Comparison of the Three Phenotypic Methods to Detect Biofilm Production in Coagulase Negative Staphylococci

Suvarna Vaibhav Sande[1], Abhishek Santosh Debnath[2]

[1]Department of Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, Meghe, Wardha, Maharashtra, India.
[2]Department of Microbiology, Jawaharlal Nehru Medical College, Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, Meghe, Wardha, Maharashtra, India.

ABSTRACT

BACKGROUND
Coagulase Negative Staphylococci species (CoNS) have been recognized as etiological agents in wide variety of infections, patients with implants and devices. CoNS has the ability to produce biofilm which is responsible for resistance to host defense mechanisms and to the antibiotics. Hence this study was undertaken to study biofilm production by the isolated species of CoNS by three different phenotypic methods and compare these methods for biofilm production.

METHODS
In this cross-sectional study, 200 CoNS isolates from clinically significant samples were identified up to species level by conventional phenotypic methods. Biofilm production was detected by Tissue Culture Plate method (TCP), Standard Tube method (ST) and Congo Red Agar Plate method (CRA)· Biofilm production in test strains were compared with reference strains.

RESULTS
Out of 200 CoNS isolates, 122 were positive by TCP method, 106 by ST method & 67 by CRA method for biofilm production. Considering TCP method as gold standard**,** sensitivity, specificity, PPV and NPV of ST & CRA method were found to be 86.06%, 98.71%, 99.05%, 81.91% and 54.09%, 98.71%, 98.50% and 57.89% respectively. Analysis of Kappa agreement between TCP and ST method showed good agreement while between TCP & CRA, moderate agreement. Comparison of ST and CRA method with TCP by Pearson correlation coefficient test showed strong association between ST and TCP method ($p= 0.006$) & poor association between TCP and CRA method. ($p<0.01$).

CONCLUSIONS
Biofilm production by TCP method was higher compared to the other methods. ST method is comparable to TCP but CRA alone cannot be considered for biofilm detection in CoNS. ST method can be used in routine as it gives reliable results with good sensitivity & specificity and is easy to perform.

KEY WORDS
CoNS, Biofilm Production, Phenotypic Methods

Corresponding Author:
Dr. Suvarna Vaibhav Sande,
Professor,
Department of Microbiology,
Jawaharlal Nehru Medical College,
Datta Meghe Institute of Medical Sciences (Deemed to be University), Sawangi, Meghe, Wardha, Maharashtra, India.
E-mail: suvarnasande@yahoo.co.in

DOI: 10.14260/jemds/2020/299

Financial or Other Competing Interests: None.

How to Cite This Article:
Sande SV, Debnath AS. Comparison of the three phenotypic methods to detect biofilm production in coagulase negative staphylococci. J. Evolution Med. Dent. Sci. 2020;9(16):1373-1378, DOI: 10.14260/jemds/2020/299

Submission 14-02-2020,
Peer Review 03-04-2020,
Acceptance 10-04-2020,
Published 20-04-2020.

BACKGROUND

Coagulase Negative Staphylococci (CoNS) are found as part of the normal flora of skin and mucous membrane of humans and animals. Earlier they were considered as non- pathogenic. Healthy human skin or mucous membrane normally support from 10^1 to 10^6 colony forming units (CFU)/cm^2 of CoNS, depending on the anatomical site. There are around more than 47 species and 23 subspecies of CoNS of human importance. Growth requirements of CoNS are not specific. CoNS can grow in wide range of temperature, pH. Hence CoNS can survive easily in varied circumstances. Increased importance of CoNS may be attributed to elderly, morbid patients, increased use of implants and devices and increased number of immunocompromised patients. They are also transmitted by various health care related procedures. Inserted implants and devices are colonized by CoNS and are responsible for failure of medical procedures resulting in economic burdens.[1]

The important characteristic of CoNS is ability to colonize the surfaces of biomaterials by adhering in biofilm-structured communities of cells encased in a self-produced polymeric matrix known as slime which is responsible for resistance to host defense mechanisms and to the antibiotics. In pathogenesis of infections and drug resistance in CoNS, biofilms play an important role.[2] Because of increasing antimicrobial resistance in CoNS, few therapeutic options are available for treatment.

Biofilm production is a complex process comprising a number of steps and involving several surface components. Stages of biofilm formation includes first step of primary attachment of cells to a surface by direct adhesion to the polymer surface, or adhesion to host matrix proteins that cover the polymer surface as a “conditioning film” (often associated with medical device-related infections) through interaction with specific bacterial binding proteins.[3] Second step is accumulation of cells in multiple layers. This step consists of nonspecific and hydrophobic interactions and after this stage, bacteria multiply forming microcolonies. This stage requires intercellular adhesion, which is achieved by the production of extracellular matrix in which the bacteria become surrounded**.** Third step is maturation of the biofilm structure with formation of channels for water, ion, and nutrient exchange giving rise to the characteristic three-dimensional appearance of biofilms**.** Last step is detachment and dispersal of single bacterial cells or large cell clusters, which then may initiate a new cycle of biofilm formation elsewhere.[3]

Biofilm provides the microorganisms with an excellent protective environment and favouring the exchange of genetic material between cells as well as intercellular communication, a process known as quorum sensing (QS).[4] Biofilm structures comprises mainly bacterial cells and an extracellular polymeric substance (EPS) provided by the polysaccharide intercellular adhesion (PIA) and synthesis of PIA is associated with the biosynthetic enzyme-coding genes intercellular adhesion operon (ica ADBC), an operon that contains the icaRADBC, four biosynthetic genes (ica ADBC) and one regulation gene (icaR).[5,6]

The icaA and icaD, when co-expressed, will show better activity for the assemblage of β (1-6)- linked glycosaminoglycans that comprise PIA. The absence of icaD is associated with a decrease in PIA production, the likely icaC functions in the extrusion of PIA from the bacterial cell, whereas the icaB appears to function as a deacetylase. The expression of icaAD or icaADC genes stimulates an increased production of biofilm.[5,6]

Biofilms are notoriously difficult to eradicate and are often resistant to systemic antibiotic therapy. Number of tests are available to detect biofilm production. The methods include quantitative method like tissue culture plate method, qualitative methods like tube adherence method, Congo red agar method. Other methods like bioluminescent assay, light or fluorescence or confocal microscopic examination are also available. [7] Hence this study was undertaken to study biofilm production by the isolated species of CoNS by three different phenotypic methods and to compare these methods.

METHODS

This cross-sectional study was carried out in department of Microbiology of Jawaharlal Nehru Medical College and Acharya Vinoba Bhave Rural Hospital, Sawangi (Meghe), Wardha which is a tertiary care hospital from September, 2016 to September, 2018 after obtaining approval from Institutional Ethics Committee.

Sample Size

In this study, the sample size was calculated by using Open EPI software based on the following assumption: Based on the finding from previous study **[8]**, slime positive CoNS isolates – 25 out of 45 CoNS isolates, Proportion was 55.56%, expecting 5% margin of error and confidence interval (CI) of 90%, the sample size was calculated as 198. The sample size was rounded up to 200. 200 CoNS isolates from clinically significant samples like blood, pus, urine, body fluids etc. received in department of Microbiology were processed. CoNS isolates from sputum, stool, wound swabs, throat and vaginal swabs were excluded.[9] The isolates were considered clinically significant when isolated in pure culture from infected sites or body fluids or if the same strain was isolated from repeat samples.[9,10]

Processing of Sample

Direct microscopy which includes wet mount preparation and gram staining were carried out on appropriate samples. Samples were cultured on Nutrient agar, Blood agar and MacConkey agar and incubated overnight at 37^0C. The organisms isolated were identified according to standard procedures on the basis of colony characters, Gram staining, catalase and coagulase test and various other biochemical tests[11,12] Biofilm production was detected by different phenotypic methods. Tissue culture plate method (TCP)[8] (quantitative method), Standard Tube method (ST)[13] and Congo Red Agar Plate method (CRA)[13] (qualitative method) Biofilm production in test strains were compared with reference strains.[8] *S. epidermidis* ATCC 35984 (strong biofilm producer) and *S. epidermidis* ATCC 12228 (non-biofilm producer).

Tissue Culture Plate Method (TCP)[8]

The quantitative method of adherence to polysterene plates proposed by Christensen et al was used. Overnight growth of test organisms in 10 ml of trypticase soy broth was diluted 1in 100 and 200µl were inoculated in sterile 96 well flat bottom polystyrene tissue culture plate with positive and negative control strains and incubated overnight at 37 ^{0}C. The test organism diluted in trypticase soy broth was inoculated in triplicate. The contents of the well were removed and washed 4 times with 0.2 ml of phosphate buffer saline (pH 7.2). Sodium acetate (2%) was used to fix adherent bacteria in the wells and allowed to dry. Crystal violet (0.1%) was used to stain the wells. Excess stain was removed and air dried. Reading was taken at wavelength 490 nm by micro ELISA auto reader (ELISA). As the bacteria forms biofilm and adhere to the wells, these OD values were taken as an index of bacterial adherence to the wells. The data was then averaged, and standard deviation was calculated.

Interpretation[8]

Calculation for Optical Density for Detection of Biofilm:

Cut off OD=Negative control (3 standard deviation +mean).
Positive control= Average.
Sample= Average.
Weak biofilm= 0 cut off OD up to 2 cut off OD value.
Moderate =2 cut off OD up to 4 cut off OD value.
Strong biofilm= more than 4 cut off OD value.

Standard Tube (ST) Method:

Biofilm production was investigated by tube adherence test proposed by Christensen et al.[13] Test strains along with positive and negative control were inoculated in trypticase soy broth and incubated overnight at 37°C. After incubation, the tubes were decanted and washed thrice with phosphate buffer saline (pH 7.3). The tubes were dried in air and stained with 0.25% safranin. After incubation for 10 min, the stain was decanted and washed with phosphate buffer saline. The tubes were dried in inverted position and observed for biofilm formation. Positive result was defined as presence of layer of stained coating on inner wall and bottom of the tube. Strains that developed stains in the form of rings at the air-liquid boundary were excluded. Tubes were then examined, and the amount of biofilm was scored as strong (+++), moderate (++), weak (+), absent (0).

Congo Red Agar (CRA) Method

Biofilm production was detected by culture of CoNS isolates on CRA plates proposed by Freeman et al **[14]**. The CRA medium was prepared with brain heart infusion broth 37 g/l, sucrose 50 g/l, agar No 1 10 g/l and Congo red 0.8 g/l. Congo red stain was prepared as a concentrated aqueous solution and autoclaved (121 °C for 15 minutes) separately from the other medium constituents, and was added when the agar had cooled to 55 °C. Plates of the medium were inoculated with test strains along with positive and negative control strains and incubated aerobically for 24 hours at 37 °C. A positive result was indicated by black colonies with dry crystalline consistency.

Strong
Isolates producing black colonies were considered as strong biofilm producers.

Moderate
Dark colonies without dry crystalline colony morphology indicted moderate biofilm production.

Weak
Weak biofilm producers produced dark pink colonies. Non slime producers mostly turned out as dry red colonies.

Statistical Analysis

Statistical analysis was carried out by relevant statistical methods like percentage, sensitivity, specificity, positive predictive value and negative predictive value, Pearson correlation coefficient test and Measurement of kappa agreement.

RESULTS

Biofilm production in CoNS was detected by phenotypic methods such as Tissue culture plate (TCP), Standard tube (ST) and Congo red agar (CRA) method. Table 1. Shows biofilm production by different phenotypic methods amongst 200 CoNS isolates.

No of CoNS (n=200)	Biofilm	TCP Method	ST Method	CRA Method
	Positive	122(61%)	106(53%)	67(33.5%)
	Negative	78(39%)	94(47%)	133(66.5%)

Table 1. Biofilm Production by Phenotypic Methods (n=200)

Out of 200 CoNS isolates, 122 (61%) CoNS isolates were positive and 78 (39%) CoNS isolates were negative for biofilm production by TCP method. 106 (53%) CoNS isolates were positive and 94 (47%) CoNS isolates were negative for biofilm production by ST method. 67 (33.5%) CoNS isolates were positive and 133 (66.5%) CoNS isolates were negative for biofilm production by CRA method. Table 2 shows grading of positive biofilm production (strong, moderate, weak) by three different phenotypic methods. Table 3 shows species wise biofilm production by TCP method, ST and CRA method.

Biofilm Production	TCP Method (n=122)	ST Method (n=106)	CRA Method (n=67)
Strong	50 (40.98%)	32 (30.18%)	7 (10.44%)
Moderate	53 (43.44%)	39 (36.79%)	9 (16.41%)
Weak	19 (15.57%)	35 (33.01%)	51 (76.11%)
Total	**122 (61%)**	**106 (53%)**	**67 (33.5%)**

Table 2. Grading of Positive Biofilm Production by Three Different Phenotypic Methods

Species	TCP Method	ST Method	CRA Method
S. epidermidis (n=80)	52 (65%)	45 (54.87%)	29 (36.25%)
S. haemolyticus (n=50)	31 (62%)	29 (58%)	18 (36%)
S. schleiferi (n=22)	13 (59.09%)	12 (54.54%)	8 (36.36%)
S. lugdunensis (n=20)	12 (60%)	10 (50%)	6 (30%)
S. saprophyticus (n=11)	7 (63.63%)	6 (54.54%)	4 (36.36%)
S. xylosus (n=7)	3 (42.85%)	2 (28.57%)	1 (14.28%)
S. intermedius (n=6)	3 (50%)	2 (33.33%)	1 (16.66%)
S. warneri (n=3)	1 (33.33%)	0	0
S. hominis (n=1)	0(0%)	0	0
Total (n=200)	**122**	**106**	**67**

Table 3. Species Wise Biofilm Production by TCP, ST and CRA Methods

Among CoNS isolates, maximum biofilm production was shown by *S. epidermidis* followed by *S. haemolyticus*. Table 4 shows comparison of ST and CRA method with TCP, considering TCP as the gold standard.

		TCP Method	
		Positive	**Negative**
ST method (n=200)	Positive	105	1
	Negative	17	77
		TCP Method	
CRA method (n=200)		**Positive**	**Negative**
	Positive	66	1
	Negative	56	77

Table 4. Comparison of ST and CRA Method with TCP Method

Comparison of ST method with TCP showed that out of 200 CoNS isolates, 105 CoNS isolates were positive by both TCP and ST method and 1 CoNS isolate was negative by TCP but weak positive by ST method. Out of 200 CoNS isolates, 17 CoNS isolates were positive by TCP but negative by ST method and 77 CoNS isolates were negative by both TCP and ST method. Comparison of CRA method with TCP showed that out of 200 CoNS isolates, 66 CoNS isolates were positive by both TCP and CRA method and 1 CoNS isolate was negative by TCP but positive by CRA method. Out of 200 CoNS isolates, 56 CoNS isolates were positive by TCP but negative by CRA method and 77 CoNS isolates were negative by both TCP and CRA method.

Table 5 shows comparison of ST and CRA method with TCP by Pearson correlation coefficient test which showed strong association between ST and TCP method (p 0.006) & poor association between TCP & CRA method. ($p<0.01$). Statistical evaluation of ST and CRA method considering TCP as gold standard showed that Sensitivity, specificity, PPV and NPV of ST method were 86.06%, 98.71%, 99.05% and 81.91% respectively. Sensitivity, specificity, PPV and NPV of CRA method were found to be 54.09%, 98.71%, 98.50% and 57.89% respectively. Table 6 shows analysis of Kappa agreement between TCP, ST and CRA method.

	Pearson Correlation Coefficient Test	Value	Dif.	P Value	Association
ST method	Pearson correlation coefficient test	2.63	1	0.006	Strong
CRA method	Pearson correlation coefficient test	12.106	1	<0.01	Poor

Table 5. Comparison of ST and CRA Method with TCP Method by Pearson Correlation Coefficient Test

	Measurement of Agreement	Value	Standard Error	Approx. Significance
ST method	Kappa	0.8	0.023	Good agreement
CRA method	Kappa	0.59	0.112	Moderate agreement

Table 6. Analysis of Kappa Agreement between ST Method, CRA Method Considering TCP as Gold Standard Method

Analysis of Kappa agreement showed good agreement between TCP & ST method & moderate agreement between TCP & CRA. (kappa value <0.20=poor agreement, 0.21-0.40=fair agreement, 0.41-0.60=moderate agreement, 0.61-0.80=good agreement, 0.81-1.00=very good agreement) It was observed that ST method is comparable to TCP method for detection of biofilm formation with respect to sensitivity, specificity, PPV and NPV with strong association and Kappa agreement was good agreement. Analysis of Kappa agreement between TCP and CRA method showed moderate agreement (kappa=0.59) so, it was observed that statistically association of CRA method with TCP method was found to be poor and kappa agreement was moderate agreement. So, CRA method alone cannot be recommended for detection of biofilm formation.

DISCUSSION

In present study, 200 CoNS were isolated from clinically significant samples & biofilm production in CoNS was detected by phenotypic methods like TCP, ST and CRA method. In present study, 61% CoNS isolates were positive, and 39 % CoNS isolates were negative for production of biofilm by TCP method (table 1). This finding correlates with a study done by Devapriya F et al.[15] where 64.4 % CoNS isolates were positive, and 35.6 % CoNS isolates were negative for biofilm production by TCP method. In present study, 53% and 33.5% CoNS isolates were positive for biofilm production byST method and CRA method respectively (table 1). This is in accordance with study done by Saumya Singh et al. [16] where 41.56% and 28.57% CoNS isolates were positive for biofilm production by ST method and CRA method respectively.

In present study, 40.98%, 30.18% and 10.44% of CoNS showed strong biofilm production by TCP method, ST method and CRA method respectively (table 2). This finding correlates with a study done by Deka N et al. [17] where 36%, 21% and 5% of CoNS showed strong biofilm production by TCP method, ST method and CRA method respectively. In present study, amongst all the CoNS isolates, biofilm production by TCP method was seen in 65% *S. epidermidis* isolates followed by 62% *S. haemolyticus* isolates, 60% *S. lugdunensis* isolates and 63.63% *S. saprophyticus* isolates (table 3). This observation of present study correlates with a study done by L.E. Alcarez et al[18] where biofilm production by TCP method was seen in 57.14% *S. epidermidis* isolates followed by 37.5 % *S. haemolyticus* isolates, 100% *S. lugdunensis* isolates and 83.83% *S. saprophyticus* isolates.

In present study, among all the CoNS isolates biofilm production by ST method was seen in 54.87% *S. epidermidis* isolates followed by 58% *S. haemolyticus* isolates, (table 3). This finding of present study correlates with a study done by L.E. Alcarezet al.[18] where 57.14% *S. epidermidis* isolates followed by 37.5 % *S. haemolyticus* isolates were biofilm producers by ST method.

TCP method is the gold standard method for biofilm detection and when we compared ST method with TCP method, we found that out of 94 non-biofilm producing CoNS isolates by ST method (table 1), 17 CoNS isolates were positive by TCP method but with different degrees of production while 77 CoNS isolates were negative by both methods (table 4). ST method showed strong association with TCP method with the P value of 0.006 (table 5) and kappa value was 0.8 (good agreement) (table 6). The sensitivity, specificity, PPV and NPV of ST method were found to be 86.06%, 98.71%, 99.05% and 81.91 % respectively. These findings of present study correlate with a study done by Thilakavathyet al.[18] So, ST method is comparable to TCP method. In present study, 53% CoNS isolates were biofilm producers by ST method while 33.5% CoNS isolates were biofilm producers by CRA method. (table 1) So we can recommend ST method is better compared

to the CRA for biofilm detection. This finding of present study correlates with a study done by Knobloch et al. [19] where CRA method was not recommended for biofilm detection.

When we compared CRA method with TCP method, out of 133 non-biofilm producing CoNS isolates by CRA method (table 1), 56 CoNS isolates were positive by TCP method but with different degrees of production while 77 CoNS isolates were negative by both methods (table 4). CRA method showed poor association with TCP method with the P value of <0.1 (table 5) and kappa value was 0.59 (moderate agreement) (table 6). The sensitivity, specificity, PPV and NPV of CRA method were found to be 54.09%, 98.71%, 98.5% and 57.89% respectively. Though, CRA method was easy to perform and less time consuming, but statistically association of CRA method with TCP method was found to be poor and kappa agreement was moderate agreement. So, CRA method alone cannot be recommended for detection of biofilm formation by CoNS. These observations of present study correlate with study done by Mathur et al.[20]and Thilakavathy et al[18] where they also recommended that CRA method alone cannot be used for detection of biofilm production.

CONCLUSIONS

From our study, it can be inferred that for detection of biofilm production by CoNS, TCP method is an accurate & reliable quantitative tool. ST method is comparable to TCP method as it gives reliable results with good sensitivity and specificity. It is easy to perform, with no need of special instruments or special training of laboratory staff and can be routinely used. CRA method alone cannot be considered for detection of biofilm production by CoNS. Biofilm production by CoNS helps to differentiate between pathogenic and commensal CoNS and should be carried out in routine which will help clinicians for planning appropriate antibiotic therapy.

ACKNOWLEDGEMENT

Authors are thankful to DMIMS (DU) for the help while conducting the study.

REFERENCES

[1] Becker K, Heilmann C, Peters G. Coagulase-negative Staphylococci. Clinical Microbiology Reviews 2014;27(4):.870-926.

[2] Gotz F. Staphylococcus and biofilms. Mol Microbiol 2002;43(6):1367-78.

[3] Schoenfelder SMK, Lange C, Eckart M, et al. Success through diversity - how Staphylococcus epidermidis establishes as a nosocomial pathogen. Int J Med Microbiol 2010;300(6):380-6.

[4] Donlan RM, Costerton JW. Biofilms: survival mechanism of clinically relevant microorganisms. Clin Microbiol Rev 2002;15(2):167-93.

[5] Heilmann C, Thumm G, Chhatwal GS, et al. Identification and characterization of a novel autolysin (Aae) with adhesive properties from Staphylococcus epidermidis. Microbiology 2003;149(Pt 10):2769-78.

[6] Nilsdotter-Augustiinsson A, Claesson C, Lindgren PE, et al. Adherence of Staphylococcus epidermidis to extracellular matrix proteins and effects of fibrinogen-bound bacteria on oxidase activity and apoptosis in neutrophils. APMIS 2005;113(5):361-73.

[7] Alper EM. Bacterial biofilm detection methods in the laboratory. Antimicrobial research: novel bioknowledge and educational programs (A. Mendez-Vilas, Ed) 2017: p. 289-93.

[8] Alcaráz LE, Satorres SE, Lucero RM, et al. Species identification, slime production and oxacillin susceptibility in coagulase-negative staphylococci isolated from nosocomial specimens. Braz J Microbiol 2003;34(1):45-51.

[9] Badampudi SSV, Surya KRLS, Gunti R. Speciation and biofilm production of coagulase negative Staphylococcal isolates from clinically significant specimens and their antibiogram. Journal of Krishna Institute of Medical Sciences University 2016;5(2):69-78.

[10] Bhatt P, Tandel K, Singh A, et al. Species distribution and antimicrobial resistance pattern of coagulase-negative Staphylococci at a tertiary care centre. Medical Journal Armed Forces India 2016;72(1):71-4.

[11] Colle JG, Miles RS, Watt B. Tests for identification of bacteria. Chap - 7. In: Colle JG, Fraser AG, Marmion BP, et al. eds. Mackie and McCartney Practical medical microbiology. 14th edn. New Delhi: Indian Reprint 2008: p. 131-49.

[12] Washington CW Jr, Stephen DA, William MJ, et al. Koneman's Color Atlas and Textbook of diagnostic microbiology in Gram positive cocci. Ch – 12. 6th edn. USA: Lippincott Williams and Williams 2006: p. 623-71.

[13] Christensen GD, Simpsom WA, Bisno AL, et al. Adherence of slime producing strains of Staphylococcus epidermidis to smooth surfaces. Infect Immun 1982;37(1):318-26.

[14] Freeman DJ, Falkiner FR, Keane CT. New method for detecting slime production by coagulase negative Staphylococci. J Clin Pathol 1989;42(8):872-4.

[15] Devapriya F, Sajith P, Ranganathan R, et al. Prevalence of biofilm and beta-lactamase producing Staphylococcus in nasal and throat isolates from healthy volunteers: a medical alert. NJMS 2014;3(2):79-83.

[16] Singh S, Asthana AK, Madan M, et al. Study of biofilm formation among clinical Staphylococcal isolates. National Journal of Laboratory Medicine 2015;4(4):24-7.

[17] Deka N. Comparison of tissue culture plate method, tube method and Congo red agar method for the detection of biofilm formation by coagulase negative Staphylococcus isolated from non-clinical isolates. Int J Curr Microbiol App Sci 2014;3(10):810-5.

[18] Thilakavathy P, Priyan RMV, Jagatheeswari PAT, et al. Evaluation of Ica gene in comparison with phenotypic methods for detection of biofilm production by coagulase negative staphylococci in a tertiary care hospital. J Clin Diagn Res 2015;9(8):DC16-DC9.

[19] Knobloch JKM, Horstkotte MA, Rohde H, et al. Evaluation of different detection methods of biofilm formation in Staphylococcus aureus. Med Microbial Immunol 2002;191(2):101-6.

[20] Mathur T, Singhal S, Khan S, et al. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. IJMM 2006;24(1):25-9.