

Prevalence of biofilm-producing *Staphylococcus epidermidis* in the healthy skin of individuals in Tamil Nadu, India

CA EL Farran¹, A Sekar², A Balakrishnan³, S Shanmugam³, P Arumugam³, J Gopalswamy³

¹ Research Core Facility GM 01/01, Faculty of Medicine, Kuwait University, Jabriya, P.O. Box 24923, Safat 13110, Kuwait

² Department of Microbiology, Yong Loo Lin School of Medicine, National University of Singapore, 5 Science Drive 2, 117597, Singapore

³ Department of Genetics, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani, Chennai, India

Date of Submission 01-Aug-2012

Date of Acceptance 15-Oct-2012

Date of Web Publication 15-Mar-2013

Correspondence Address:

C A EL Farran

Research Core Facility GM 01/01, Faculty of Medicine, Kuwait University, Jabriya, P.O. Box 24923, Safat 13110

Kuwait

DOI: 10.4103/0255-0857.108712

~ Abstract

Purpose: *Staphylococcus epidermidis* is a major commensal bacteria. Various strains of *S. epidermidis* are capable of forming biofilms by attaching to several surfaces. Biofilm-forming ability of this organism is found to be associated with many hospital-acquired infections and can even impair wound healing. *S. epidermidis* strains producing polysaccharide-biofilms possess the intercellular adhesion (*ica*) operon while strains forming the protein adhesion-mediated biofilms possess the accumulation associated protein (*aap*) gene. We screened for biofilm-forming *S. epidermidis* in the skin of healthy individuals in Tamil Nadu in order to determine the risk of acquiring *S. epidermidis* infections in hospital settings. **Materials and Methods:** Skin swabs were taken from seventy two subjects residing in Chennai with healthy skin who showed no visible signs of skin lesions or allergies. *S. epidermidis* was isolated from 58 samples out of the 72 collected. The presence of *ica* operon in *S. epidermidis* isolates was determined by PCR and biofilm production was examined using quantitative tissue culture plate assay. **Results:** Majority of the samples (47/72; 65.3%) showed pure *S. epidermidis* growth, (14/72; 19.4%) showed pure *Staphylococcus aureus* growth and the remainder (11/72; 15.3%) showed mixed growth. Biofilm-forming *S. epidermidis* were found in the majority of samples (53/58; 91.4%) and *ica* operon was detected in 19 samples out of 58 (32.8%) which is a significantly higher percentage when compared to other studies conducted at different parts of the globe ($P = 0.0003$). **Conclusion:** We inferred that *ica* operon and biofilm-forming *S. epidermidis* are common in the healthy skin of individuals in Tamil Nadu. Measures have to be taken to reduce the risk of hospital-acquired *S. epidermidis* infections.

Keywords: Biofilms, hospital-acquired infections, *ica* operon, *Staphylococcus epidermidis*, skin flora

How to cite this article:

EL Farran C A, Sekar A, Balakrishnan A, Shanmugam S, Arumugam P, Gopalswamy J. Prevalence of biofilm-producing *Staphylococcus epidermidis* in the healthy skin of individuals in Tamil Nadu, India. *Indian J Med Microbiol* 2013;31:19-23

How to cite this URL:

EL Farran C A, Sekar A, Balakrishnan A, Shanmugam S, Arumugam P, Gopalswamy J. Prevalence of biofilm-producing *Staphylococcus epidermidis* in the healthy skin of individuals in Tamil Nadu, India. *Indian J Med Microbiol* [serial online] 2013 [cited 2013 Mar 20];31:19-23. Available from: <http://www.ijmm.org/text.asp?2013/31/1/19/108712>

~ Introduction

Staphylococcus epidermidis are non-motile, coagulase negative, gram-positive cocci commonly found in the human skin. It is the most commonly isolated bacteria from the skin. The organism is non-pathogenic in healthy people but people with compromised immunity are at risk for developing an infection. Various strains of *S. epidermidis* are capable of forming biofilms and that is a major concern for people with catheters, heart valves or other implants since the organism can form biofilms on these surfaces. [1] It can also form biofilms on various metals and materials other than surgical implants. [2] It has been demonstrated that biofilm formation by staphylococci impairs wound healing. [3]

Biofilm is a mode of survival for various microbes by which they form aggregates during unfavorable conditions. The bacterial cells in this mode of living are more resistant to antimicrobial agents, harsh conditions of the environment and to the immune cells. In this mode of living, the bacterial aggregates form channels which serve to diffuse nutrients into the biofilm and waste products away from the biofilm. [4]

The formation of biofilms in *S. epidermidis* proceeds in two steps. First, bacteria adhere to a surface by unspecific factors such as hydrophobicity, surface material, cell wall teichoic acids and several proteins. Then actual accumulation of biofilm occurs. Major genes involved in *S. epidermidis* biofilm formation include autolysin E (altE), biofilm associated protein (bap), accumulation associated protein (aap) and intercellular adhesion (ica). [4]

In polysaccharide-biofilms produced by *S. epidermidis*, extracellular polysaccharide adhesin play an essential role in initial bacterial adherence to surfaces and intercellular adhesion is important for the aggregation of cells. Two major polysaccharides produced by *S. epidermidis*, capsular polysaccharide adhesin (PSA) and polysaccharide intercellular adhesin (PIA) are found to have a vital role in the formation of this type of biofilms. [5] In the two step model proposed for the formation of *S. epidermidis* slimy-biofilms, initial adherence is mediated by PSA and accumulation of cells is due to production of PIA. [6] The ica locus contains an operon, icaADBC which is proposed to contain the structural genes required for PIA synthesis.

The presence of ica operon has been proposed to be associated with strong biofilm formation and it is assumed that ica operon and strong slime producers are more prevalent in *S. epidermidis* isolates associated with infections or implants than in *S. epidermidis* isolated from healthy individuals. [7],[8]

The aim of this study was to screen for biofilm-producing *S. epidermidis* and ica-positive *S. epidermidis* in isolates from normal skin of healthy volunteers in order to determine the risk of acquiring a *S. epidermidis* infections in hospitals especially that this organism is a common inhabitant of human skin and it is a major concern for people with catheters, heart valves and other implants and can even impair wound healing.

~ Materials and Methods

Collection of samples

Samples were collected from the healthy skin of 72 random subjects from Chennai, Tamil Nadu, India who did not suffer from any skin lesions, infection or allergy and who were not associated with catheters, valves or any other implantation. Forehead, anterior nares and arm joint were rubbed vigorously with sterile swabs pre-moistened with sterile physiological saline. The swabs were transferred to Tryptic Soy Broth (TSB) (Himedia Laboratories Pvt. Ltd., India) in sterile tubes and incubated at 37°C in a shaker incubator at 200 rpm for 16-18 hours. Turbidity was observed in all tubes except the sterility check tube. Samples were then sub-cultured in mannitol salt agar (MSA) for two days. *S. epidermidis* produces pink colonies on MSA.

Isolation of *Staphylococcus epidermidis*

Isolates from the samples were characterized and identified by standard microbiological techniques including gram staining, mannitol fermentation, catalase test and coagulase test. Cultures were maintained on TSB. *Staphylococcus epidermidis* ATCC 155 was used as a positive control and *Escherichia coli* XL10-Gold was used as a negative control.

Quantitative detection of biofilm production

Quantitative detection was performed using tissue culture plate assay (TCP). 48-well tissue culture plates (Greiner Bio-one, CELLSTAR®) were used. In each well 200 µl of sterile TSB was added. 2 µl of each sample was added to each well. The plate was incubated aerobically for 24 hours at 37°C. *Staphylococcus epidermidis* ATCC 155 was used as a positive control. After 24 hours, the contents of each well were gently removed by tapping the plate. Then each well was washed 4 times with 200 µl 1x PBS to remove planktonic cells. After that, 100 µl of 0.1% crystal violet was added to each well to stain the biofilm. The tissue culture plate was incubated at room temperature for 15 minutes. Each well was then washed repeatedly with autoclaved double distilled water to remove excess stain. The tissue culture plate was allowed to dry and then read at 570 nm using ELISA plate reader. The assay has been performed in triplicate for each sample. Samples that had given reading values of more than 0.12 were considered as strong biofilm producers. Samples that gave values between 0.06 and 0.12 were considered as moderate to weak biofilm producers and samples with reading values of less than 0.06 were considered as non-biofilm producers. The results were validated by using a compound microscope.

Standardization of biofilm quantification by tissue culture plate method

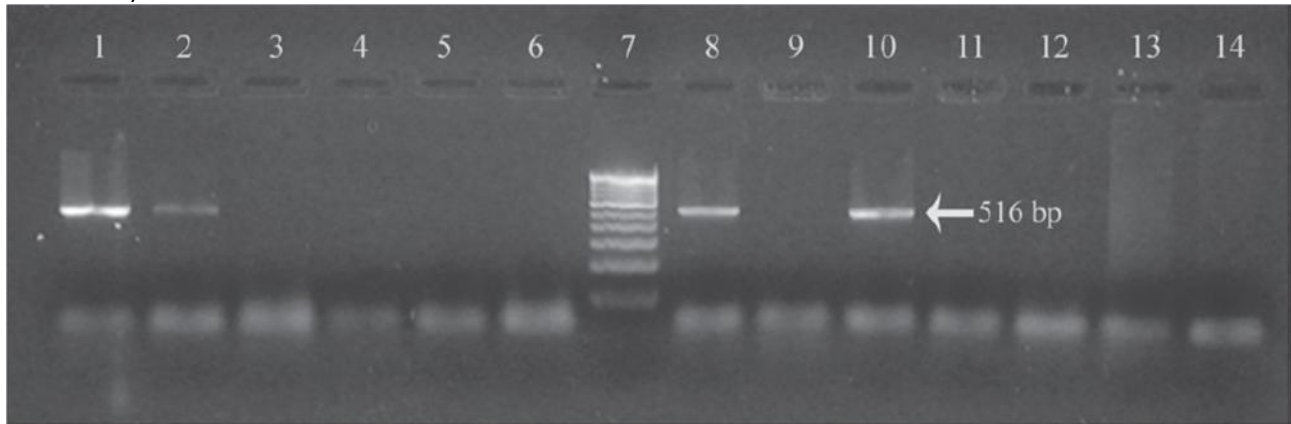
For the standardization of TCP, the effect of sucrose on biofilm formation was studied. Hence, *Staphylococcus epidermidis* ATCC 155 was grown in two tubes containing TSB with one of the tubes being enriched with 5% sucrose. Then biofilm production was quantified using the above mentioned procedure with each tube being considered as a separate sample. Biofilm quantification by TCP method was done six times for each tube and the readings obtained from each tube were averaged.

Detection of *ica* operon

Bacterial DNA was isolated using Commercial DNA extraction kit (Bangalore Genei Pvt. Ltd., India). The presence of genomic DNA was detected using agarose gel electrophoresis technique with 1% agarose. UV-transilluminator was used to view the DNA bands. Polymerase Chain Reaction (PCR) was performed to screen for *ica* operon in the isolated DNA samples. The primers used for the amplification of *ica* operon were 5'- TTATCAATGCCGAGTTGTC-3' and 5'- GTTTAACGCG AGTGCGCTAT-3'. [9] These primers which are specific for the *icaADBC* region in *S. epidermidis* were used in the PCR reaction to produce amplicon having the size of 516 bp [Figure 1]. A 20 µl reaction mix was prepared with PCR grade water, 10x buffer, primers, dNTP mix, Taq Polymerase and DNA template. The PCR cycling conditions used were 1 cycle of 2 minutes of initial denaturation at 94°C then 35 cycles each of 45 seconds of denaturation at 94°C, 45 seconds of

annealing at 60°C and 45 seconds of extension at 72°C followed by 1 cycle of final extension of 5 minutes at 72°C. The amplicon were analyzed by 2% agarose gel electrophoresis using a UV transilluminator. The PCR was performed in triplicate for each sample to confirm the result. PCR was performed by using Eppendorf Mastercycler Gradient, Germany. *Staphylococcus epidermidis* ATCC 155 was used as a positive control and *Escherichia coli* XL10-Gold was used as a negative control.

Figure 1: PCR for detection of *ica* operon, Lane 1— Positive control (*Staphylococcus epidermidis* ATCC 155); Lane 2— An *ica*-positive sample (amplicon size is 516 bp); Lanes 3, 4, 5, 6— Samples which are *ica*-negative; Lane 7— Molecular mass marker (100 bp DNA ladder); Lane 8— An *ica*-positive sample (amplicon size is 516 bp); Lane 9— An *ica*-negative sample; Lane 10— An *ica*-positive sample (amplicon size is 516 bp); Lanes 11, 12, 13— Samples which are *ica*-negative; Lane 14— Negative control (*Escherichia coli* XL10-Gold)



Statistical analysis

Chi-squared test was performed to ensure that the results obtained from the screening of *ica* operon were significantly different than those obtained in studies conducted at different parts of the globe. Unpaired two-sample t-test was performed to confirm that the results obtained from the standardization of TCP were significantly different for each sample.

~ Results

Top

Bacterial isolates

Majority of the samples (47/72; 65.3%) showed pure *Staphylococcus epidermidis* growth while (14/72; 19.4%) showed pure *Staphylococcus aureus* growth and (11/72; 15.3%) showed mixed growth. *Staphylococcus epidermidis* was isolated successfully from 58 samples and isolates were confirmed using standard microbiology techniques.

Standardization of biofilm quantification by tissue culture plate method

The optical density obtained from the sample grown in TSB without sucrose was 0.302 while it was 0.217 for the sample grown in TSB enriched with 5% sucrose. Samples grown in TSB enriched with sucrose showed significantly weaker biofilm formation than those grown in TSB without sucrose ($P = 0.02$).

Biofilm quantification by tissue culture plate method

Seven samples out of 58 (12%) showed very strong biofilm formation in the wells. Forty-six samples out of 58 (79.4%) showed moderate biofilm growth while five samples out of 58 (8.6%) showed no biofilm formation [Table 1]. These values are much higher compared to values obtained in other studies. [8],[9]

Table 1: Results obtained from the screening of biofilm production and ica possession in *S. epidermidis* from healthy skin isolates

Biofilm producing	Non-biofilm producing	Moderate biofilm producing	Strong biofilm producing	<i>ica</i> -positive	<i>ica</i> -negative
53/58 (91.4%)	5/58 (8.6%)	46/58 (79.4%)	7/58 (12%)	19/58 (32.8%)	39/58 (67.2%)

Presence of ica operon

Intercellular adhesion (*ica*) operon was detected in 19 samples out of the 58 samples screened for *ica* operon (32.8%). This is a significantly higher percentage when compared with other studies ($P = 0.0003$).

Correlation between the results obtained from the screening of *ica* operon and quantification of biofilm production by TCP method

Out of the 19 samples in which *ica* operon was detected, only six samples were found to be strong biofilm producers and the remaining 13 were found to be moderate biofilm producers. Out of the 39 samples in which *ica* operon was not detected, only five sample were found to be non-biofilm producers. In the remaining 34 samples, one strong biofilm producer and 33 moderate biofilm producers were observed.

~ Discussion

Staphylococcus epidermidis is a common normal flora inhabiting the skin. It has the ability to form potent biofilms on adherent surfaces thereby giving way to catheter related infections and heart valve associated infections as medical devices can get easily contaminated with these bacteria from the skin of hospital staff and visitors. In this study, *S. epidermidis* was isolated from volunteers with healthy skin and who are not associated with hospital setting in Chennai city, India. A high percentage of *S. epidermidis* was obtained.

The intercellular adhesion (*ica*) operon had been associated with strong biofilm production since it encodes proteins necessary for the formation of polysaccharide intercellular adhesin (PIA). [4] It has been reported in previous studies that *ica* operon is detected very rarely in *Staphylococcus epidermidis* isolated from healthy skin of individuals when compared to isolates from patients or individuals associated with catheters or implants. [8],[9],[10] In one study 30% of the samples collected from patients were *ica* positive compared to 8% of the samples collected from healthy individuals. [10] In another study, 53% of the samples isolated from patients were *ica* positive compared to 0% of samples collected from healthy volunteers. [8]

Similarly biofilm production is considered to be less in case of samples collected from healthy skin when compared to those collected from people associated with infections or implants. [11],[12],[13] One study reported that 44.2% of the samples that were collected from patients were strong biofilm-producers compared to 0% of the samples that were isolated from healthy volunteers. [11] Biofilm production is considered to be a virulence marker for *S. epidermidis*. [12]

In our study, it was noted that samples grown in TSB enriched with sucrose showed significantly weaker biofilm formation than those grown in TSB without sucrose. This observation suggests that there might be a relation between the amount of biofilm production and the amount of time the organism was exposed to sucrose. The molecular mechanism behind this reduction in biofilm formation is still unknown and needs further studies.

Out of the 58 samples tested for the ability to form biofilms, 53 samples (91.4%) were capable of producing biofilms. This percentage is higher when compared with the percentages obtained in various studies across the globe [8],[10],[11],[12],[13] in which in some cases it was as low as 8.6% of the samples. [13] This leads to an inference that climatic conditions and environmental factors might play a significant role in the behavior of normal flora and in producing biofilms. The percentage of biofilm producing strains among volunteers with healthy skin from Chennai city is highly alarming. Studies on hospital staff as well as patient by-standers would shed light on the incidence of implant related infections and conjunctivitis. The relevance of this study is very high as a significant number of implants related infections and conjunctivitis are being reported in Chennai city. [14] It is also highly alarming because it has been demonstrated that wound healing can be impaired by the presence of staphylococci biofilms [3] and that is a harmful situation in a hospital setting.

PCR-based assay was also used to screen for prevalence of ica operon among the samples. The presence of ica operon was significantly higher (32.8%) when compared to other works done across the globe. [8],[9],[10],[11],[13] This finding serves as an evidence for the fact that healthy skin harbours a high percentage of ica positive *S. epidermidis* in Chennai population which is an alarming finding since ica operon is associated with strong biofilm formation. The study in a relatively large population may be useful to ascertain the incidence and prevalence of biofilm producing *S. epidermidis* in Chennai city and also the screening for the presence of aap gene in healthy skin isolates will also be useful for such assertion.

When the results obtained from tissue culture plate method and PCR-based assay were analyzed and compared, it was noted that the presence of ica operon is always associated with biofilm formation but the absence of ica operon is not always associated with absence of biofilm formation. In fact, one sample in which ica operon was not detected showed a strong biofilm forming capability. This confirms the fact that multiple genes are involved in biofilm production and ica operon alone is not a crucial cluster for causing an implant associated infection.

~ Conclusion

This study has demonstrated the high prevalence of biofilm-forming *S. epidermidis* strains in the healthy skin of individuals in Chennai city of Tamil Nadu. It has also demonstrated that ica operon can be detected in a significant number of healthy skin isolates in Chennai and thus measures to prevent hospital-acquired *S. epidermidis* infections have to be taken. It was also noted in this study that the presence of ica operon is associated with biofilm formation but the absence of ica operon doesn't necessarily mean the absence of biofilm production.

~ Acknowledgment

Sekar has contributed equally to this work.

~ References

1. O'Gara JP, Humphreys H. Staphylococcus epidermidis biofilms: Importance and implications. J Med Microbiol 2001;50:582-7. Back to cited text no. 1 [PUBMED]
2. Sheehan E, McKenna J, Mulhall KJ, Marks P, McCormack D. Adhesion of Staphylococcus to orthopaedic metals, an in vivo study. J Orthop Res 2004;22:39-43. Back to cited text no. 2

[PUBMED]

3. Schierle CF, De la Garza M, Mustoe TA, Galiano RD. Staphylococcal biofilms impair wound healing by delaying reepithelialization in a murine cutaneous wound model. *Wound Repair Regen* 2009;17:354-9. Back to cited text no. 3

[PUBMED]

4. Ziebuhr W, Hennig S, Eckart M, Kränzler H, Batzilla C, Kozitskaya S. Nosocomial infections by *Staphylococcus epidermidis*: How a commensal bacterium turns into a pathogen. *Int J Antimicrob Agents* 2006;28:S14-20. Back to cited text no. 4

5. Heilmann C, Schweitzer O, Gerke C, Vanittanakom N, Mack D, Götz F. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol Microbiol* 1996;20:1083-91. Back to cited text no. 5

6. Mack D, Davies AP, Harris LG, Rohde H, Horstkotte MA, Knobloch JK. Microbial interactions in *Staphylococcus epidermidis* biofilms. *Anal Bioanal Chem* 2006;387:399-408. Back to cited text no. 6

[PUBMED]

7. de Silva GD, Kantzanou M, Justice A, Massey RC, Wilkinson AR, Day NP, et al. The *ica* operon and biofilm production in coagulase-negative *Staphylococci* associated with carriage and disease in a neonatal intensive care unit. *J Clin Microbiol* 2002;40:382-8. Back to cited text no. 7

[PUBMED]

8. El-Din SS, El-Rehewy MS, Ghazaly MM, Abd-Elhamid MH. Biofilm formation by blood stream *Staphylococcal* isolates from febrile pediatric cancer patients at South Egypt Cancer Institute. *J Am Sci* 2011;7:674-86. Back to cited text no. 8

9. Frebourg NB, Lefebvre S, Baret S, Franc J, Lemeland O. PCR-Based Assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J Clin Microbiol* 2000;38:877-80. Back to cited text no. 9

10. Eftekhari F, Mirmohammadi Z. Evaluation of biofilm production by *Staphylococcus epidermidis* isolates from nosocomial infections and skin of healthy volunteers. *Int J Med Med Sci* 2009;1:438-41. Back to cited text no. 10

11. Mateo M, Maestre JR, Aguilar L, Giménez MJ, Granizo JJ, Prieto J. Strong slime production is a marker of clinical significance in *Staphylococcus epidermidis* isolated from intravascular catheters. *Eur J Clin Microbiol Infect Dis* 2007;27:311-4. Back to cited text no. 11

12. Arciola CR, Baldassarri L, Montanaro L. Presence of *icaA* and *icaD* genes and slime production in a collection of *Staphylococcal* strains from catheter-associated infections. *J Clin Microbiol* 2001;39:2151-6. Back to cited text no. 12

[PUBMED]

13. Galdart JO, Allignet J, Tung HS, Rydén C, El Solh N. Screening for *Staphylococcus epidermidis* markers discriminating between skin-flora strains and those responsible for infections of joint prostheses. *J Infect Dis* 2000;182:351-5. Back to cited text no. 13

14. Murugan K, Usha M, Malathi P, AL-Sohaibani AS, Chandrasekaran M. Biofilm forming multi drug resistant *Staphylococcus* spp. among patients with conjunctivitis. *Pol J Microbiol* 2010;59:233-9. Back to cited text no. 14

[PUBMED]