Staphylococcus aureus **isolation and identification.**

Milk samples were inoculated on mannitol salt agar and incubated at 37°Cfor 24hrs. All colonies from primary cultures were purified by subculture on brain-heart infusion (BHI) agar and then inoculated onto MSA and incubated at 37°C for 24 h. (15).

Suspected colonies on mannitol salt agar were identified by coagulase test(15) , chromogenic agar (CHROMagar™ Staph aureus) (16,17) and VITEK 2 compact system according to its manufactures instructions.

Antibiotics susceptibility test

The antimicrobial susceptibility patterns of isolates to different antimicrobial agents was determined and interpreted according to (18). Nine antibiotics were chosen for the study. The antibiotic tested were from (Bioanalyse/ Turkey), as it was shown in table (1).

Table (1): Zone diameter interpretation standards according to (18)

N0.	Antimicrobial disc	Disc concentration µg or U/dis	Zone Diameter		
			R	I	S
1	Penicillin 10 units	10 units	≤ 28	-	≥ 29
2	Cefoxitin	30 µg	≤24	-	≥25
3	Vancomycin	30 µg	-	-	≥15
4	Gentamicin	10 µg	≤12	13-14	≥15
5	Clarithromycin	15 µg	≤13	14-18	≥18
6	Tetracycline	30 µg	≤14	15-18	≥19
7	Ciprofloxacin	5 µg	≤15	16-20	≥21
8	Clindamycin	2 µg	≤14	15-20	≥21
9	Chloramphenicol	30 µg	≤12	13-17	≥18

Biofilm formation assay

Biofilm formation was assayed phenotypically by the ability of cells to adhere to the wells of 96-well microtiter plate as described by (19).Briefly, the inoculum was prepared from bacteria grown in TSB broth, the culture was diluted 1:100 in TSB supplemented with 1% glucose, and 200 µl was poured into each wells. The negative control wells contained 200 µl of TSB supplemented with 1% glucose. The tissue culture plates were incubated at 37°Cfor 24hours. After incubation, the content of each well was gently removed by tapping the plates. The wells were washed 3 times with 0.2 ml of phosphate

buffer saline (PBS), fixed by methanol (0.2 ml) for 20 min, dried at room temperature and finally stained with 0.1% crystal violet. The crystal violet dye bound to the adherent cells was dissolved with 200 µl 95% ethanol per well, and the plateswere read at 490nm (A490) using ELISA reader. Optical density cut-off (ODc) was determined. It is defined as average OD of negative control + 3× standard deviation (SD) of negative control. Biofilm production is considered;

(Non-biofilm producer (OD < ODc), 0**) (**Weak biofilm producer (ODc < OD < 2×ODc), +**)(** Moderate biofilm producer (2×ODc < OD < 4×ODc), ++**) (** Strong biofilm producer (4×ODc < OD). +++**)**

Bacterial "DNA extraction" and PCR Method:

PCR technique was performed for detection ,icaA gene and icaD gene in "*Staphylococcus aureus*" isolated from mastitis milk samples by following steps:-

1-DNA extraction: Genomic DNA of *S.aureus* isolates were extracted by using Genomic DNA Kit (Geneaid . U S A) according to manufacturing instructions

2-Nano drop: The extracted DNA was estimated by "nanodrop device" at 260 /280 n m, and then kept at deep freezer until used in PCR method.

3-Primers: The PCR primers that used in this study for detection icaA and icaD genes were design by (20).These primers were provided by (Bioneer company, Korea) (Table 2).

Table (2): Primers for amplification *icaA* and *icaD* genes .

Primer	Sequence		Product size (bp)
icaA gene	F	5-GAGGTAAAGCCAACGCACTC-3	151
	R	5-CCTGTAACCGCACCAAGTTT-3	
icaD gene	F	5-ACCCAACGCTAAAATCATCG-3	211
	R	5-GCGAAAATGCCCATAGTTTC-3	

4- The "PCR master mix preparation"The reaction mixture was prepared by adding 1µl of both forward and reverse of the primers specific for the each gene, 3µl of DNA template to AccuPower ® PCR PreMix(20 µl reaction volume) and the volume was completed to 20 µl by adding nuclease free water.After that, all the PCR tubes transferred into "vortex and centrifuged" for 3 minutes. Then transferred into thermo cycler (Bioneer. Korea).

5- PCR thermo cycler conditions: -thePCR thermo cycler conditionslisted in table (3) .

Table (3): PCR thermo cycler conditions

Step	Temperature, °C	Time	Cycle
Initial denaturation	95	5 min	1
Denaturation	95	20 s	40
Annealing	60	20 s	
Extension	72	20 s	
Final extension	72	3min	1

6- PCR product analysis: The PCR products (151 b p and 2011 b p) were examined by electrophoresis in a 1.5% "agarose gel" using "1X TBE buffer", stained with "ethidium bromide", and conceive under "gel documentary".

RESULTS

Bacterial isolation and identification

According to the results of isolation and identification there were(٣٠2٠%) isolates of*S.aureus*(Table 4). The percentage of *S.aureus* isolates observed in sheep was 21.33% while in the goat (18.66%). There as no significant differences (P>0.05) in the percentage of *S.aureus* isolates between these mastitc milk samples .

Table 4. Numbers and percentages of *S .aureus* isolates recovered from mastitic milk of goat and sheep.

Sample	No. of sample	*S. aureus* isolates No.(%)	Other *staphylococci* No. (%)
Goat	75	14 (18.66)	39 (52)
Sheep	75	16 (21.33)	42 (56)
Total	150	30 (20)	81 (54)

(P>0.05)

All *S. aureus* isolates convert the medium of mannitol salt agar from red to yellow color (fig.1.A), form pink to mauve colonies on chromogenic agar (fig.1.B).and give positive result for coagulase test.

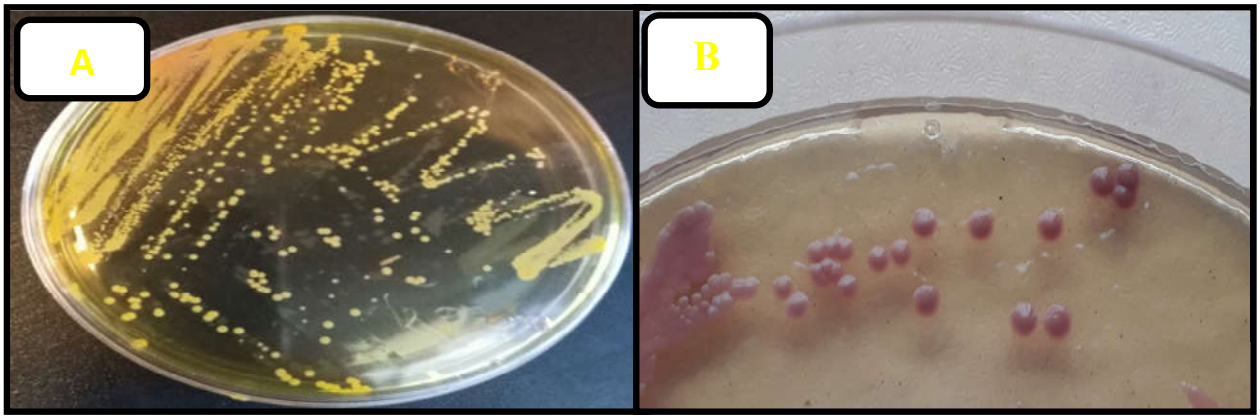

Fig .1. A-*S. aureus* colonies on MSA, B-*S. aureus* colonies on chromogenic agar

The identification was confirmed with automated VITEK-2 compact system using GP cards with ID massage confidence level as excellent (probability percentage from 95-99).

Antibiotics susceptibility test

After the identification of *S. aureus*, susceptibility test was performed for all *S. aureus* (30 isolates) by disk diffusion method to examine 9 different antibiotics as clarified in table (5). The results showed that, the highest resistant rate was against pencillin (100%) followed by cfoxitine (50%), tetracycline (30%), clindamycin , ciprofloxacin (22%) and clarithromycin (4%) .On the other hand, all the tested isolates showed 100% sensitivity to

vancomycin, gentamycin and Chloramphenicol. There was a significant difference among the antibiotics resistancy (P< 0.01).

Table 5.Antimicrobial susceptibility of *S.aureus* isolates from mastitic milk ofgoat and sheep to ward nine antimicrobials.

Antibiotic	Sheep (14)	Goat (16)	Total (69)
Penicillin 10 units	R (14)100% I (0) 0% S (0) 0%	R (16)100% I (0) 0% S (0) 0%	R (30)100% I (0) 0% S (0) 0%
30µg cefoxitin	R (6) 43% S (8) 57%	R (8) 50% S (8) 50%	R (14) 47% S (16) 53%
Vancomycin 30 µg	R (0) 0% I (0) 0% S (14)100%	R (0) 0% I (0) 0% S (16)100%	R (0) 0% I (0) 0% S(30)100%
Gentamicin 10 µg	R(0) 0% I (0) 0% S (14) 100%	R (0) 0% I (0) 0% S (16) 100%	R (0) 0 % I (0) 0 % S (30) 100%
clarithromycin 15 µg	R (0) 0% I (0) 0% S (14) 100%	R (0) 0% I (0) 0% S (15) 94%	R (0) 0% I (0) 0% S (30) 100%
Tetracycline30 µg	R (2) 14% I (2) 14% S (10) 72%	R (3) 19% I (2) 13% S (11) 68%	R (5) 16.5% I (4) 13.5% S (21) 70%
Ciprofloxacin 5 µg	R (0) 0% I (2) 14 % S (12) 86%	R (0) 0% I (4) 25% S (12) 75%	R (0) 0% I (6)20% S (39) 80%
B Clindamycin 2 µg	R (1) 7% I (1) 7% S (12) 76%	R (1) 6% I (2) 12% S (13) 82%	R (2) 6.6% I (3) 10% S (25) 83%
C Chloramphenicol 30 µg	R (0) 0% I (0)0% S (0)0%	R (0) 0% I (0)0% S (0)0%	R (0) 0% I (0) 0% S (0) 0%

P< 0.01

Biofilm formation assay by micro titer plat.

The ability of *S. aureus* isolates to produce biofilm were evaluated by using pre-sterilized 96-well polystyrene microtiter plates and then absorbance was determined at 580 nm in an ELISA reader for the determination of the degree of biofilm formation for studied isolates that adhered on the surface of the microtiter well.Absorbance values represented the degree of the biofilm thickness that formed by the studied isolates on the surface of the microtiter well. All *S. aureus* isolates assayed for the production of biofilm, and the results obtained are categorized into four groups based on statistical analysis of biofilm forming capacity: weak or non-producers (OD 490 nm < 0.064), modrate producers (OD490nm 0.064– 0.128), strong producers (OD490nm ≥ 0.128) .The results of the present study revealed that, all the tested isolates were found to be biofilm producer at different level (Fig 2). As shown in (table 6), out of a total 30 tested isolate, 6 (20%) isolates were moderate biofilm producer and the remaining isolates 24 (80%) were weak producer,.There were significant differences (P<0.05) in the percentage of weak and moderate biofilm formation between sheep and goat isolates.Moreover, There were no significant differences (P>0.05) in the percentage of weak and moderate biofilm formation between MRSA and MSSA isolates (Table 7).

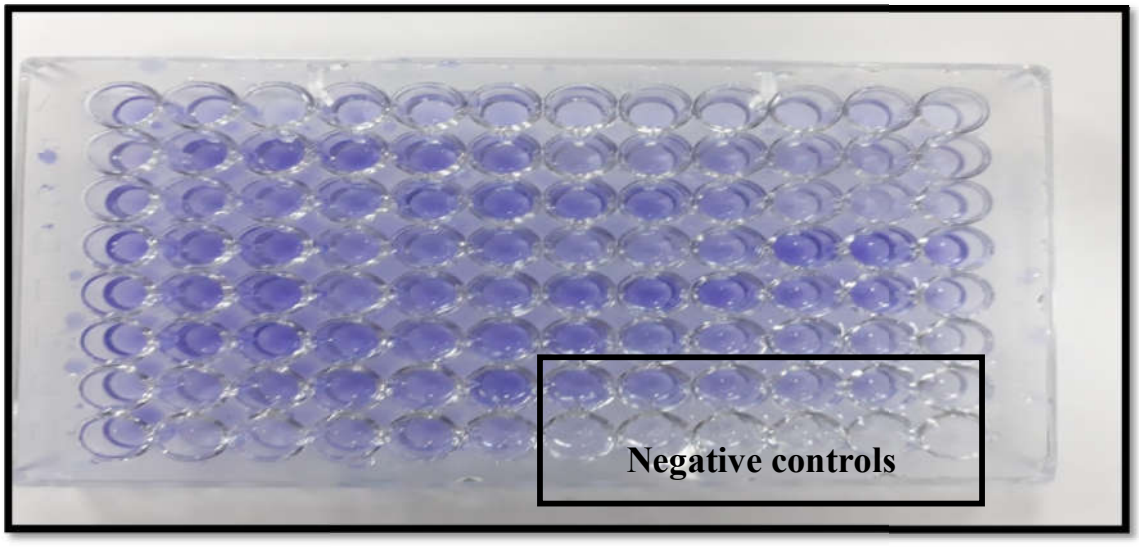

Fig 2: Biofilm formation of *S.aureus* on microtiter plate after staining with 1%crysal violet

Table 6: Biofilm producing ability of *S.aureus* on microtiter plate

Source of milk isolate	NO. of isolates	Biofilm producer			
		None NO. (%)	Weak NO. (%)	Moderate NO. (%)	Strong NO.(%)
Goat	14	0	14(100)	0 (0)	0
Sheep	16	0	10(62.5)	6(37.5)	0
Total	30	0	24 (80)	6 (20)	0

(P<0.05)

Table 7: Relationship between biofilm producing abilities and resistance to methicillin.

	NO.of isolate (%)	Biofilm producer			
		NoneNO.(%)	Weak NO. (%)	Moderate .NO. (%)	Strong .NO. (%)
MRSA	14 (47)	0	9(64)	5(36)	0
MSSA	16 (53)	0	10(62.5)	6(37.5)	0
Total	30	0	19 (63.4)	11 (36.6)	0

(P>0.05)

Detection of *icaA* and *icaD* gene.

The PCR analysis was applied to DNA extracted from *S.aureus* isolates from milk samples and the results of PCR assay revealed that all *S.aureus* isolates gave positive results for both icaA and icaD genes (100%) with Product size 151 and 211 bp, respectively (Fig 3 and 4).

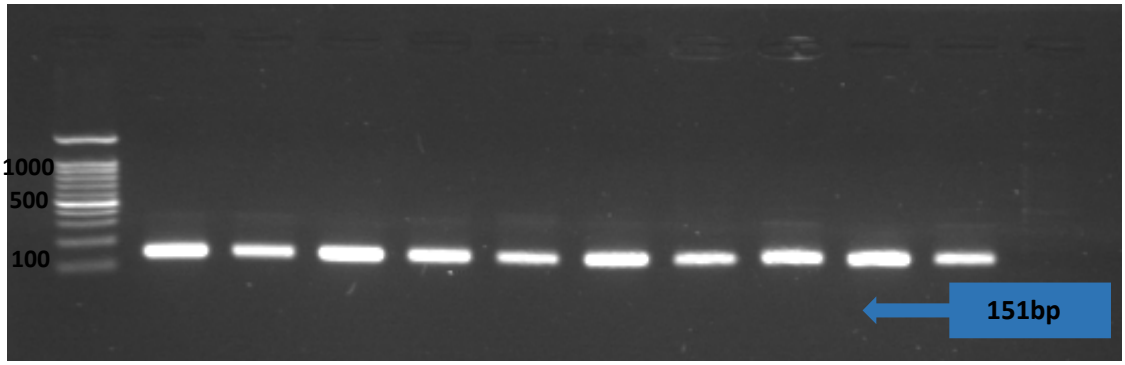

Fig 3 : Agarose gel electrophoresis of icaA gene amplification, M:ladder, 11:negative control , 1-10:positive results.

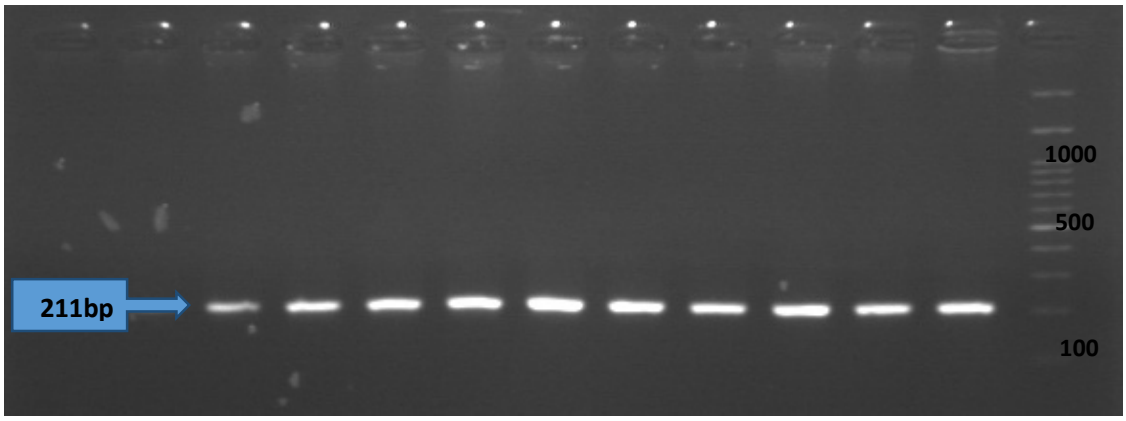

Fig 4: Agarose gel electrophoresis of icaD gene amplification, where M:ladder, 12:negative control , 1-11:positive results.

DISCUSSION

Distribution of *S. aureus*

S.aureus is one of the main etiological agents of mastitis in different mammalian species (2).Different works from different parts of the world give varying frequency of *S.aureus* isolation rate from mastitis milk of dairy animals , some of which agree while others disagree with the findings of the present study.

In the present study the isolation rate of *S.auresus* froim mastitis cases in ewes was 21.33%.This finding in agreement with the previous study(21) in which the isolation rate

of *S.aureus* from ovine mastitis was 26 %.It has been reported that*S. aureus*of intramammary infections in sheep ranged from 3 - 37%(22).

On the other hand ,the current result may appear higher than the result of(23–25), who isolate *S.aureus* from ovine mastitis in percentage 6.6%, 11.8% and 6.2 % respectively, and lower than results of (26,27), who recorded a percentage 90%, 40%, respectively. Mastitis milk samples of goat have lower percentage (18.66%) of *S.aureus* isolation rate in the present study ,these percentage an agreement with study of (28) who report low prevalence(4.1-23.7%) of *S.aureus* in caprine herds. Moreover ,our result also agreed with (29) who report the prevalence of *S.aureus* inframammary infections in goats was 17%.On the other hand ,higher rate of isolation were detected by (30,31) as 60 and 23% respectively and lower rate of isolation were detected by (32,33)as, 4.9% and 5.5% respectively.

Staphylococcal mastitis prevalence in dairy animals varies widely between different countries and may reflect the fact that different policies for infection control. A comparison of the results of the present study and those reported by other authors is difficult because the occurrence of *S.aureus* as a causative agent of mastitis varies according to the area, handling practices of the animals and hygienic conditions during milking(34)

Antibiotic susceptibility test

All the *S.aureus* isolates were resistance to penicillin and sensitive to vancomycin ,gentamicin ,clarithromycin and chloramphenicol .

This finding in agreement with the previous studies(35–39)in which that*S.aureus* isolated from mastitis were resistance to penicillin 100% and sensitive to Gentamicin ,clarithromycin chloramphenicol and vancomycin 100% .

On the other hand ,VRSA have been reported by (6,8,40,41) in a percentage 8.6% ,21% ,50%,76% respectively and chloramphenicol resistance were detected in a percentage of 17% by(41) , 12 % by (8) and 42% by (6).

The high sensitivity rate twoard vancomycin and chloramphenicol in the current result may belong to low rate of usage in the animals and human host.

In the present study, cefoxitin was used for detection MRSA strains. According to (18) , oxacillin or cefoxitin replace methicillin as this antibiotics is stable under storage conditions, and methicillin actually is an excellent inducer of the mecAgene. However, methicillin is not the agent of choice for MRSA recognition and its not preferred to evaluate methicillin resistance, so it should be replaced by oxacillin or cefoxitin for detection of MRSA isolates. Moreover the cefoxitin disk test is easier to read and thus is the preferred method in comparison with oxacillin and methicillin (18).

The current result revealed that, the resistance to methicillin was 43% and 50% for sheep and goat, respectively. This finding in agreement with the previous studies dealing with mastitis milk of dairy animals (42,43) who isolate MRSA in percentage of 52% and 53.3%.respictivily.On the other hand, higher results were obtained by (21)who recorded the occurrence of MRSA was 88% and lower results were detected by (36) who found only 10 % of *S.aureus* was MRSA.

Methicillin resistance is clinically the most important, since single genetic element can convers resistance to most commonly prescribed class of antimicrobials-the beta lactam antibiotics, which include penicillins, cephalosporin and carbapenems(44,45).

The reason behind continuous increasing in resistant to β-lactam antibiotics is caused by the overuse or misuse of these antibiotics and by the use of poor quality antibiotics. It also results from natural genetic changes, or mutations, within the organisms that cause diseases. Different classes of antibiotics such as vancomycin, linezolid, quinupristin/dalfopristin (streptogramin) and newer fluoroquinilones were used for treatment of severe MRSA infection caused by multidrug resistant strain (44). However, since 1990, MRSA strains with intermediate resistance to vancomycin (MIC, 8-16 µg/ml) and strains fully resistant to vancomycin (MIC ≥ 32 µg/ml) have been reported (46)

The results of the present study showed that the susceptibility of animals isolate against chloramphenicol, gentamicin,clarithromycin and ciprofloxacin were 100% while, the sesnsitivity against tetracycline 70%Ciprofloxacin 80% and clindamycin83% .This

finding in agreement with the previous study (21) who found the sensitivity of *S.aureus* isolated from dairy animals to clarithromycin , ciprofloxacin ,gentamicin were 100% ,100% and 94.1%.Moreover ,the present result were compatible with local study of (35)who found the sensitivity rate of *S.aureus* was 100% to gentamicin and ciprofloxacin , 94.5% to erythromycin and clindamycin and 89.9% to doxycycline . However , the local study of (47) showed slightly higher rate of resistance to gentamicin,clarithromycin and ciprofloxacin in a percentage of 29% ,12% ,9% ,respectively.

Biofilm Formation

The isolated *S. aureus* were evaluated for biofilm formation capability using phenotypic screening as well as molecular detection of icaA and ica D genes. Microtiter plate (MTP) showed that, 30/30 isolates (100%) were able to form biofilm .In addition,all *S.aureus* isolates were investigated for biofilm associated genes, icaA and icaD. Molecular investigation revealed that both *icaA* and *icaD* genes were present in the 100% of isolates.

These data are in accordance with those reported by (48)who detected *icaA* and *icaD* in all *S. aureus* isolates by PCR techniques. Similar results were obtained by (49) who found that all the isolates were biofilm producing and contain ica locus .

The current results also were compatible with the studies of (50), (51).Whom found all clinical isolates of *S. aureus* were biofilm producer and positive for both *icaAand icaD* genes .In addition ,the present study are in line with local study of (8)who found 94.117%of biofilm production in strains of *S. aureus* isolates from bovine mastitis. On the other hand , slightly lower percentage of biofilm production were reported by the study of (35) reported that, 80.6% of *S.aureus* isolates were biofilm positive when tested by MTP method.

The present result close to many worldwide studies such as (52) who found all strains tested were biofilm producer by MTP and 97% of them harboring icaA and icaD gene.(53) who found all 32 *S. aureus* isolates harbored the *icaA* and *icaD* genes. However, our results are in contrast with the data reported by (54), who detected icaA and *icaD* genes in only (12.5%) of 23 *S. aureus* isolates and (55) who detected *icaA* and

icaD genes in 70% of *S. aureus* isolates. The variations in the presence of *icaAD* genes from different studies might be due to the heterogeneity in the genetic origins of *S.aureus*(55)

In the present study, a high percentage of agreement (100%) was observed between the genotypes and phenotypes of isolates, determined by PCR and MTP, respectively. Broad applicability, reliability and high reproducibility of the MTP were previously verified for bacterial biofilms (56).

On the other hand , failure of *S. aureus* strains that possess the ica locus to form biofilm has been reported *in vitro*(53) and biofilm producing *S.aureus* that lack ica operon also reported by many studies such as (57,58).These results suggest that biofilm production is regulated by the interaction of different regulatory mechanisms and the expression of ica genes is strongly influenced by environmental factors such as glucose, temperature, osmolarity, and growth in anaerobic conditions (59). Indeed, transcriptional regulation of the ica operon is complex, involving the interdependent and independent activity of several activators and repressors. Differential transcriptional regulation of the locus and/or putative ica-independent biofilm mechanisms can influence biofilm production phenotype (60). Insertional inactivation and point mutations in the ica locus were reported as other plausible mechanisms to give rise to biofilm-negative variants in *S.aureus*(61). Thus, the difference between phenotypic and genotypic characterization may be due to the heterogeneity in the genetic origins, and not because of the presence or absence of genes required for the biofilm formation.Therefore, a combination of phenotypic and genotypic assays should be employed for improved confidence in identifying biofilm-producing *S. aureus* isolates.

In the present study the statistical analysis showed there was no significant differences ($P>0.05$) in the percentage of weak and moderate biofilm formation between MRSA and MSSA isolates.The current result agreed with the study of (62) who reported there is no significant differences in biofilm production between MRSA and MSSA. in contrast, biofilm producing ability was higher in MRSA isolates according to the study of (63).on the other hand, lower level of biofilm production in MRSA than MSSA were reported by (64).The lower level of biofilm production in MRSA strain may be due to the biofilm

phenotype expressed by clinical isolates of *S. aureus* is influenced by acquisition of the methicillin resistance gene mecA which cause repression of PIA-mediated biofilm production (65). This differences in the results may relate to the most commonly isolated genotypes of MRSA included in each study.

الكشف عن جينات الالتصاق الداخل خلوية وتكوين الغشاء الحيوي في جرثومة المكورات العنقوديه المعزولة من حالات التهاب الضرع في الأغنام والماعز

حسن كريم ادبيس ،محمد حسن خضر

فرع الاحياء المجهريه ، كلية الطب البيطري، جامعة البصره ،البصره ، العراق.

الخلاصة

تم في هذه الدراسة جمع ١٥٠ عينة من الحليب بواقع ٧٥ عينه لكل من النعاج والماعز المصابه بالتهاب الضرع،تم جمع العينات خلال فترة سبعة اشهر . تم تحليل هذه العينات لغرض التحري عن وجود بكتريا المكورات العنقوديه الذهبيه واظهرت النتائج ان هذه البكتريا لوحظت بنسبة 20٪ من مجموع هذه العينات .اعلى نسبه من بكتريا المكورات العنقوديه كانت في النعاج 21.33%ثم الماعز١٨.٦٦%. أظهرت دراسة اختبار الحساسية للمضادات الحيوية على ٩ مضادات حيوية مختلفة أن بكتيريا المكورات العنقوديه كانت مقاومه ١٠٠٪ للبنسلين و ١٠٠٪ حساسة للفانكوميسين ، جنتاميسين ، كلاريثروميسين و الكلورامفينيكول في حين كانت المقاومة للسيفوكسيتين (بديل للميثيسيلين) ٤٧٪ .وكانت نسبة المقاومة متغيرة لباقي المضادات الحيوية كمايلي: ، تتراسيكلين (٣٠ ٪) ، سيبروفلوكساسين (٢٠ ٪) ، الكليندامايسين (٢٣ ٪) ، تم تقييم مقدرة المكورات العنقودية الذهبية على تكوين الغشاء الحيوي عن طريق (microtiter plates)وكشفت النتيجة أن جميع العزلات المدروسة كانت إما منتجه للغشاء الحيوي بصوره معتدله او ضعيفه ولا وجود للعزلات الغيرمنتجه او المنتجه بصوره قويه للغشاء الحيوي . أظهرت العلاقة بين تكوين الأغشية الحيوية والمقاومة الميثيسيلين عدم وجود فروق معنوية بين انتاج الغشاء الحيوي بصوره ضعيفه او معتدله مع العزلات المقاومه او الحساسه للمثسيلينتم استخلاص الحمض النووي من جميع عزلات المكوورات العنقوديه الذهبيه واستخدم اختبار تفاعل البلمرة المتسلسل من اجل التحري عن الجينات المنتجه للبايوفيلم icaA وicaD وأظهرت نتائج الاختبارأن جميع العزلات أعطت نتائج إيجابية لكلا الجينين100) ٪ (مع حجم المنتج ١٥١ و ٢١١ bp، على التوالي

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