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CORRELATION BETWEEN INTRAVENOUS CATHETER RELATED INFECTIONS AND BIOFILMS IN *STAPHYLOCOCCUS EPIDERMIDIS*

PRACHI SHAW* [1], CHANDAN KUMAR SHAW [2] AND K. SAILEELA[3]

[1] Department of Microbiology. Sri Manakula Vinayagar Medical College and Hospital, Pondicherry, India.
[2] Department of Pediatrics, Sri Manakula Vinayagar Medical College and Hospital, Pondicherry, India.
[3] Department of Microbiology. Kamineni Institute of Medical Sciences, Narketpally. India.

ABSTRACT

S. epidermidis is commonly associated with catheter related sepsis with a unique property of biofilm formation. This genetically determined property aids its survival by the phenomenon of immune evasion. In all, IV catheter tips and two synchronous blood samples from 297 patients with intravenous catheters and ≥ 48 hours stay in KIMS were subjected to culture and biofilm formation by *S. epidermidis* strains thus isolated. Biofilm formation was correlated with the clinical presentation of the cases grouped as "septicemia" or "asymptomatic" - with or without thrombophlebitis. The results were compared using the χ^2 test. Out of these 297 samples, *S. epidermidis* was isolated from 62 catheter tips (20.9%) and simultaneously from blood cultures (septicemia) and I.V. catheter tips from same patients in 38 cases (12.8%) all of which were associated with septicemia. Biofilm was demonstrated in 63.2% of 38 cases of culture proven septicemia as well as I.V. catheter tip positivity, significantly higher, compared to 25% of 24 isolates from catheter tip only. (p value < 0.005). The incidence of biofilm forming *S. epidermidis* in cases with thrombophlebitis was 17.1% not significantly different than in the cases without thrombophlebitis was 18.5%. (p value > 0.05). Thirty out of the 62 isolates (48.4%) from I.V. catheter tips from cases in the "septicemia group" were biofilm formers while 20.8% of the isolates from "asymptomatic group" were biofilm formers (p value < 0.05) whereas 25.0% were positive for biofilm formation exclusive I.V. Catheter tip positivity.(p value >0.05). Biofilm formation by isolates of *S. epidermidis* was found to be significantly more common in cases with culture proven sepsis than with those with only I.V. catheter tip positivity. Biofilm formation by *S. epidermidis* isolates does correlate significantly with cases with clinical signs and symptoms of sepsis but not so with local signs like thrombophlebitis.

KEY WORDS; ***Staphylococcus epidermidis,*** **Biofilms, Antimicrobial Resistance,**

PRACHI SHAW
Department of Microbiology. Sri Manakula Vinayagar Medical College and Hospital, Pondicherry, India.

***Corresponding author**

INTRODUCTION

Indwelling catheters are an integral part of medical management especially in "in-patient care". The rate of colonization of such catheters by organisms which may be skin commensals or even nosocomial is proportional to the duration for which the catheter remains in situ at a site apart from other factors site of insertion, the catheter material and the use of aseptic techniques. [1,2] The common organisms which colonise catheters and also produce biofilms are *Staphylococococcus epidermidis, Staphylococcus aureus*, *Stenotrophomonas maltophilia*, *Pseudomonas* and *Acinetobacter* species and fungi like *Candida albicans* and *Candida parapsilosis*.[3] In other words some organisms have found a rather unnatural yet favourable ecological niche for themselves as a part of their survival instinct.

These organisms are usually resistant to standard antibiotics and result in amplification of cost of treatment and prolonged hospital stay ultimately contributing to increased morbidity and mortality. The cost of treating a single episode of catheter-related bloodstream infection (CRBI) has been estimated to be in excess of $ 28000.[4] Biofilm identification in such isolates could predict the virulent nature of such isolates. However it would be incorrect to assume all that organisms isolated from indwelling catheters are pathogenic or lead to biofilm production or vice versa and hence need not warrant the use of higher and costlier antibiotics in every case where such organisms are isolated from blood culture or catheters. *Staphylococococcus epidermidis is* the commonest organism causing catheter related sepsis. So we decided to study the correlation between catheter related sepsis and biofilm formation by *Staphylococococcus epidermidis* in our hospital which is a tertiary care centre and often caters to patients requiring long term indwelling catheters.

MATERIAL AND METHODS

The study was carried out in our hospital from October 2007 to September 2009. The following samples were randomly taken from patients with intra-venous catheters (central or peripheral) for ≥ 48 hours stay in the hospital from various wards and ICU's irrespective of the primary diagnosis, signs and symptoms of sepsis or evidence of local thrombophlebitis-

1. Intravenous catheter (IVC) tips duly transported in a sterile container subjected to culture by the roll plate technique.
2. Two blood samples, 5 mL each, collected with full aseptic precautions taken < 10 minutes apart from two separate venipuncture sites for culture by inoculation into BHI broth and subsequent plating on nutrient agar and blood agar.

Culture of catheter segment was done by the roll plate method.[5,6] In this semi-quantitative technique, the IVC tip was rolled across on agar plate and after 24 hours incubation, the colony forming units (CFU's) were counted. A yield of 15 CFUs or higher was considered significant for further processing and testing for biofilm formation.
Isolation and identification of *Staphylococcus epidermidis* from catheter tip and simultaneously 2 sets of blood culture was done by conventional methods like gram staining, catalase test, slide and tube coagulase test and Novobiocin Disk sensitivity. Organisms isolated other than *S epidermidis* were not included in the study.

Biofilm formation in these isolates was demonstrated by the Tissue Culture Plate Method.[7] Isolates from fresh agar plates were inoculated in Brain Heart Infusion (BHI, Difco) with 2% sucrose and incubated for 24 hrs at 37ºC in stationary condition and diluted in 100 with fresh medium. Individuals wells of sterile polystyrene, 96 well- flat bottom tissue culture

plates were filled with 0.2ml aliquots of diluted culture and only broth as control to check sterility and non-specific binding of media. The tissue culture plates were incubated for 24 hrs at 37ºC. After incubation content of each well was gently removed by tapping plates. The wells were washed 4 times with 0.2 mL of phosphate buffer saline to remove free floating planktonic bacteria. Biofilms formed by adherent sessile organisms in plate were fixed with 2% sodium acetate and stained with crystal violet (0.1% w/v). Excess stain was rinsed off. Adherent Staphylococcal cells usually form biofilm on all side wells and were uniformly stained with crystal violet. Optical density of strain adherent bacteria was determined with a micro ELISA autoreader at wavelength of 570 nm. These O. D values were considered an index of bacteria adhering to surface and forming biofilms. The χ^2 test was used to compare the results of biofilm formers by the isolates from blood and IVC's as well as those associated with asymptomatic, septicemia and thrombophlebitis cases.

OBSERVATIONS AND RESULTS

A total of 297 cases were included in the study of which 189 (63.6%) were males. The mean duration of IVC placement from all cases was 61.6 ± 12.4 hours with 88.2% of the IVC's being peripheral while rest were central lines. The mean duration of prior antibiotic therapy was 52.4 ± 9.8 hours. 24.1 % of the cases were neonates, while those in the age group 1 month to 14 years were 27.6%, the rest being > 14 years age.

Irrespective of the primary diagnosis for the purpose of "relevant clinical correlation" to our study we grouped the clinical findings into three main groups: (Table – 1)

1. Those with local thrombophlebitis with or without clinical signs and symptoms of septicemia – accounting for 105 (35.4%) cases.
2. Those with clinical signs of septicemia: consisting of two or more of the following present simultaneously: Increased (fever) or decreased (hypothermia) body temperature, Tachycardia or Bradycardia, Tachypnoea or Bradypnoea, Hypotension and/ or Leucocytosis – accounting for 261 (87.9%) cases.
3. Those cases with symptoms not fitting into septicemia, or totally asymptomatic (hereafter labeled as "Asymptomatic" group), which included samples from cases like: Cerebrovascular accidents (CVA), Post operative cases of Caesarian section, appendicectomy and others admitted in the orthopedics, ENT or Dermatology wards, Trauma patients etc. accounting for 36 (12.1%) cases.

Table 1

Comparison of the distribution of isolates with clinicalmanifestations: A Clinico - microbiological correlate. [N = 297]

Clinical Manifestation	*S. epidermidis* Isolates (%)
Local thrombophlebitis (n = 105)	35 (33.3)
Septicemia (n = 261)	38 (14.5)
Asymptomatic (n = 36)	24 (66.7)

The purpose of classifying the clinical features in the above mentioned three categories was to correlate the biofilm formation property of the isolates, more precisely *S. epidermidis*, with the incidence of local (Thrombophlebitis) and systemic (septicemia) manifestations. *S. epidermidis* accounted for 33.3 %, 14.5% and 66.7% of the cases with local thrombophlebitis, septicemia and asymptomatic cases. (Table – 1)

Out of these 297 samples, the isolation rate of all organisms from blood in cases of septicemia was 43.7%. *Staphylococcus epidermidis* was isolated from 62 catheter tips. (20.9%) [Table 2] *Staphylococcus epidermidis* was isolated simultaneously from blood cultures (septicemia) and I.V. catheter tips from same patients in 38 cases (12.8%) while in 24 (8.1%) cases *Staphylococcus epidermidis* was isolated from IVC tips only. In 6 cases *S. epidermidis* was isolated from only one of the blood sample but not from I.V. catheter tips and none of these cases were biofilm formers and hence not included in the study as they could be regarded as skin contaminants.

Table 2
Distribution of isolates from clinical samples (I.V. catheter tips and Blood) (N = 297)

Organism	I.V Catheter tip Only	I.V Catheter + 2 Blood Cultures	(single) Blood Culture Only	Total I.V. catheter tip isolates (%)	Total isolates (%)
S. epidermidis	24 (8.1)	38 (12.8)	6 *	62 (20.9)	68 (22.9)

**** = single blood culture sample isolates (probably non pathogenic/ not included in study)***
Six out of 24 isolates (25.0%) of Staphylococcus epidermidis from catheter tip only without simultaneous blood culture positivity were biofilm producers – significantly lower than 24 isolates (63.2%) of Staphylococcus epidermidis from 38 cases of culture proven septicemia as well as I.V. Catheter tip positivity. (p value < 0.005) [Table 3]

Table 3
Comparison of Biofilm formation by S. epidermidis isolates from I.V. Catheter Tip only versus Septicemia cases with catheter tip positivity also. [n=62]

S. epidermidis [n= 62]	I.V. Catheter tip only [n =24] (%)	IV Catheter tip + Both Blood culture [n=38] (%)
Biofilm +	6 (25.0)	24 (63.2)
Biofilm -	18 (75.0)	14 (36.8)

Optical Density (O.D. value) in the ELISA reader was used to classify the biofilm formation by *S. epidermidis* into “strong” (O.D. > 0.240 after 24 hr. incubation in BHI with 2% sucrose media) moderate (O.D. 0.120 - 0.240 after 24 hr. incubation in BHI with 2% sucrose media) and weak/no biofilm formers. Strong *S. epidermidis* biofilm producers were seen in 2

(33.3%) out of the 6 total biofilm producers from I.V. catheter tips exclusively compared to 19 (79.2) of the 24 biofilm producers from both Blood and I.V. catheter tips. [Table 4] (p value < 0.05). (The value of cells in Table IX being less than 5, "Yate's correction" was applied to calculate the value of χ^2).

Table 4
Comparison of strong and moderate biofilm formers of *S. epidermidis amongst IV catheter tips and septicemeia cases.*

S. epidermidis	I.V. Catheter tips only [n = 6]	Simultaneous IV catheter tips and Blood [n = 24]
Strong Biofilm Producers *	2 (33.3)	19 (79.2)
Moderate Biofilm Producers **	4 (66.7)	5 (20.8)

- **** = O.D. > 0.240 after 24 hr. incubation in BHI with 2% sucrose media.***
- ***** = O.D. 0.120 - 0.240 after 24 hr. incubation in BHI with 2% sucrose media.***

Out of the 62 strains of S. *epidermidis* isolated 35 (56.5%) were associated with local thrombophlebitis while the rest were not. The incidence of biofilm forming *S. epidermidis* in cases with thrombophlebitis was 17.1% while that in the cases without thrombophlebitis was 18.5%. (Table 5) The values were not statistically significant (p value > 0.05).

Table 5
Comparison of biofilm formation by S. epidermidis isolates from cases with or without thrombophlebitis. [N= 62]

S. epidermidis [N = 62]	Thrombophlebitis + [n = 35] (%)	Thrombophlebitis – [n = 27] (%)
Biofilm +	6 (17.1)	5 (18.5)
Biofilm -	29 (82.9)	22 (81.5)

Twenty four out of 38 (63.2%) of the isolates from cases with septicemia were associated with biofilm production while 20.8% of the "asymptomatic" cases were associated with the latter. (p value < 0.001).This shows that biofilm formation was significantly associated with septicemia cases by *S. epidermidis* and could be one of the virulence factors. [Table 6]

Table 6
Comparison of biofilm formation by S. epidermidis isolates from asymptomatic cases versus cases with culture proven sepsis. *[N= 62]*

S. epidermidis **[N = 62]**	Asymptomatic cases **(38.7%)** [n = 24] (%)	Symptomatic Cases with culture proven sepsis **(61.3%)** [n = 38] (%)
Biofilm +	5 (20.8)	24 (63.2)
Biofilm -	19 (79.2)	14 (36.8)

The total number of isolates of *S. epidermidis* from I.V. catheter tips was 62. Thirty out of the 62 isolates (48.4%) from I.V. catheter tips (including the cases I.V. catheter tips and blood culture positive) were biofilm formers while 20.8% of the isolates from asymptomatic group were biofilm formers. [Table 7] (p value < 0.05) Thus it can be concluded that biofilm formation was significantly more common in cases with I.V. Catheter tip positivity than those in the "asymptomatic" group. However biofilm formation by exclusive IVC isolates (25.0%) of *S. epidermidis* was not significantly different compared to the asymptomatic group (20.8%). [Table 8].

Table 7
Comparison of biofilm formation by S. epidermidis isolates from asymptomatic cases versus cases with Catheter tip positivity.

S. epidermidis	Asymptomatic cases [n = 24] (%)	Symptomatic Cases with Catheter tip positive. [n = 62] (%)
Biofilm +	5 (20.8)	30 (48.4)
Biofilm -	19 (79.2)	32 (51.6)

Table 8
Comparison of biofilm formation by S. epidermidis isolates from asymptomatic cases versus cases with exclusive I.V. Catheter tip positivity.

S. epidermidis	Asymptomatic cases [n = 24] (%)	Cases with exclusive I.V. Catheter tip positivity. [n= 24] (%)
Biofilm +	5 (20.8)	6 (25.0)
Biofilm -	19 (79.2)	18 (75.0)

DISCUSSION

Catheter related blood-stream infections (CRBI) form a major portion of nosocomial infections. Coagulase negative staphylococci (CNS), predominantly *S. epidermidis*, along with other notorious pathogens like *Pseudomonas aeruginosa*, *S. aureus*, *Klebsiella spp.* etc are the commonest offenders. The spectrum of infections associated with I.V catheters/devices (IVC/IVD) ranges from local colonization (asymptomatic infection) to bacteremia (or candidemia) with septic shock. Clinical findings are unreliable for diagnosing IVD-related bloodstream infection because they have poor specificity and sensitivity. [8, 9] The most common clinical findings have poor specificity (for example, fever with or without chills), and inflammation or purulence around the intravascular device has high specificity but poor sensitivity.[8] Here we have tried to elucidate the correlation between the biofilm formation by *S. epidermidis* and the clinical presentations in the cases studied. Biofilm formation was more significantly associated with blood stream infections (septicemia) than mere colonization of I.V. catheter tips.

In a similar study by Safdar N et al[8] correlating the local signs and symptoms with CRBI's in case of short-term, noncuffed central venous catheters, no significant differences was noted among mean scores for each inflammatory variable examined or overall score among colonized CVCs, catheters causing

CVC-related BSI, and noncolonized CVCs. The sensitivity of local inflammation for diagnosis of CVC-related BSI was dismal (0-3%). Similar results were obtained by us when we compared the biofilm formation by *S. epidermidis* wherein we did not observe any significant differences between the strains associated with local thrombophlebitis (p value > 0.05) as well as in cases with exclusive catheter tip positivity without any clinical signs of sepsis. (p value > 0.05).

Francisco Diaz Mitoma et al (1987)[10] noted that shunt obstruction and abdominal pain occurred more frequently when infectious episodes were due to slime-producing CNS than to non-slime-producing CNS (P < 0.05). Despite appropriate antimicrobial therapy, the mean duration of fever was longer and the failure to eradicate the infecting organisms was more frequent when the infectious episodes were due to slime-producing CNS than to non-slime-producing CNS (P < 0.025). However the findings cannot be equated with that from IV catheters, as the VP shunt lies primarily in a subcutaneous tunnel while a part of IV catheter is always exposed to both in-vivo and ex-vivo factors.

In our study biofilm formation was found to be significantly more common in cases with culture proven sepsis than with those with only I.V. catheter tip positivity. This shows that mere colonization of I.V. catheter tips by S. epidermidis does not necessarily mean that the strain is virulent, but those associated with sepsis are more likely to be biofilm producers. Thus one may need to be more aggressive in the treatment regimen adopted for such cases.

Pirkko Kotilainen (1990)[11] in his retrospective analysis of 64 strains from 62 adult septicemias reported 34 (53%) adherent slime producers. In comparison, only 142 (29%) of 489 single blood culture isolates were adherent slime producers. (p < 0.001). However out of the 44 IV catheter samples studied, 25 (57%) were slime producers. (p value > 0.05). The difference of results between their study and ours can be explained by the fact that they retrospectively studied the strains isolated from two different hospitals during two different periods of time and also the fact that IV catheter tips were not examined from all septicemia cases. The variations in secular trends of nosocomial flora are an established fact, resulting from selection pressure on the prevalent microbial flora by the antibacterial agents being used during that period in that region/ hospital. Amita Jain et al (Lucknow, India 2009)[12] noted that the difference in biofilm production by commensal, colonizing and invasive strains was statistically significant (p < 0.0001) wherein 3 out of 3 (100%) invasive strains of *S. epidermidis* were biofilm formers while 12 out of 17 (70.6%) strains from peripheral IV catheters were positive for the latter. The number of *S. epidermidis* isolates studied by them is also much less than that studied by us in the present study and the samples were all obtained from suspected nosocomial sepsis cases from the Pediatrics ward only. M. G. Ammendolia et al (1999 Italy)[13] noted in their study that out of 115 isolates of *S. epidermidis*, 24 (20.9%) were isolated from blood out of which 16 (66.7%) were biofilm producers compared to 63.2% from IV catheter tips. (p value > 0.1). However they did not consider the number of isolates obtained simultaneously from both Blood and IV catheter tips. Hence their result also is not truly comparable to ours due to methodological differences. Arciola CR et al (Italy 2001)[14] reported 49% of *S. epidermidis* strains from catheters and, surprisingly, 61% of *S. aureus* strains were *icaA* and *icaD* positive and slime forming. All the *S. epidermidis* strains from the skin and mucosa turned out to be negative for both *icaA* and *icaD* and also non-slime forming.

De Silva G D I et al (U.K, 2002)[15] reported that quantitative biofilm production was significantly greater in strains isolated from either the blood or the skin of neonates with *S. epidermidis* bacteremia. The mean O.D. value for blood strains of neonates with sepsis was 0.25 (0.07–0.56) while that of skin controls of sick babies was 0.19 (0.13–0.27) and well babies was 0.04 (0.01–0.07) (p value < 0.0001). These findings correlate with that of our study

wherein we found that strong *S. epidermidis* biofilm producers are more likely to be associated with septicemia.

Physiological changes in *S. epidermidis* biofilms thus protect the bacteria by two mechanisms. First, they lower the sensitivity toward harmful molecules, such as antibiotics, antibacterial peptides, and cytokines due a physical barrier created by the exopolysaccharides controlled by novel transcriptional regulators like SarZ. [16] Second, they cause a shift to a nonaggressive state, reducing inflammation and the attraction of immune cells to the site of infection. Decreased production of the proinflammatory PSMs,[17] increased production of specific protective factors and low activity of the quorum-sensing system *agr*, (for ***a***ccessory ***g***ene ***r***egulator)[18] which controls expression of several aggressive virulence factors, including the PSMs.

There are however a few drawbacks in the present study such as - Isolation of *S. epidermidis* by roll plate technique identify extraluminal colonization only and not the intraluminal ones. So the actual estimates may be higher than that observed in the study. We have not excluded the cases receiving prior empirical antibiotics. Ideally the paired blood culture samples must be drawn before the initiation of empirical antibiotic therapy. In many of our cases organisms were isolated despite > 48 hours of prior empirical antibiotic therapy. However one must bear in mind that antibiotics do exert a selection pressure on the organisms and strains and there is a chance that the most resistant organisms would be isolated in such cases, such as those producing biofilms.

CONCLUSIONS

Biofilm formation by isolates of *Staphylococcus epidermidis* was found to be significantly more common in cases with culture proven sepsis than with those with only I.V. catheter tip positivity. Biofilm formation by *S. epidermidis* isolates does correlate significantly with cases with clinical signs and symptoms of sepsis but not so with local signs like thrombophlebitis. Strong *S. epidermidis* biofilm producers are more likely to be associated with septicemia than weaker ones. However as other studies have findings contrary to that of ours, further multicentric studies with larger database and meta-analysis of the existing studies is needed to come to concrete conclusions on the basis of "evidence based medicine". It would be appropriate to conclude with a few recommendations and future strategies to prevent "Catheter" Related Blood stream infections associated with "biofilm formation" by various organisms. Based on the above study we can say that *S. epidermidis* isolates from blood of septicemic patients must not always be disregarded as contaminants. Instead such isolates must be tested for biofilm formation. Infections with biofilm forming *S. epidermidis* strains from blood must be treated aggressively with higher antibiotics from the outset to decrease the mortality and morbidity from septicemia. Biofilm forming *S. epidermidis* isolated from blood must necessitate removal of such colonised catheters. Studies correlating the sensitivity pattern of biofilm forming *S. epidermidis* must be carried out to aid in the antibiotic policy in all tertiary care hospitals.

REFERENCES

1. Mermel LA, McCormick RD, Springman SR, Maki DG. The pathogenesis and epidemiology of catheter-related infection with pulmonary artery Swan-Ganz catheters: a prospective study utilizing molecular subtyping. Am J Med. 1991;91(suppl):197S-205S.
2. Sitges-Serra A, Puig P, Linares J. Hub colonization as the initial step in an outbreak of catheter-related sepsis due to coagulase negative staphylococci during parenteral nutrition. J Parenter Enteral Nutr. 1984;8(6):668-72.
3. Seifert H, Strate A, Schulze A, Pulverer G. Vascular catheter-related bloodstream

infection due to *Acinetobacter johnsonii* (formerly *Acinetobacter calaoaceticus var. lwoffi*): report of 13 cases. Clin Infect Dis. 1993;17:632-6.

4. Pittet D, Tarara D, Wenzel RP. Nosocomial bloodstream infection in critically ill patients: excess length of stay, extra costs, and attributable mortality. JAMA. 1994;271(20):1598-601.
5. Maki DK, Stolz SM, Wheeler S, Mermel LA. Prevention of central venous catheter–related bloodstream infection by use of an antiseptic-impregnated catheter: a randomized, controlled trial. Ann Intern Med. 1997;127(4):257-266.
6. Raad, II. Intravascular-catheter-related infections. Lancet 1998;351:893-898.
7. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of Biofilm Formation among the Clinical Isolates of Staphylococci: An Evaluation of Three Different Screening Methods. Ind J Med Microbiol. 2006;24(1):25-9.
8. Safdar N and Maki DG. Inflammation at the insertion site is not predictive of catheter-related bloodstream infection with short-term, noncuffed central venous catheters. Crit Care Med. 2002;30:2632-5. [PMID: 12483050].
9. Kluger DM, Maki DG. The relative risk of intravascular device related bloodstream infections in adults [abstract]. In: Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. San Francisco, CA: American Society for Microbiology; 1999:514.
10. Mitoma FD, Hardina GKM, Hoban DJ, Roberts RS, Low DE. Clinical significance of a test for slime production in ventriculoperitoneal shunt infections caused by coagulase negative Staphylococci. J Infect Dis. 1989;156(4):555 - 9.
11. Kotilainen P. Association of Coagulase-Negative Staphylococcal Slime Production and Adherence with the Development and Outcome of Adult Septicemias. J Clin Microbiol.1990;28(12):2779-85.
12. Amita Jain, Astha Agarwal. Biofilm production, a marker of pathogenic potential of colonizing and commensal staphylococci. J Microbiol Method 2009;76:88–92.
13. Ammendolia MG, Di Rosa R, Montanaro L, Arciola CR, Baldassarri L. Slime Production and Expression of the Slime-Associated Antigen by Staphylococcal Clinical Isolates. J Clin Microbiol.1999;37(10):3235–8.
14. Arciola CR, Baldassarri L B, Montanaro L. Presence of ica A & ica D genes & slime production in a collection of staphylococcal strains from catheter-associated infections. J Clin Microbiol. 2001;39(6):2151-56.
15. de Silva GDI, Kantzanou M, Justice A, Massey RC., Wilkinson AR, Day NPJ et al. The ica Operon & Biofilm Production in Coagulase-Negative Staphylococci Associated with Carriage & Disease in a Neonatal Intensive Care Unit. J Clin Microbiol. 2002;40(2):382-8.
16. Wang L, Li M, Dong D, Bach THL, Sturdevant DE, Vuong C et al**.** SarZ Is a Key Regulator of Biofilm Formation and Virulence in *Staphylococcus epidermidis*. J. Infect. Dis. 2008;197:1254–62.
17. Yao Y, Sturdevant DE, Otto M. Genomewide Analysis of Gene Expression in *Staphylococcus epidermidis* Biofilms: Insights into the Pathophysiology of *S. epidermidis* Biofilms and the Role of Phenol-Soluble Modulins in Formation of Biofilms. J. Infect. Dis. 2005;191:289–98.
18. Vuong C, Gerke C, Somerville GA, Fischer ER, Otto M. Quorum-sensing control of biofilm factors in *Staphylococcus epidermidis*. J Infect Dis. 2003;188:706–18.