Effect of growth medium on hydrophobicity of
Staphylococcus epidermidis

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The present study was undertaken to evaluate the effect of growth medium on expression of hydrophobicity of Staphylococcus epidermidis. A total of 24 hydrophobic isolates of S.epidermidis, determined by n-hexadecane adherence assay (HAA) earlier were included. Five different growth media: horse blood agar (HBA), brain heart infusion agar (BHIA), brain heart infusion broth (BHIB), tryptic soy broth (TSB) and proteose peptone broth (PPB) were used. All 24 isolates exhibited the reproducible hydrophobicity when grown on HBA; however, 20 (83.33%), 19 (79.16%), 15 (62.50%) and 13 (54.16%) isolates were found to be hydrophobic when grown in BHIA, BHIB, TSB and PPB, respectively. HBA was found to be the most suitable medium for detection of hydrophobicity of S.epidermidis followed by BHIA or BHIB.

Key words  Cell surface hydrophobicity - growth medium - n-hexadecane adherence assay (HAA) - Staphylococcus epidermidis

Till 1980s the coagulase negative staphylococci (CONS) were not considered as pathogen in clinical cases but as saprophyte being ubiquitous in nature and negative for coagulase production1. The CNS, particularly the Staphylococcus epidermidis has gained clinical importance in recent years specially in surgical cases viz. corneal opacity correction2, thoracic aorta graft3, mediastinitis after cardiac surgery4, penile prostheses5, and breast implant6 and has been recognised as a consequence of nosocomial infection7. Adherence of bacteria followed by colonization onto the susceptible host is one of the essential pre-requisites for establishing pathogenesis8 and hydrophobic interaction has been recognized as one of the determinants to identify the pathogenic strains of S. epidermidis9. Certain environmental factors may greatly influence the expression of cell surface components of bacterial pathogens10 which influence the adhesion onto the susceptible host cell through the hydrophobic cell surface protein interaction between bacteria and host cell. In laboratory diagnosis, optimum expression of surface protein of S.epidermidis relates to its growth in suitable environment, otherwise the test strain may be detected false negative for hydrophobicity. Not much work has been done on this aspect, particularly in Indian context. The present study was undertaken to evaluate the role of growth medium, if any, on determination of hydrophobicity of S.epidermidis isolates.

A total of 24 hydrophobic isolates of S.epidermidis, characterised earlier in our laboratory11 by n-hexadecane adherence assay (HAA) with growth on horse blood agar, were included in this study. The isolates were obtained from both man and animal sources. The human hand and nasal isolates were from healthy milkers and butchers (6), hospital admitted pre-operative patient (2)
and the clinical isolates (4) from the post-operative patients (blood sample between days 5 to 7 post operation). The animal isolates were from healthy sources (8) [raw milk (2), udder surface (3), pre-operative skin (dog) surface (3)], and clinical isolates (blood samples between days 5 to 7) of post-operative dogs (4). These isolates were characterised for slime layer production (in vitro: quantitative microtitration method) and 16 found positive for slime production were from healthy professional (4), pre-operative patients (2), post-operative patient (4), healthy animals (2) and operated dogs (4). Milkers and butchers were employed in the Dairy Farm, Division of Livestock Production and Research, Indian Veterinary Research Institute (IVRI), and Central Avian Research Institute (CARI), Izatnagar, UP.

The hydrophobicity was assessed by the n-hexadecane adherence assay (HAA)\textsuperscript{12}. Briefly, the isolates were cultured in the following media to observe the effect of medium on the expression of cell surface protein associated to hydrophobicity: (i) horse blood agar (HBA) (Hi-media, Mumbai) supplemented with 5% horse blood; (ii) brain-heart-infusion agar (BHIA) (Hi-media); (iii) brain-heart infusion broth (BHIB) (Hi-media); (iv) proteose peptone broth (PPB) (Hi-media); (v) tryptic soy broth (TSB) (Difco, USA). The late exponential phase growth was washed and suspended in phosphate-buffer saline (PBS, pH 7.3) to an absorbance of 1.0 at 540 nm (A\textsubscript{540}) (Spectrophotometer; Systronics, Ahmedabad, India). To 3 ml of this suspension, 0.8 ml n-hexadecane was added and mixed thoroughly for 30 seconds and left at room temperature for 20 min for phase separation. The hexadecane phase was aspirated, and absorbance (A\textsubscript{540}) of the remaining aqueous phase was measured. A\textsubscript{540} of the bacterial suspension without hexadecane was measured for the initial optical density (OD). Samples were run in duplicate and the final adherence value was calculated using the mean. The percentage of bacteria that adhered to n-hexadecane was calculated as follows:

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\text{% of adherence} = \frac{A_{540}(\text{initial O.D.}) - A_{540}(\text{final O.D.})}{A_{540}(\text{initial O.D.})} \times 100
\]

Initial O.D. = A\textsubscript{540} of bacterial suspension without adding hexadecane
Final O.D. = A\textsubscript{540} of bacterial suspension on adding hexadecane

The result was graded as strong positive (++) where adherence was over 75 per cent; intermediate (+), if 25 to 75 per cent and negative, if under 25 per cent.

The isolates found non-autoaggregating were selected for the study. Such screening was done as one drop each of bacterial suspension (in PBS, pH 7.0) was mixed with one drop of 0.15 M sodium chloride solution placed on a clean glass slide and mixed by rocking. The aggregates appear (particulate appeared on slide field) within 2 min was considered as auto-aggregating and excluded from the count. \textit{S. aureus} Cowan I and Wood 46 strains (on courtesy kindly provided by the Department of Microbiology, Maulana Azad Medical College, New Delhi) were employed as positive (hydrophobic) and negative (hydrophilic) controls, respectively.

Of the 24 hydrophobic isolates screened for hydrophobicity in different growth media, 24, 20, 19, 15 and 13 were found to be hydrophobic by HAA with the growth in HBA, BHIA, BHIB, TSB and PPB, respectively. The result demonstrated that the level of hydrophobicity of \textit{S. epidermidis} isolates varied when grown in different media. The result were similar to the findings of previous studies\textsuperscript{13,14} showing difference in hydrophobicity of staphylococci when grown in different media. It was also noted that all isolates exhibited the positivity in HAA when grown on HBA which in turn indicated the 100 per cent reproducibility of the HAA test in determining the hydrophobicity of \textit{S. epidermidis} if grown in HBA. On the contrary, 83.33, 79.16, 62.50 and 54.16 per cent isolates were detected to be hydrophobic when grown in BHIA, BHIB, TSB and PSB, respectively. It revealed that hydrophobicity could be better expressed when the bacteria were allowed to grow in blood based agar medium. Our finding corroborated the observation of Galliani \textit{et al}\textsuperscript{15} who observed the highest hydrophobicity of \textit{S.aureus} by HAA when the cultures were grown in blood agar. However, the present results were different from that of Mamo \textit{et al}\textsuperscript{16} who did not find any hydrophobic strains of \textit{S. epidermidis} when tested by salt aggregation test (SAT). This could be due to difference in sensitivity of the test employed and heterogeneity of strains tested as in the earlier study\textsuperscript{16} wherein \textit{S.epidermidis} strains of bovine mastitis origin were tested by the SAT.

The hydrophobicity status of the isolates remained almost the same when grown in BHIA and BHIB, with exception of one isolate from healthy professionals (milker and butcher) failed to exhibit the hydrophobicity when grown in BHIB. It seems that state of the BHI medium (\textit{i.e.} agar or broth) does not matter significantly.
for growth and expression of hydrophobicity of S. epidermidis. The result suggested that next to the blood agar, either BHIA or BHB may be used as a growth medium for determining hydrophobicity of S. epidermidis that reaffirm the observation of Mamo et al. It was observed that 15 (62.50%) and 13 (54.16%) isolates were hydrophobic when grown in TSB and PPB, respectively which was relatively low in comparison to BHIA. The TSB is the standard medium used for growth towards detection of slime layer (psuedocapsule) of S. epidermidis and stimulation of slime layer production in TSB may have the inhibitory effect on expression of the surface proteins responsible for hydrophobicity. Similar findings were reported earlier by Hegt et al. who explained that TSB was the ideal medium for production of slime layer with S. epidermidis but not suitable for expression of cell surface hydrophobicity. Mamo et al. also found that staphylococci grown in PPB or TSB possessed relatively weak hydrophobic cell surface. In conclusion, the results of this study suggested that among the growth media used, HBA was the most suitable medium for expression of hydrophobicity of S. epidermidis followed by BHIA or BHB, TSB and PPB were not to be recommended for the purpose.

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References


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