MANSOURA JOURNAL OF BIOLOGY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-492X

Phenotypic and Genotypic Characterization of Biofilm Formation among Staphylococcus aureus Isolates

Alkubaisi, A.R.[1] El Degla, H.E.[2], Abdel-Fattah, G.M.[1], Zeid, M.S.[3]

[1]*Department of Botany, Faculty of Science, Mansoura University, Egypt*

[2]*Department of Medical Microbiology and Immunology, Faculty of Medicine, Mansoura University, Egypt.*

[3]*Lecturer of pediatrics Infectious disease and Malnutrition Unit*

[*]*Corresponding Author: Prof. Dr. Gamal Abdel-Fattah*

E-mail: abdelfattaham@yahoo.com

Received: 24 / 7 /2019
Accepted: 6/ 8 /2019

Abstract: (The main objective of this study was the characterization of S. aureus clinical isolates collected from different patients admitted to Mansoura hospitals. Then, this study was extended to detect the ability of these isolates to form biofilm by genotypic and phenotypic methods. Fifty-two clinical S. aureus isolates were collected from patients admitted in different intensive care units (ICU) of Mansoura University Hospitals. Antibiotic susceptibility pattern of S. aureus was tested by disc diffusion method. The antibiotic susceptibility of different isolates of S. aureus to different tested antibiotics showed that the tested isolates were highly resistant to vancomycin (92.30%) and ciprofloxacin (61.5%) and the lowest sensitivity was detected with amoxicillin, penicillin, cefotaxime and meropenem. Results of the PCR technique on 18 S. aureus strains on 9 strains were biofilm producer and other 9 were non produced biofilm based on both CRA and MTP methods showed that 9 strains were positive for icaA and icaD. The PCR product bands for all positive strains were 188-bp band for the icaA gene and 198-bp band for the icaD while bap was not detected in any strain.

keywords: Antibiotic sensitivity, Staphyllococcus aureus,Biofilm formation,IcaA and IcaD genes

1.Introduction

S. aureus is widely spread in nature although it basically lives on the skin glands and mucous membranes of mammals and birds. They may be found in the mouth and mammary glands, intestinal, urinary tract and the higher respiratory regions of these hosts, *S. aureus* generally has benign or symbiotic relationship with their host, however they may develop pathogenic life style if enters the host tissue through the break of the skin by needles or direct implantation of medical devices. Infected host tissues support large clusters of Staphylococci and in some cases lasts for long periods. The presence of intestinal Staphylococcus strains in different food products is a public health hazard because of the capacity for the production of food poisoning. **[1].**

Current therapy for *S. aureus* biofilm-mediated infections involves surgical removal of the infected device followed by antibiotic treatment. Conventional antibiotic treatment alone is not effective in eradicating such infections **[2].** An alternative to postsurgical antibiotic treatment is using antibiotic-loaded, dissolvable calcium sulfate beads, which are implanted with the medical device. These beads can release high doses of antibiotics at the desired site to prevent the initial infection **[3].**

It has been shown that *S. aureus* carries the *ica* operon responsible for slime production. In the operon, coexpression of *icaA*, *icaD* and bap genes are required for full slime synthesis. DNA isolation from *S. aureus* is performed. Strains which are positive for *icaA* are also positive for *icaD* **[4].** The aim of this study to characterize staphylococcus aureus (S. aureus) isolated from human clinical cases for their biofilm formation ability by genotypic and phenotypic methods.

Materials and methods

Collection of samples and identification of *S. aureus*

Clinical samples (blood, wound, urine, sputum) were collected from patients admitted to different Mansoura University Hospitals. These samples were cultured using the standard media Blood agar and MacConkey's agar and incubated aerobically at 37^0C overnight. The identification of *S. aureus* isolates was done by colony morphology, microscopic examination after Gram staining, and biochemical tests.

Antimicrobial susceptibility test

Antibiotic susceptibilities of *S. aureus* isolates were done by Kirby Bauer disc diffusion method **[5].** Using Muller-Hinton agar medium. The tested antibiotics include: Ciprofloxacin, CIP (10 µg); Meropenem, MEM (30 µg); Cefepime, FEP, (30 µg), Gentamycin, CN, (10 µg) and Vancomycin, VA (30 µg). The clear zones were measured and compared with the standard recommendation of Clinical Laboratory Standard Institute [**6].**

Biofilm formation assay

Biofilm was performed in 96 well microtitere plate as described by some modification. In order to test for biofilm production by the different strains of *S. aureus* isolated from clinical samples, two different methods were used namely; the microtiter plate method **[7]**. And the Congo red agar-based method **[8].**

PCR of biofilm genes of *S. aureus*

In this study, molecular characterization of *S. aureus* biofilm genes depends on detection of (*icaA, icaD* and bap) genes in *S. aureus* according to the method of **[9].**

Results

Isolation of *S. aureus* strains

In this study, fifty-two clinical *S. aureus* isolates were isolated from patients admitted to different Mansoura University Hospitals. Each sample was cultured on blood agar media except urine samples were cultured on CLED agar medium. Results recorded in **Table (1)** show that urinary samples 25 (48%) were the commonest samples giving positive *S. aureus* growth while throat swab and vaginal swab gave the lowest number 1 (2%) of *S. aureus.*

Table (1): Distribution of S. aureus strains among different clinical samples.

Samples	Number of *S. aureus* Total = 52	Percentage (%)
Blood	3	6
Wound	12	23
Urine	25	48
Throat swab	1	2
Breast discharge	2	4
Vaginal swab	1	2
Knee effusion	2	4
Sputum	6	12
Total	52	100

Antimicrobial susceptibility

Fifty-two *S. aureus* isolates were tested. Data indicated that the high sensitivity was detected with vancomycin (92.30%) and ciprofloxacin (61.53%) and the lowest sensitivity was detected with amoxicillin, penicillin, cefotaxime and meropenem (0%). **(Fig. 1).**

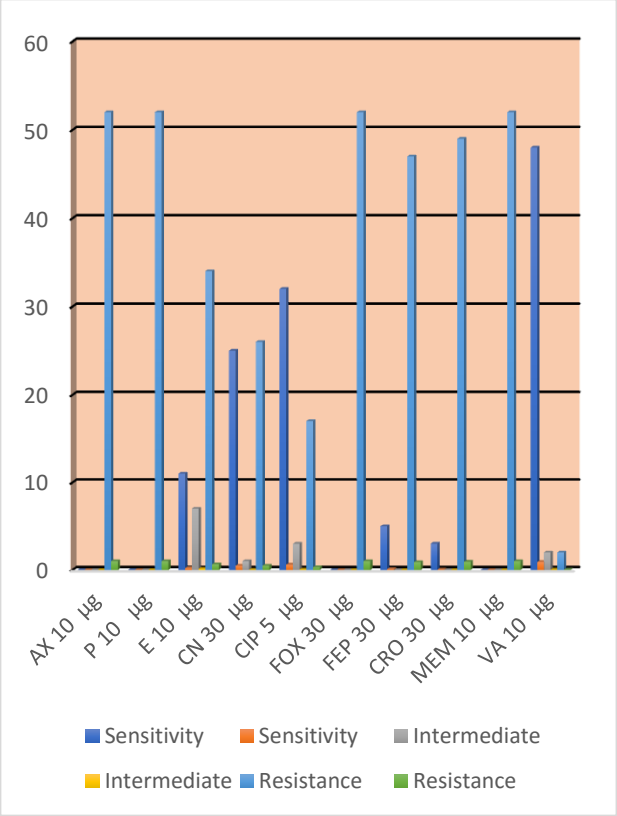

Fig. (1): Response of the tested clinical *S. aureus* isolates to antibiotics

Biofilm formation by phenotype

Fifty-two clinical *S. aureus* strains isolated in our study were tested for their ability to form biofilms by Microtiter Plate method (MTP)**.**

The data obtained was classified based on the OD value, in which less than 0.120 was considered as non- biofilm producers, 0.120-0.240 as moderate biofilm producers, and more than 0.240 as strong biofilm producers **(Plate 1).**

Results depected in **Plate (2)** show that *S. aureus* isolates revealed high (34.59%) moderate (15.36%) and non (49.98%) biofilm formation, While by Congo red agar method (CRA) the results were interperated according to that described by **[8].** The *S. aureus* isolates showed that 73% were biofilm producers, and 27% were non-biofilm producers. This explains most of isolates were resistance to antibiotics. When compared with the MTP, CRA truly identified 26 (50%) isolates biofilm producers and 26 (50%) isolates non-biofilm producers as showing in Table (2).

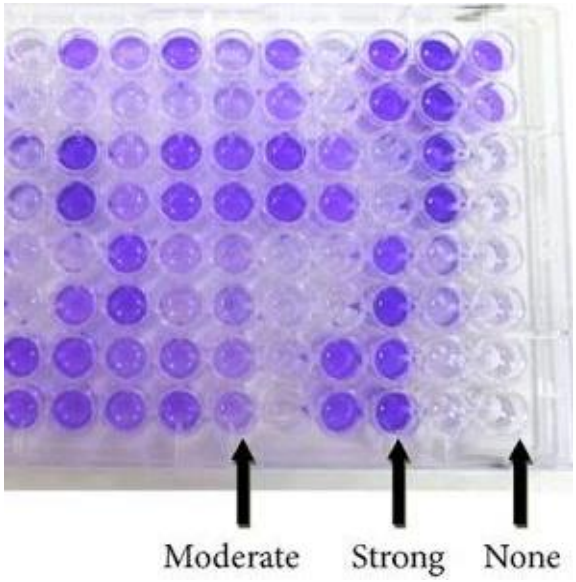

Plate (1). Phenotypic detection of biofilm production by *S. aureus* isolates using the quantitative Microtiter plate method**.**

Plate (2): Phenotypic detection of biofilm production by *S. aureus* isolates using the quantitative Congo red agar plate test. Biofilm producing *S. aureus* strains on right produce black colonies while non-producers and on left produce pink colonies.

Table (2): Different in sensitivity of the MTP

when compared with CRA.Test	MTP		Total
CRA	Positive	Negative	
Positive	19(36.53)	19(36.53)	38(73.07)
Negative	**7**(13.46%)	**7**(13.46)	14(26.92)
Total	26(50%)	26(50%)	52(100%)

Detection of *icaA, icaD* and bap genes by PCR

The PCR technique was applied to 18 *S. aureus* strains. 9 strains were biofilm producer and other 9 were non-biofilm producer by both CRA and MTP methods. 9 strains were positive for *icaA* and *icaD*. The PCR product bands for all positive strains were 188-bp band for the *icaA* gene and 198-bp band for the *icaD*, while bap was not detected in any strain as illustrated in **Plate (3)** and recorded in **Table (3).**

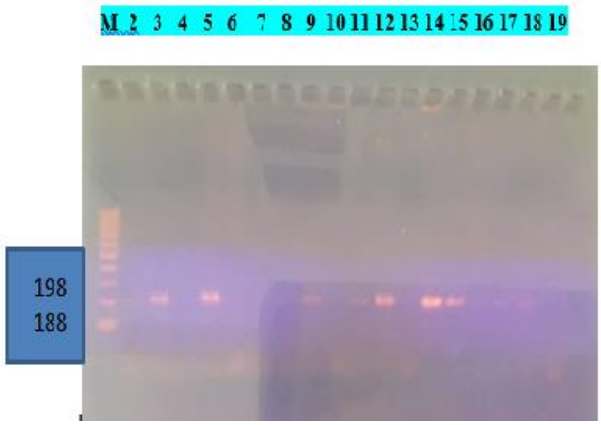

Plate (3): PCR bands of *ica*A and *ica*D genes of 18 isolates of *S. aureus* [3,5,9,11,12,14,15,17 and 18 (biofilm isolates)] and [2, 4, 6, 7 ,8 ,10, 13 and 19 (non- biofilm], where M is the marker.

Table (3): Results PCR for samples isolates

ica A				***ica D***			
Positive		**Negative**		**Positive**		**Negative**	
NO	%	NO	%	NO	%	NO	%
9	50	9	50	9	50	9	50

PCR results with primers for icaA and icaD. Lane 1, molecular size markers 100bp; lanes 3,5,9,11,12,14,15,17 and 18 show bands of 188 and198-bps from biofilm producing *S. aureus*

strains, lanes 2,4,6,7,8,10,13,16 and 19 show absence of PCR bands from non-biofilm producing *S. aureus* strains

When compared of CRA and MTP with PCR for Biofilm Detection of the tested 18 *S. aureus* strains by PCR, 9 strains were positive by both CRA and MTP, while the other 9 strains were negative by both CRA and MTP. OF the 9 positive strains by CRA and MTP, 6 were positive for *icaA* and *icaD* genes by PCR while 3 were negative **(Table 4)**. OF the 9 negative strains by CRA and MTP 6 were negative for *icaA* and *icaD* genes by PCR while 3 were positive**.**

Table (4): Comparison of CRA and MTP with PCR for Biofilm Detection.

PCR ica A and ica D			**CRA and MTP**			
			Positive		**Negative**	
Positive	NO	%	NO	%	NO	%
	9	50	6	66.66	3	33.33
Negative	9	50	3	33.33	6	66.66

Discussion

S. aureus is the most pathogenic species of the genus Staphylococcus, being implicated in both community-acquired and nosocomial infections. Present in the nares, but also in the throat, axilla, groin, perineum, and vagina **[10,11].** *S. aureus* is the most commonly isolated bacterial pathogen in humans. It is responsible for a number of infections **[12].** ranging from uncomplicated skin and soft-tissue infections such as boils, carbuncles, and abscesses, to more severe invasive illnesses, including empyema, septic arthritis, pyomyositis, osteomyelitis, necrotizing fasciitis, pneumonia, endocarditis, and septicemia **[13,14].**

Staphylococci are recognized as the most frequent causes of biofilm-associated infections **[15,16].** This exceptional status among biofilm-associated pathogens is due to the fact that *Staphylococci* are frequent commensal bacteria on the human skin and mucous surfaces (and those of many other). Thus, *Staphylococci* are among the most likely germs to infect any medical device that penetrates those surfaces, **[17,18].**

Biofilm-associated protein (Bap) and the fibronectin-binding proteins FnbpA and B, were implicated in matrix formation. These findings suggest that other surface proteins may also be involved in biofilm development. In some strains, biofilm formation may be mediated additionally or exclusively by specific surface proteins (*Bap/Bhp* and *Aap*) **[33].** So, the present work was planned to isolate and identify of different isolates *S. aureus* from different clinical samples. In addition, the susceptibility and resistance of *S. aureus* isolates to antibiotic by disc diffusion method of clinical isolates were tested. Detection of phenotypic biofilm by Congo red agar and Micro-titration plate. Finally, genotypic biofilm by PCR of *ica* A, *ica* D and bap genes were detected.

In this study, fifty-two clinical *S. aureus* isolates were isolated from patients admitted to different Mansoura University Hospitals. Each sample was cultured on blood agar media except urine samples were cultured on CLED agar medium. Results recorded in the present study showed that urinary samples 25 (48%) were the commonest samples giving positive *S. aureus* growth while throat swab and vaginal swab gave the lowest number 1 (2%) of *S. aureus*. Our results are in agreement with previous studies **[19,15].** Showing that the majority of *S. aureus* isolates were isolated from urinary samples followed from vaginal swab samples.

In this study, the antibiotic susceptibility of different isolates of *S. aureus* to different tested antibiotics showed that the tested isolates were highly resistant to vancomycin (92.30%) and ciprofloxacin (61.53%) and the lowest sensitivity was detected with amoxicillin, penicillin, cefotaxime and meropenem. These results are in agreement with previous investigations by **[20,21,12,22].** They reported that the highest resistance of *S. aureus* isolates was detected with vancomycin followed by ciprofloxacin. In addition**, [23]** showed that 60% of clinical *S. aureus* isolates collected from different hospitals exhibited high level of resistance to vancomycin and ciprofloxacin respectively **[24,33].**

Data of this study demonstrated that biofilms are involved in a multitude of different infections and often contribute significantly to the difficulties encountered in treatment.

Developing anti-biofilm drugs aims to combine these drugs with conventional antibiotics, thus restoring the efficacy that the latter show to bacteria in a non-biofilm status **[25].** Congo red agar method that is a qualitative assay for detection of biofilm producer microorganism, as a result of color change of colonies inoculated on CRA medium **[8].** The results are supported by **[16,26].** Who reported that *S. aureus* can form biofilms.

Biofilms are aggregates of microbial cells surrounded by a matrix of exopolymers **[27,24].** Besides the production of exotoxins and surface proteins, the formation of these highly organized multicellular complexes is increasingly recognized as an important virulence factor in *S. aureus* **[28,23].**

Biofilm formation can lead to persistent contamination or infection because the cells within the biofilm are very resistant to sanitation procedures and to the action of the host immune system and antimicrobial agents **[29].** Although some researchers have studied the ability of members of the Staphylococcus genus to adhere to surfaces and form biofilm, most studies have addressed the clinical aspects related to biofilm formation by Staphylococcus intermedius on medical implants and materials **[20,30].** Moreover, few studies have reported biofilm formation by *S. aureus* isolated from ready-to-eat-foods **[26].**

Results from the present investigation that the PCR technique was applied on 18 *S. aureus* strains (9 strains were biofilm producer and other 9 were non-biofilm producer) by both CRA and MTP methods. 9 strains were positive for *icaA* and *icaD*. The PCR product bands for all positive strains were 188-bp band for the *icaA* gene and 198-bp band for the *icaD* while bap was not detected in any strain. This observation agrees with the results of **[31,32].** They reported that all *S. aureus* carried and were able to express icaA and icaD genes. Biofilm development was glucose- and NaCl-induced (5 *S. aureus* and 1 *S. epidermidis*) or glucose-induced (the remaining strains). Proteinase K and sodium metaperiodate treatment had different effects on biofilms dispersion revealing that the strains studied were able to produce chemically different types of extracellular matrix mediating biofilm formation. While several studies **[26,29]** have extensively described the distribution of genes involved in biofilm formation and virulence in Staphylococcal strains causing orthopaedic peri-implant infections. Recent studies have begun to highlight the existence of PIA/PNAG-independent biofilm mechanisms in both species [9]. Accumulation-associated protein (Aap) independently or together with *ica* operon, has also been suggested to be important in coagulase-negative staphylococci **[32].** In *S. aureus* more additional surface proteins such as SasG, extracellular matrix binding protein (Embp), biofilm-associated protein (*Bap*) and the fibronectin-binding proteins FnbpA and B, were implicated in matrix formation. These findings suggest that other surface proteins may also be involved in biofilm development. In some strains, biofilm formation may be mediated additionally or exclusively by specific surface proteins (Bap/Bhp and Aap) **[23,25].**

Furthermore, it has been shown that *S. aureus* carries the *ica* operon responsible for slime production. In the operon, expression of *icaA, icaD* and *Bap* genes are required for full slime synthesis. DNA isolation from *S. aureus* is performed. Strains which are positive for *icaA* are also positive for *icaD* **[15,28].**

Conclusion

S. aureus cause severe diseases that are characterized by biofilm formation and resistance to several antibiotics. Based on the results in this study, we found that 38 *S. aureus* strains (73%) were biofilm producers, while, 14 ones (27%) were non producers as detected by the CRA plate test and by MTP method. 26 *S. aureus* strains (49.98%) were non- biofilm producers while, 18 (34.59%) were strong biofilm producer and 8 (15.36 %) were moderate producer.

It was found that icaA,icaD genes are present in 9 from 18 isolates of *S.aureus*, of this 9 isolates 6 were biofilm producers and 3 isolates were non-biofilm producers by CRA and MTP, while Bap gene were not detected in these isolates. Therefore icaA, icaD could be used as indicators for biofilm forming of *S. aureus.* However, further studies are needed to evaluate the mechanism of these genes for infection of *S. aureus* isolates.

References

1. Murray, P.R., Baron, E.J., and Jorgensen, J.H., (2003): Manual of clinical microbiology. 8th, ed. Washigton, UAS, P: 304-404.
2. Nandakumar, V., Chittaranjan, S., Kurian, VM. and Doble, M., (2013): "Characteristics of bacterial biofilm associated with implant material in clinical practice". *Polymer Journal*.,**45:** 137–152.
3. Archer, NK., Mazaitis, MJ., Costerton, JW., Leid, JG., Powers, ME., and Shirtliff ME (2011): "Staphylococcus aureus biofilms: properties, regulation, and roles in human disease". Virulence., **2**: 445–59.
4. Arciola, C.R., Baldassarri, I., and Montanaro, L. (2001): "Presence of icaA and icaD Genes and Slime Production in a Collection of Staphylococcal Strains from Catheter-Associated Infections" *J. Clinic. Microbiol.*, **39**: 2151-2156.
5. Bauer, A.W.; Kirby, W.M.; Sheris, J.C. and Turck, M. (1966): Antibiotic susceptibility testing by a standardized single disc method. Am. *J. Clin. Pathol*., **45**:149-158.
6. CLSI, (2013): Performance Standards for Antimicrobial Susceptibility Testing; Twenty- Third Informational Supplement. CLSI document M 100-S23. Wayne, PA: Clinical and Laboratory Standards Institute.
7. Kwon. A. S., Park, G. C., Ryu, S. Y., Lim, D. H., Lim, D. Y., Choi, C. H. *et al.* (2008): Higher biofilm formation in multidrug-resistant clinical isolates of Staphylococcus aureus. *Int. J. Antimicrob. Agents***.,** **32**: 68–72.
8. Freeman J, Falkiner FR, Keane CT. (1989). New method for detecting slime production by coagulase negative staphylococci. *J Clin Pathol*. **(42):** 872-4.
9. Mariana, N.; Salman, S.; Neela, V.; Zamberi S. (2009): Evaluation of modified congo red agar for detection of biofilm produced by clinical isolates of methicillin resistance Staphylococcus aureus. *Afr. J. Microbiol*. Res., **3:** 330–338.
10. Wootton M, Bennett PM, MacGowan AP, Walsh TR. (2005): Reduced expression of the *atl* autolysin gene and susceptibility to autolysis in clinical heterogeneous glycopeptide-intermediate Staphylococcus aureus(hGISA) and GISA strains. *J Antimicrob Chemother*., **56**: 944–7.
11. Montanaro L, Poggi A, Visai L, Ravaioli S, Campoccia D, Speziale P, *et al*. (2011): Extracellular DNA biofilms. *Int J Artif Organs*., **34**:824–31.
12. Kamal, R.M., Mohamed, A., Bayoumi Salah, F.A., and Abd, E.A. (2013): MRSA detection in raw milk, some dairy products and hands of dairy workers in Egypt, a mini-survey. Food Contr**., 33**: 49–53.
13. Lowy FD. (1998): *Staphylococcus aureus* Infections. N *Engl J Med*., **339**: 520-532.
14. Kav, K., Col, R., and Ardic, M. (2011): Characterization of *S.* aureus isolates from white-brined Urfa cheese. (22054178) *J. Food Prot.*, **74**: 1788–1796.
15 .Houston, P., Rowe, SE., Pozzi, C., Waters, EM., and O'Gara, JP., (2011): Essential role for the major autolysin in the fibronectin-binding protein mediated Staphylococcus aureus biofilm phenotype. Infect Immun., **79**: 1153–1165.
16 .Prenafeta, A., Sitja, M., Holmes, M.A., and Paterson, G.K. (2014): Short communication: Biofilm production characterizationof mecA and mecC methic illin-resistant *S. aureus* isolated from bovine milk in Great Britain. *J. Dairy Sci.*; **97**: 4838–4841.
17. Rohde, H., Knobloch, JK., Horstkotte, MA. and Mack D. (2001): Correlation of Staphylococcus aureus icaADBC genotype and biofilm expression phenotype. *J Clin Microbiol*., **12**: 4595–4596.
18 .Vuong, C. and Otto, M. (2002): Staphylococcus epidermidis infections. Microbes Infect., **4**: 481–489.
19 .O'Neill, E., Pozzi, C., Houston, P., Smyth, D., Humphreys, H., Robinson, DA., *et al.* (2007): Association between methicillin susceptibility and biofilm regulation in Staphylococcus

aureus isolates from device-related infections. *J Clin Microbiol*.,**5:** 1379–88.

20 .Lozano, C., Aspiroz, C., Lasarte, J.J., Gómez-Sanz, E., Zarazaga, M., and Torres, C. (2011): Dynamic of nasal colonization by methicillin-resistant *Staphylococcus aureus* ST398 and ST1 after mupirocin treatment in a family in close contact with pigs. (20663559) Comp. Immunol. Microbiol. Infect. Dis., 34: e1–e7.

21 .Pozzi, C., Waters, E.M., Rudkin, J.K., Schaeffer, C.R., Lohan, A.J., Tong, P., Loftus, B.J., Pier, G.B., Fey, P.D., Massey, R.C., and O'Gara, J.P. (2012): Methicillin resistance alters the biofilm phenotype and attenuates virulence in Staphylococcus aureus device-associated infections. PLoS Pathog., 8: e1002626.

22. Marti, R., Schmid, M., Kulli, S., Schneeberger, K., Naskova, J., Knøchel, S., Ahrens, C.H., and Hummerjohann, J. (2017): Biofilm formation potential of heat resistant Escherichia coli dairy isolates and complete genome of MDR heat resistant strain FAM21845. Appl. Environ. Microbiol.; **83:** e00628-17.

23. Lee, S.H.I., Mangolin, B.L.C., Gonçalves, J.L., Neeff, D.V., Silva, M.P., Cruz, A.G., and Oliveira, C.A.F. (2014): Biofilm-producing ability of *Staphylococcus aureus* isolates from Brazilian dairy farms. (24440248) *J. Dairy Sci.*; **97**: 1812–1816.

24. Normanno, G., Corrente, M., La Salandra, G., Dambrosio, A., Quaglia, N.C., Parisi, A., Greco, G., Bellacicco, A.L., Virgilio, S., and Celano, G.V. (2007): Methicillin resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *Int. J. Food Microbiol.*,**117**: 219–222.

25 .Kaplan, JB., Ragunath, C., Velliyagounder, K., Fine, DH., and Ramasubbu, N., (2004): Enzymatic detachment of Staphylococcus epidermidis biofilms. Antimicrob Agents Chemother.,**48:** 2633–2636.

26 Oniciuc, E.A., Cerca, N., and Nicolau, A.I. (2016): Compositional analysis of biofilms formed by Staphylococcus aureus isolated from food sources. (27065962) *Front.Microbiol.*;7: 390.

27 .Costerton, J.W., Stewart, P.S., and Greenberg, E.P. (1999): Bacterial biofilms: A common cause of persistent infections. Science., **84**: 1318–1322.

28. Tang, J., Chen, J., Liu, J., Zhang, R., Yang, R., and Chen, L. (2012): Effects of different cultivation conditions on Staphylococcus aureus biofilm formation and diversity of adhesin genes. *J. Food Saf.*, **32**: 210–218.

29 .Song, M., Li, Q., Zhang, Y., Song, J., Shi, X., and Shi, C. (2016): Biofilm formation and antibiotic resistance pattern of dominant Staphylococcus aureus clonal lineages in China. *J. Food Saf.* **44**: 254-260.

30. Souza, E.L., Meira, Q.G.S., de M. Barbosa, I., Athayde, A.J.A.A., da Conceição, M.L., and de Siqueira, J.P. (2014): Biofilm formation by Staphylococcus aureus from food contact surfaces in a meat-based broth and sensitivity to sanitizers. Braz. *J. Microbiol*., **45**: 67–75.

31 .Silva, GD., Kantzanou, M., Justice, A., Massey, RC., Wilkinson, AR., Day, NP., *et al*. (2002): The *ica* operon and biofilm production in coagulase-negative Staphylococci associated with carriage and disease in a neonatal intensive care unit. *J Clin Microbiol*., **40**: 382 – 388.

32. Thiran, P.A., Di Ciccio, H.U., Graber, E., Zanardi, A., Ianieri, J., (2018): Biofilm formation of *Staphylococcus aureus* dairy isolates representing different genotypes. *Journal of Dairy Science*., **101:** 1000–1012.

33 Ugolotti, E., Bandettini, R., Marchese, A., Gualco, L., Vanni, I., Borzi, L., *et al.* (2011): Molecular characterization of hospital-acquired methicillin-resistant Staphylococcus aureus strains in pediatric outbreaks using variable tandem repeat analysis with spa and ClfB typing. Diagn Microbiol Infect Dis.,**69:** 213 –217.