Int.J.Curr.Microbiol.App.Sci (2016) *5*(5): 33-44

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 5 Number 5 (2016) pp. 33-44**
Journal homepage: http://www.ijcmas.com

Original Research Article http://dx.doi.org/10.20546/ijcmas.2016.505.004

Biofilm Production by *Staphylococcus aureus* isolated from Bovine Mastitis Related with Resistance to the Antibiotics

Sahar Mahdi H. Al-Rubaye[1], Essam F. Al-Jumaily[2*] and Hassan A. Abdul-Ratha[3]

[1]Microbiology Department, College of Veterinary Medicine/Baghdad University, Iraq
[2]Biotechnology Department, Genetic Engineering and Biotechnology Institute Postgraduate/Baghdad University, Iraq
[3]Agriculture College / Baghdad University, Iraq
**Corresponding author*

Keywords

Staphylococcus aureus, Biofilm, Bovine Mastitis, Antibiotics resistant.

Article Info

Accepted:
06 April 2016
Available Online:
10 May 2016

A B S T R A C T

The objective of this study is by focusing on the use of biofilm and probiotics on prevention and treatment of mastitis in cows. Fifty one isolates of *Staphylococcus aureus* were collected from different local areas in Iraq. In this study, forty eight *Staphylococcus aureus* were producing biofilm in different thickness while the remaining three isolates couldn't produce biofilm. Six of *Staphylococcus aureus* isolates (from the forty eight) had higher thickness (3mm) of biofilm in which the protein concentration were 62,66,70,72,80 and 85 mg /ml., subsequentially. *Staphylococcus aureus* isolates that produce high thickness of biofilm appeared to be more resistant to most common antibiotics and had significant differences at 18 ±0.25 with the level $p< 0.05$.

Introduction

One of the defense mechanisms of *S. aureus* is the capacity to form biofilm. A biofilm is an aggregate of microorganisms in which cells adhere to each other on a surface, these adherent cells are frequently embedded within a self- produced matrix of extracellular polymeric substance (Medora *et al.*, 2010).

Biofilm EPS, which is also referred to as slime (although not everything described as slime is a biofilm), is a polymeric conglomeration generally composed of extracellular DNA, proteins and polysaccharides, biofilms may form on living or non-living surfaces and can be prevalent in natural, industrial and hospital settings (Hall-Stoodley *et al.*, 2004; Lear and Lewis, 2012). The microbial cells growing in a biofilm are physiologically distinct from planktonic cells of the same organism, which by contrast are single-cells that may float or swim in a liquid medium, microbes from a biofilm in response to many factors, which may include cellular recognition of specific or non- specific

attachment sites on a surface, nutritional cues, or in some cases, by exposure planktonic cells to sub-inhibitory concentrations of antibiotics (Karatan and Watnick, 2009).

Adhesion of bacterial cells (e.g. *Staphylococcus aureus*) to the mammary gland epithelium has been considered the primary step in the pathogenesis of mastitis (Cifrian *et al.,* 1994).

Initial biofilm formation mastitis cases occur proximately 24 hours after exposure to the infecting microorganism, bacterial clusters appear in the mammary alveoli and lactiferous ducts and also within the interstitial tissue (Hensen *et al.,* 2000).

Research on the molecular and genetic basis of biofilm development has demonstrated that when cells switch from planktonic to community mode, they also undergo a shift in behavior that involves alteration in the activity of numerous genes, there is evidence that specific genes must be transcribed during the attachment phase of biofilm development, in many cases, the activation of these genes is required for synthesis of the extracellular matrix that protects the pathogens inside (Costerton *et al.,* 1999).

Materials and Methods

Milk Sample Collection for *Staphylococcus aureus* Isolation

One hundred forty two (142) milk samples were collected from Abu Ghraib cow fields, Al-Rashedia cow field, field of College of Veterinary Medicine–University of Baghdad, field of College of Agriculture-University of Baghdad, Radwanyya fields from cows suffering acute clinical and sub clinical mastitis.

California mastitis test is an indirect test used to determine the somatic cells amount in milk. A field test was conducted by put equal amounts of milk in California lotion paddle and mixed well light circular motion and horizontal for 10 seconds according to Schalm *et al.,* 1971.

Results and Discussion

Table (1) shown that the results out of (51) *S. aureus* isolates, 48 isolates produce biofilm (94.117%), and these results were in agreement with Vasudevan *et al.* (2003), who demonstrated that (32) of (35) *S. aureus* isolates were slime positive, while Zmantar *et al.* (2010) found that (26) out of (46) isolates of *S.aureus* with mean (56.5%) were slime producers.

The results showed that these isolates different in its biofilm producing efficiency, the thickness of biofilm which measured in these isolates ranged between 0.2-3 mm, these results nearby Yarmorade (2013) showed that thickness of biofilm produced by *S.aureus* ranged between 0.2-1.5 mm., and agreement with Al-Tabakchally (2015) that found the thickness of biofilm produced by *E. coli* between 0.2 -2 mm,but disagreement with Al- Ilthawe (2010) found that the thickness of biofilm produced by *Pseudomonas aeruginosa* ranged between 1.1- 6.5 mm.

Gondogan *et al.* (2006) found that(58) out of (110) *S.aureus* isolates were slime producers, while Fox *et al.,* (2005) found a higher percentage (41%) of biofilm-positive isolates from milk, but Baselga *et al*. (1993) demonstrated a lower percentage of 12% of biofilm-positive producer isolates in (92) bovine isolates tested. *Staphylococcus aureus* is the main etiological organism responsible for bovine mastitis, while ability of *S. aureus* to form biofilms plays an

important role in the pathogenesis of mastitis, biofilm formation in *S. aureus* is associated with the production of polysaccharide intercellular adhesion (PIA) protein and several other proteins, several environmental factors, including glucose, osmolarity, oleic acid and temperature, have been reported to affect biofilm formation in *S. aureus* on the other hand, previous studies showed that lactose increased biofilm formation in *S.aureus* predominantly by inducing PIA production, whereas milk increased biofilm formation through PIA as well as by increasing the production of other biofilm-associated proteins, which might be mediated by the transcriptional regulators intercellular adhesion regulator (icaR) and repressor of biofilm (rbf) (Xue, 2014).

Staphylococcus aureus and coagulase-negative staphylococci (CoNS), are characterized by the ability of the causative microorganisms to colonize surfaces of biomaterials by adhering to their surface in biofilms structured communities of cells encased in self produced polymeric matrix, the capacity of Staphylococci to form biofilms allowing it to evade host immune defense mechanisms and antibiotic therapy is considered to be crucial in colonizing the surfaces of the implants, an understanding of the composition of staphylococcal biofilms and the detailed chemical structure of biofilm components is essential for a clear understanding of the pathogenesis of these bacteria, and the designing of new therapeutic tools against staphylococcal infections (Evgueny *et al.,* 2005).

Internalization is an important step in staphylococcal mastitis pathogenesis (Hensen *et al.,* 2000). *In vitro* studies have shown that Staphylococci are able to adhere to and invade bovine mammary epithelium, biofilm formation is a potential virulence factor (Almeida and Oliver, 2001). During intramammary infection bacterial clusters may develop within the udder and biofilm structures may facilitate Staphylococci adherence and colonization of the mammary gland epithelium (Hensen *et al.,* 2000).

In other bacterial genera, the ability to form biofilm appears to be associated with invasiveness (Latasa *et al.,* 2005). In *S. aureus*, this relationship has not yet been clarified although it has been shown that capsule and other exoproducts may inhibit internalization, the abilities of Staphylococci to be internalized and form a biofilm can contribute to host immunological defense evasion that subsequently impairs antimicrobial therapy (Manuela *et al.*, 2011).

Most studies of biofilm biology have taken a reductionist approach, where single-species biofilms have been extensively investigated. However, biofilms in nature mostly comprise multiple species, where interspecies interactions with shape the development, structure and function of these communities differently from biofilm populations (Lee *et al.,* 2014). *S.aureus* isolates results showed differences in the thickening ring formed into test tube in the laboratory, after measuring thickened episode turned out to be ranging from (0.2 to 3) mm Table (2).

Biofilm of Protein Concentration

Direct contact of *S. aureus* with the surface is required for attachment and subsequent colonization by produce extracellular polymeric substance (EPS) that will glue the cell to the surface and form the biofilm matrix, this material composed of polysaccharides, and may also proteins, nucleic acid and polymeric lipophilic compounds. (Neu *et al.,* 2001).

Zulfiqar *et al.,* (2013) found that many strains of methicillin sensitive *S. aureus* MSSA isolates were biofilm positive and

majority were found to carry agr type II, also found that isolates of methicillin resistant *Staphylococcus aureus* (MRSA) carry *icaA* gene, polymerase chain reaction studies suggested that all of the biofilm positive MRSA isolates belong to SCC*mec* type IV and carry agr type II, this showed the strong association of SCC*mec* IV agr type II and biofilm formation in food borne.

Six isolates which gave the highest thickness of biofilm that belong to local numbers (11, 22, 30, 33, 37. and 45) were used to measure the protein concentration in its crude biofilm according to Biuret method (Fenk *et al.*, 2007). Table (3) shown the results showed the highest protein concentration of biofilm produced by *S aureus* isolate number 30, was 85 mg/ml., while the lower one by *S.aureus* isolate number 37 was 62 mg/ml.

Figure (1a,b)shown the results were in agreement with (AL-Tabakchally 2015), who found that the protein concentration of the biofilm for *E.coli* isolates was 92 mg/ml, while Yarmorad (2013) showed that *Staphylococcus aureus* produced Biofilm with low protein concentration 9 mg/ml for one *S.aureus* isolates and 18 mg/ml from another caused mastitis cases, and Al-Ithawy (2010) found that the highest protein concentration of biofilm extract produced by *Pseudomonas aeruginosa* was 0.12 mg/ml only.

A biofilm can be defined as a microbially-derived sessile community, typified by cells that are attached to a substratum, interface or to each other are embedded in a matrix of extracellular polymeric substance, and exhibit an altered phenotype with regard to growth, gene expression and protein production, biofilm thickness can range from a single cell layer to a substantial community encased by a viscous polymeric milieu, structural analyses have shown that in some cases unique pillar or mushroom-shaped structures can be formed by the micro-colony architecture of these dense biofilms, however other structures do form depending on the environmental conditions (Nathan *et al.*, 2011).

In vitro, strains differ in their ability to withstand killing by neutrophils, form biofilms or invade mammary epithelial cells (Hensen, 2000). Biofilm formation and invasion into mammary cells can be expected to protect *S. aureus* from the host immune response and from antibiotics by making the bacteria inaccessible (Cucarella *et al.*, 2004).

The ability to survive phagocytosis by neutrophils would protect the bacteria even if they were exposed to the host immune response, except maybe in the case of antibiotics that penetrated intracellularly, such as macrolides (any of a class of antibiotics containing a lactone ring of which the first and best known is erythromycin) (Janosi *et al.*, 2001).

Susceptibility tests were conducted for the 48 isolates of *S.aureus* against different 12 antibiotics to assess the prevalence of antibiotic resistance. These isolates were divided into two categories, first one the *S. aureus* isolates produced biofilm thickness between 0.2 -0.9 mm. and the second, *S. aureus* isolates produced biofilm thickness between 1-3 mm., each isolates was tested against (12 antibiotics. The zone of inhibition of bacterial growth around the antibiotic disc were measured to check isolates susceptibility. The inhibition zone measured by HiAntibiotic Zone Scale™C, (Figure 2).

Table (3) showed the highest sensitivity of *S.aureus* that produced biofilm (0.1-0.9 mm) isolates to Ciprofloxacin (84%), then Chloramphenicol, Azithromycin and

Kanamycin (76%), while the Nitrofurantion and Vancomycin (52%) and less sensitivity were Piperacillin (12%), Trimethoprim+ Sulphamethoxazole (28%) and Gentamycin (36%), also the results were showed the higher resistant to piperacillin(56%), Cloxacillin (28%) whilst Nitrofurantin, Amoxicillin+Clavulanic acid, Chloramphenicol, Azithromycin and Kanamycin were 12 %.

The results showed the highest sensitivity of *S.aureus* that produced biofilm (1-3 mm) isolates to Azithromycin and Ciprofloxacin were (78.2%), Chloramphenicol (73.9%), Kanamycin (65.2%), while Gentamycin and Trimethoprim +Sulphamethoxazole (52.1%), in addition the highest resistant of *S. aureus* to Cloxacillin (47.8%), Amoxicillin+ Clavulanic acid, Polymyxin B, and Piperacillin (26%), the lowest antibiotics were Vancomycin, Chloramphinicol, Azithromycin, Gentamycin and Trimethoprim + Sulphamethoxazole (8.6%) Table (4).

Table.1 The Biofilm Production by Different *S.Aureus* Isolates

No. of isolates	Result	No. of isolates	Result	No. of isolates	Result
1	+	20	+	39	+
2	+	21	+	40	+
3	+	22	+	41	+
4	+	23	+	42	+
5	+	24	+	43	+
6	+	25	+	44	+
7	+	26	+	45	+
8	+	27	+	46	+
9	+	28	+	47	+
10	+	29	+	48	+
11	+	30	+	49	-
12	+	31	+	50	-
13	+	32	+	51	-
14	+	33	+		
15	+	34	+		
16	+	35	+		
17	+	36	+		
18	+	37	+		
19	+	38	+		

(+) produce Biofilm;
(-) notproduceBiofilm

Table.2 Thickness of Biofilm Produced by Different Isolates of *S. aureus*

Number of isolates	Thickness of biofilm	Number of isolates	Thickness of biofilm
1	0.2	25	1.2
2	0.2	26	0.8
3	0.4	27	2.6
4	0.8	28	2.4
5	0.7	29	0.4
6	0.7	30	3.0
7	0.2	31	0.6
8	0.2	32	0.8
9	0.3	33	3.0
10	0.9	34	1.8
11	3.0	35	2.2
12	0.6	36	0.3
13	0.4	37	3.0
14	0.2	38	0.8
15	0.8	39	2.6
16	2.0	40	2.0
17	2.6	41	0.6
18	1.4	42	1.4
19	2.8	43	1.0
20	0.2	44	0.5
21	0.9	45	3.0
22	3.0	46	1.0
23	2.2	47	1.4
24	0.4	48	1.2

Table.3 *S. aureus* Isolates Producing Biofilm 3mm Thickness

No. of *S.aureus* isolates	Protein Concentration (mg/ml)
11	66
22	70
30	85
33	80
37	62
45	72

Table.3 The Antibiotic Sensitivity Test of (25) *S. aureus* Isolates Produce Thin Layer (0.2-0.9) mm of Biofilm

Number of *S.aureus* isolates produced biofilm (0.1-0.9)mm						
Antibiotics	Resistant		Intermediate		Susceptible	
	Number	%	Number	%	Number	%
Nitrofurantion	3	12	9	36	13	52
Amoxicillin/ Clavulanic acid	3	12	10	40	12	48
Vancomycin	4	16	8	32	13	52
Polymyxin B	4	16	10	40	11	44
Chloramphenicol	3	12	3	12	19	76
Cloxacillin	7	28	8	32	10	40
Azithromycin	3	12	3	12	19	76
Piperacillin	14	56	8	32	3	12
Gentamycin	4	16	12	48	9	36
Trimethoprim/ sulphamethoxazole	4	16	14	56	7	28
Ciprofloxacin	2	8	2	8	21	84
Kanamycin	3	12	3	12	19	76

Table.4 Susceptibility Test of Antibiotics against (25) *S. aureus* Isolates Produced Thick layer (0.2-0.9) mm of Biofilm

Thickness of biofilm (0.2-0.9) mm / Antibiotics µg	Sensitive	Intermediate	Resistance
Nitrofurantion 300	0.4154±0.06186b	0.5300±0.07000Bb	0.8667±0.03333Aa
Amoxicillin+ Clavulanic acid 30	0.5000±0.08842b	0.4818±.06983Cb	0.7000±0.05774Ba
Vancomycin 30	0.5000±0.07110b	0.4714±0.07781 Cb	0.6500±0.15000 Ba
Polymyxin B 300	0.5333±0.07521b	0.4300±0.07753Cb	0.7333±0.06667Ba
Chloramphenicol 30	0.4789±0.06145b	0.6000±0.05774Ba	0.6667±0.13333Ba
Cloxacillin 1	0.5200±0.08537	0.5000±0.09063B	0.5286±0.09932B
Azithromycin 15	0.4882±0.06580	0.5750±0.10308B	0.6750±0.11087B
Piperacillin 100	0.5667±0.11450	0.4429±0.07514B	0.5333±0.08009BC
Gentamycin 20	0.5300±0.08699	0.5167±0.07671B	0.4240±0.08539C
Trimethoprim+ Sulphamethoxazole 25	0.5000±0.09063	0.5000±0.06887B	0.6000±0.15811B
Ciprofloxacin 10	0.5143±0.05744b	0.3500±0.05000Cb	0.7000±0.10000Ba
Kanamycin 30	0.4722±0.05648b	0.7333±0.03333Aa	0.6333±0.21858Bb

Different Capital letters denote significant ($p<0.05$) differences among antibiotics.
Different small letters denote significant ($p<0.05$) differences among characters(thickness of biofilm)

Table.5 The Antibiotics Sensitivity Test for 23 *S.aureus* Isolates Produce Thick Layer (1-3)mm of Biofilm

Number of *S.aureus* isolates produced biofilm (1-3)mm						
Antibiotics	Resistant		Intermediate		Susceptible	
	Number	%	Number	%	Number	%
Nitrofurantion	4	17.3	8	34.7	11	47.8
Amoxicillin/ Clavulanic acid	6	26	9	39.1	8	34.7
Vancomycin	2	8.6	11	47.8	10	43.4
Polymyxin B	6	26	12	52.1	5	21.7
Chloramphenicol	2	8.6	4	17.3	17	73.9
Cloxacillin	11	47.8	5	21.7	7	30.4
Azithromycin	2	8.6	3	13	18	78.2
Piperacillin	6	26	14	60.8	3	13
Gentamycin	2	8.6	9	39.1	12	52.1
Trimethoprim/ sulphamethoxazole	2	8.6	9	39.1	12	52.1
Ciprofloxacin	3	13	2	8.6	18	78.2
Kanamycin	3	13	5	21.7	15	65.2

Table.6 Susceptibility Test of Antibiotics against (23) *S. aureus* Isolates Produced Thick Layer (1-3) mm of Biofilm

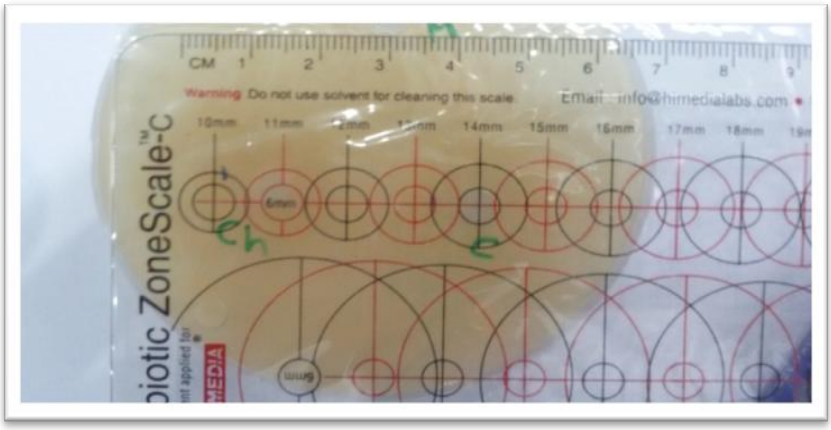
Thickness of biofilm (1-3) mm / Antibiotics µg	Sensitive	Intermediate	Resistance
Nitrofurantion 300	1.545±0.2407 Db	1.5455±0.24135 Cb	3.1000±0.25166 Aa
Amoxicillin+ Clavulanic acid 30	1.3250±0.11299 Cc	2.3778±0.1222 Cb	2.9667±0.3333 Ba
Vancomycin 30	2.1600±0.18809 Bb	2.0182±0.25075 Cb	2.9333±0.06667 Ba
Polymyxin B 300	1.2000±0.08944 Cc	2.1500±0.14381 Cb	2.9625±0.02631 Ba
Chloramphenicol 30	1.8824±0.15440 Bb	2.7000±0.3000 Ba	2.9000±0.07071 Ba
Cloxacillin 1	1.5714±0.25608 Cb	1.8000±0.24495 Db	2.7091±0.11237 Ba
Azithromycin 15	2.0222±0.16160 Bc	2.4667±0.53333 Bb	2.9667±0.03333 Ba
Piperacillin 100	2.2667±0.43716 Ab	1.7857±0.15473 Dc	2.9750±0.01637 Ba
Gentamycin 20	1.7667±0.20865 Bc	2.5111±0.14948 Bb	2.9333±0.06667 Ba
Trimethoprim+ Sulphamethoxazole 25	1.7333±0.18641 Bc	2.5556±0.17249 Bb	2.9667±0.0333 Ba
Ciprofloxacin 10	1.9333±0.15424 Bb	2.9667±0.0333 Aa	2.9500±0.05000 Ba
Kanamycin 30	2.000±0.19712 Ba	0.7333±0.0333 Eb	0.6333±0.21858 Cb

note: Different Capital letters denote significant ($p<0.05$) differences among antibiotics.
Different small letters denote significant ($p<0.05$) differences among characters (thickness of biofilm)

Figure.1a Thickness of Biofilm produced by *Staphylococcus aureus*

Figure.1b *S. aureus* produced a Dense Material of Biofilm Stick to the Internal wall of the Tube Stained by Saphranine Dye

Figure.2 Hi Antibiotic Zone Scale TMC, that measured the Thickness of Biofilm Layer Produced by *S. aureus* Isolates



The results showed in sensitivity there were no significant differences in among all of concentration of antibiotics. While the intermediate were Significant differences

among the concentration of antibiotics in which Kanamycin have been more value than the other antibiotics, and the lowest were Amoxacillin + Clavulanic acid, Vancomycin, Polymyxin, and B Ciprofloxacin, also the table showed that resistance were significance differences among Antibiotics in which the Nitrofurantion have been higher value and the lowest one were Piperacillin and Gentamycin. As the table shown, the resistance has superior value as compared with intermediate and sensitivity as Amoxicillin+ Clavulanic acid, Vancomycin and Polymyxin B. Chloramphinicol resistance and intermediate higher than sensitivity while Cloxacillin has no significant as well as Azanthromycin, Piperacillin, Gentamycin and Trimethoprim+Sulphamethoxazole. The results of resistance to *S. aureus* that produced biofilm showed were significant differences against Vancomycin that agreement with Harriott and Noverr (2009).

The sensitivity results showed there was high significant differences in Piperacillin 100 among different concentration of antibiotics, then Kanamycin, and others Vancomycin, Chloramphenicol and Ciprofloxacin and the lowest one was Nitrofurantion (Table 5).

While the intermediate were Significant differences among the concentration of antibiotics in which Ciprofloxacin have been more value than the other antibiotics, and the lowest were Kanamycin also the table showed that resistance were significance differences among Antibiotics in which the Nitrofurantion have been higher value and the lowest one was Kanamycin.

Table (6), the resistance of Nitrofurantion has superior value as compared with intermediate and sensitivity. The sensitivity of Gentamycin against *S.aureus* was (1.7667±0.20865) appeared significant differences at level $p< 0.05$, this result disagreement with (Coraca *et al.,* 2012) he showed the Gentamycin was the most effective inhibitors of *S. aureus* biofilm-related infections, but the sensitivity of *S. aureus* toward Vancomycin was (2.1600±0.18809) appeared less significant differences that result agreement with (Okuda *et al.,* 2013) who said that Vancomycin, aglycopeptide antibiotic used in the treatment of *S.aureus* infections, showed less activity against biofilm cells.

References

Al-Ithawy, A.A. 2010. Affected factors on *Pseudomonas aeruginosa* Isolated from deferent environmental sources on biofilm formation, M.Sc. University of Anbar. College of science -Iraq

AL-Tabakchally, B.N. 2015. Detection of biofilm formed by *Escherichia coli* isolated from milk and fecal animal samples and evaluation its immunogenicity. M.Sc. Thesis- College of Veterinary Medicine- Baghdad University- Iraq.

Baselga, R., Albizu, I., De La Cruz, M., Del Cacho, E., Barberan, M., Amorena, B. 1993. Phase variation of slime production in *Staphylococcus aureus*: implication in colonization and virulence. *Inf. Imm.*, 61: 4857-4862.

Cifrian, E., Guidry, A.J. 1994. Adherence of *Staphylococcus aureus* to cultured bovine mammary epithelial cells. *J. Dairy Sci.*, 77(4): 970-983.

Costerton, J.W., Stewart, P.S., Greenderg, E.P. 1999. Bacterial biofilms: acommon cause of persistent infections. *Sci.*, 284: 1318-1322.

Coraca, H.D.C., Fille, M., Hausdorfer, J.,

Pfaller, K., Nogler, M. 2012. *Staphylococcus aureus* biofilm formation and antibiotic susceptibility test on polystyrene and metal surfaces. *J. Appl. Microbiol.*, 112(6):1235-43.

Cucarella, C.M.A., Tormo, C., Ubeda, M.P., Trotonda, M., Monzon, C., Peris. 2004. Role of biofilm – associated protein bap in the pathogenesis of bovine *Staphylococcus aureus*. *Infect. Immun.*, 72: 2177-2185.

Evgueny, V., Irina, S., Jianjun, L., Said, J. 2006**.** Structural elucidation of the extracellular and cell-wall teichoic acids of *Staphylococcus aureus* MN8m, a biofilm forming strain. Institute for Biological Sciences, National Research Council, 100 Sussex Drive, Ottawa, ON, Canada KIA OR6,Carbohydrate Research 341 : 738–743.

Fenk, C.J., Kaufman, N., Gerbig, D.G.J. 2007. *Chem. Educ.*, 84: 1676-1678.

Fox, L.K., Zadoks, R.N., Gaskins, C.T. 2005. Biofilm production by *Staphylococcus aureus* associated with inflammatory infection. *Vet. Microbiol.*, 107: 295-299.

Gondogan, N., Citak, S., Turan, E. 2006. Slime production, Dnase activity and antibiotic resistance of *Staphylococcus aureus* isolated from raw milk, pasteurized milk and ice cream samples. Food Control, 17: 389-392.

Hall-Stoodley, L., Costerton, J.W., Stoodley, P. 2004. Bacterial biofilms: from the natural environment to infectious disease. Nature R.

Harriott, M.M., Overr, M.C. 2009. *Candida albicans* and *Staphylococcus aureus* form polymicrobial biofilms: effects on antimicrobial resistance. *Antimicrob. Agents Chemother.*, 53: 3914–3922.

Hensen, S.M. 2000. Bovine *Staphylococcus aureus* mastitis- bacterial adhesion and invasion in relation to pathogenesis and anti miecrobial sensitivity. PhD Thesis, Utrecht University, the Netherlands.

Hensen, S.M., Pavičić, M.J.A.M.P., Lohuis, J.A.C.M., deHoog, J.A.M., Poutrel, B. 2000. Location of *Staphylococcus aureus* within the experimentally infected bovine udder and the expression of capsular polysaccharide type 5 in situ. *J. Dairy Sci.*, 83: 1966–1975.

Janosi, S.A., Huszenicza, T., Horvath, Huszenicza, G. 2001. Bacteriological recovery after intramuscular or intracisternalspiramycin- based drying –off therapy. *Acta Vet. Hung.*, (49): 155-162.

Karatan, E., Watnick, P. 2009. Signals, regulatory networks and materials that build and break bacterial biofilms. *Microbiol. Mol. Biol. Rev.*, 73: 310-47.

Lear, G., Lewis, G.D. 2012. Microbial Biofilms: Current Research and Applications. Caister Academic Press, 978: 96-97.

Manuela, O., Ricardo, B., Sandro, F.N., Cristina, L.V. 2011. Invasive potential of biofilm-forming Staphylococci bovine subclinical mastitis isolates. *J. Vet. Sci.*, 12(1): 95-97.

Medora, J.H., Andrew, C.K., Jeff, D., Petra, L. 2010. Beta Toxin catalyzes formation of nucleoprotein matrix in *Staphylococcal* Biofilm. *PNAS*, (107) NO.32.

Nathan, K.A., Mark, J.M., William, J.C., Jeff, G.L., Mary, E.P., Mark, E.S. 2011. *Staphylococcus aureus* biofilms, properties, regulation and roles in human disease. PMC.US National library of medicine

National Institutes of Health. *J. Viru*, 2(5): 445-459.

Neu, T.R., Swehone, G.D.W., Lawrence, J.R. 2001. Assessment of lectin-binding analysis for in situ detection of glycoconjugates in biofilm system. *Microbiol.*, (147): 299-313.

Okuda, K., Zendo, T., Sugimoto, S., Iwase, T., Tajma, A., Yamada, S. 2013. Effect of bacteriocin on methecillin-resistant *Staphylococcus aureus* biofilm. *J. Nat. Library of Medicine, Antimicrobial agents Chemother*., 57(11): 5572-5579.

Schalm, O.W., Carrol, E.J., Jain, N.C. 1971. Bovine Mastitis 1st Ed. Philadelphia. Lee and Febiger Publication. Pp: 209-217.

Vasudevan, P., Nair, M.K.M., Annamalai, T., Venkitanarayanan, K.S. 2003. Phenotypic and genotypic characterization of bovine mastitis isolates of *Staphylococcus aureus* for biofilm formation. *Vet. Microbiol*., (92): 179-185.

Xue, T., Chen, X., Shang, F. 2014. Short communication: Effects of lactose and milk on the expression of biofilm-associated genes in *Staphylococcus aureus* strains isolated from a dairy cow with mastitis. US National Labrary of Medicine National Institutes of Health. *J. Dairy Sci.*, (10): 6129-6134.

Yarmorad, M.A. 2013. Study Comparative Protection of Biofilm Produced by *Staphylococcus aureus* with other its Antigens.. M.Sc. Thesis, College of Veterinary Medicine- Baghdad University- Iraq.

Zmantar, T., Kouidhi, B., Mahdouani, K., Bakhrouf, A. 2010. A Microtiter plate assay for *Staphylococcus aureus*biofilm quantification at various pH levels and hydrogen peroxide supplementation. *New Microbiologica*, (33): 137-145.

Zulfiqar, A.M., Mubashir, A., Mohammed, N.K., Irfan, l., Najam, H., Seema, I.K. 2013. Biofilm formation and dispersal of *Staphylococcus aureus* under the influence of oxacillin. In Microbial Pathogenesis, *Sci. Direct.*, (61-62): 66-72.

How to cite this article:

Sahar Mahdi H. Al-Rubaye, Essam F. Al-Jumaily and Hassan A. Abdul-Ratha. Biofilm Production by *Staphylococcus aureus* Isolated from Bovine Mastitis Related with Resistance to the Antibiotics. *Int.J.Curr.Microbiol.App.Sci.* 5(5): 33-44.
doi: http://dx.doi.org/10.20546/ijcmas.2016.505.004