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ADHERENCE CAPABILITIES OF BACTERIAL ISOLATES FROM HUMAN CONJUNCTIVITIS

E. A. OPHORI, M. ADU and C. ISIBOR

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ABSTRACT

The adherence capabilities of *Staphylococcus aureus, Streptococcus pneumoniae,Pseudomonas aeruginosa* and *Haemophilus influenzae* to liquid hydrocarbons and polystyrenes were studied as a means of assessing the cell surface hydrophobicity of these bacteria to the human conjunctiva. The bacteria were suspended in phosphate urea magnesium (PUM) and various volumes of the hydrocarbons were added to the cells suspension and the absorbance of the lower aqueous phase was read at 400nm to determine the adherence to liquid hydrocarbons. Adherence to polystyrene was by the replica method of screening for bacteria hydrophobicity. Results showed that the bacteria adherence to hydrocarbons and the level of hydrophobicity varied with the organism. The percentage mean adherence for *Staphylococcus aureus* ranged from 2.5-3.5%, that of *P.aeruginosa* was between 4-15%,*Streptococcus pneumoniae* 2.0-8.4% and *Haemophilus influenzae* was 1.7-12.6% to the liquid hydrocarbons used. There was no correlation between the two methods. *S. aureus* and *P.aeruginosa* adhered well to polystyrene while *H.influenzae* and *S.pnuemoniae* adherence was poor. Adherence was generally poor in all cases, therefore hydrophobicity may not possibly play a significant role in adherence to the conjunctiva

KEYWORDS: Hydrophobicity, polystyrene, conjunctivitis, Bacteria, cell surface .

INTRODUCTION

In any environment, bacterial infections still constitute one of the commonest causes of ill health. Adherence of microorganisms to surfaces like hydrocarbons, polystyrene, solid surfaces, eyes, hydrophobic contact lenses have been reported to have enormous clinical implications (Bitton and Marshall,1990).*Staphylococcus aureus, Streptococcus pnuemoniae,Pseudomonas aeruginosa* and *Haemophilus influenzae* have been implicated as causes of many ocular diseases (Rosental *et al.*1995;Moshe 2003)Adherence of the bacteria to an epithelia surface is accepted to be one of the essential steps in the establishment of an infection and it is affected by several factors including strain of bacteria, species of host, physiological state and developmental stage of host cases(Miller and Ahearn1987;Costerton *et al.*1978).

Hydrophobic interactions have been implicated in adherence of bacteria to phagocytes and to also play a role in the adherence of bacteria to a wide variety of surfaces and cell types (Absolom,1988) .The pronounced specificity of some bacteria that attack only a particular host tissue may well be explained by the specificity of the glycocalyx of the host tissue(Johnson *et al.*1980)

Cell surface hydrophobicity of bacterial isolates such as *Staphylococcus aureus, Enterobacter faecalis, Proteus spp.* from wound infections has been reported(Enabulele *et al.*1998).*Staphylococcus aureus* had the highest percentage of adherent cells with a maximum of 26% for xylene and a minimum of 5% for n-hexane. This study was designed to determine the adherence capabilities of bacteria isolates from human conjunctiva to liquid hydrocarbons.

MATERIALS AND METHODS

Sample Source

The samples for bacterial analysis were obtained from patients with conjunctivitis and identified as previously described(Cowan and Steel,1974).They were stored in slants of Nutrient Agar (Oxoid) at 4°C The organisms included *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenzae* and *Pseudomonas aeruginosa*

BINDING TESTS

The test hydrocarbons adherence assayed were benzene, octane and pentane.

GROWTH AND PREPARATION OF CELL SUSPENSION

Each bacterial isolate was inoculated into 50ml of nutrient broth in 100ml Erlenmeyer flask, supplemented with 0.85% NaCl. The cells were grown at 37°C for 24 hours at the end of which cells were harvested by centrifugation, washed twice and suspended in phosphate urea magnesium (PUM) buffer (pH 7.1) and absorbance read at 400nm using Spectronic 21D Milton ray spectrophotometer.

BINDING ASSAY TO LIQUID HYDROCARBONS

The optical density of the cell suspension at 400nm was adjusted to between 1.4-1.5. To 4.0ml of the cell suspension was added different volumes of 0.1 ml 0.2ml 0.3ml 0.4ml 0.5ml of the test hydrocarbons and shaken for 120 seconds to mixe the phases. After phase separation, the optical density (Light absorbance) of the lower aqueous phase was measured at 400nm and compared with that of the cell suspension (Rosenberg.1982)

ADHERENCE TO POLYSTYRENE

A flat polystyrene disc was pressed into the surface of an agar plate containing colonies to be screened as previously described (Rosenberg 1981). The sterile disc was 80mm in diameter cut from the standard polystyrene. The replica of the colonies obtained on the polystyrene surface was then washed for 2 minutes under a vigorous stream of tap water to remove all cells, which were not firmly bound. At this stage, translucent areas corresponding to colonies of adherent cells were observed on polystyrene surface. Microscopic

E. A. OPHORI, Department of Microbiology, Faculty of Science, University of Benin, P. M. B. 1154, Benin City, Nigeria. E-mail *eaophori@yahoo.com
M. ADU, Department of Microbiology, Faculty of Science, University of Benin, P. M. B. 1154, Benin City, Nigeria
C. ISIBOR, Department of Medical Microbiology, Central Hospital, Agbor Delta State Nigeria

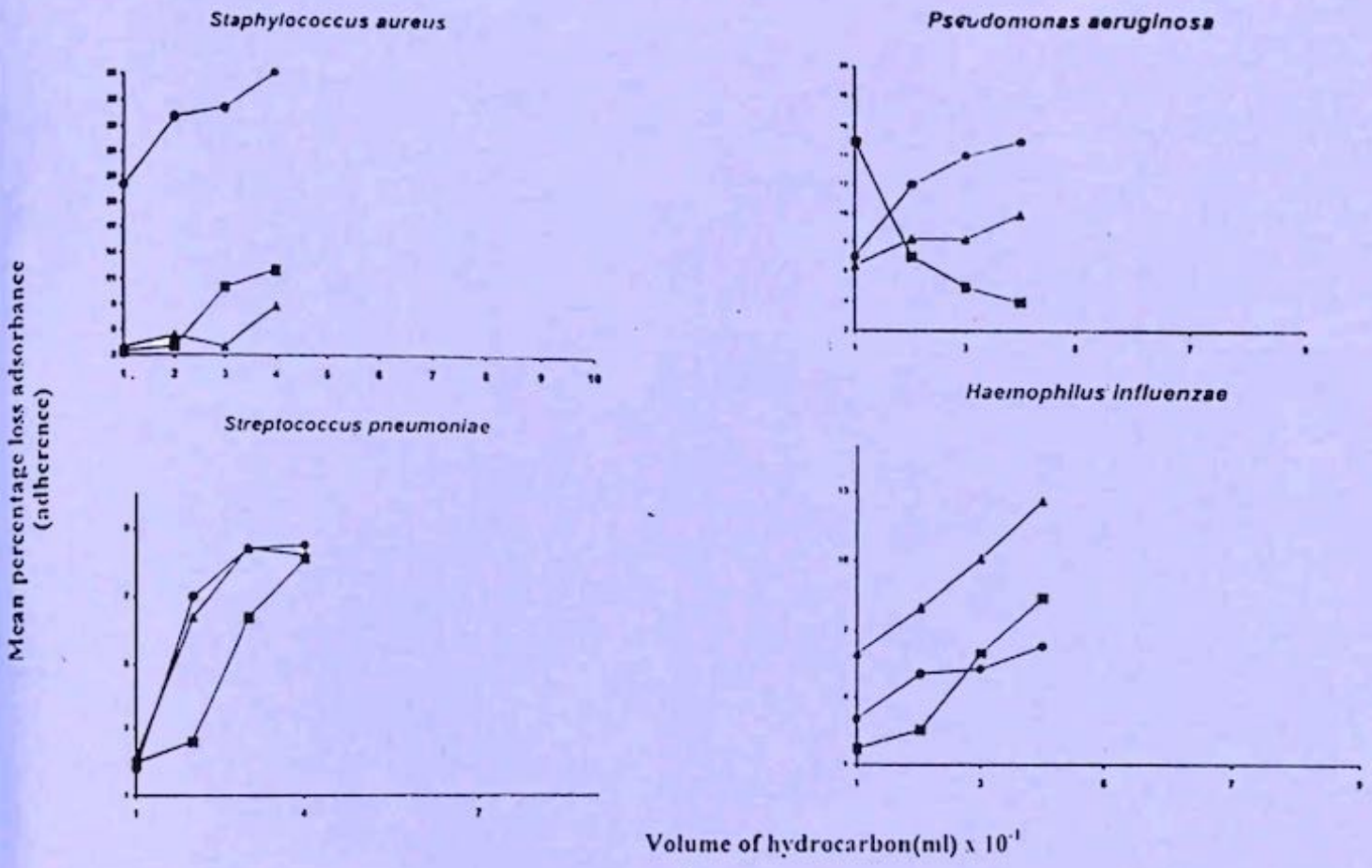

Fig 1: Adherence of isolates to liquid hydrocarbons

observation of these spots revealed a dense layer of attached cells. Visualization and comparison were enhanced by dipping the replica in methanol and staining with gentian violet. This was then viewed under the microscope (x40mm) and the average number of cells adhered per microscopic field was counted.

RESULTS

Fig 1.shows the adherence of bacterial isolates to different liquid hydrocarbons. The level of adherence varied with the organism. The adherence capability of *S.aureus*, *S. pnuemoniae* and *H. influenzae* increased with volume while that of *P.aeruginosa* varied with increase in volume. The percentage adherence of *S. aureus* was higher in pentane than in benzene and octane(2.5-12% in benzene,3.0-7.8% in octane and 22-35% in pentane).On the other hand, adherence capability of *H. influenzae* was higher in octane than in benzene and pentane(6.0-12.6%,1.7-8.4% and 3.0-6.3% respectively)

Table 1: Adherence of Bacterial isolates from conjunctiva to polystyrene

Organisms	No.of isolates	No. of styrene	isolates (%)	adherent	to	Poly-
		++++	+++	++	+	-
S.aureus	22	8(36.4)	6(27.3)	4(18.2)	2(9.1)	2(9.1)
P.aeruginosa	14	5(35.7)	3(21.4)	3(21.4)	2(14.3)	1(7.1)
S.pneumoniae	10	-	1(10.0)	2(20.0)	3(30.0)	4(40.0)
H.influenzae	6	-	-	1(16.6)	3(50.0)	2(33.4)

++++ = Microscope field over crowded
+++ > 300 cells per field
++ = Between 100 and 300 cells per field
+ = 100 cells per field
- < 10 cells per field

Table 1 shows the adherence of bacterial isolates to polystyrene. *S.aureus* and *P.aeruginosa* adhered well to polystyrene disc by forming a characteristic ring on the disc while the adherence of *H.influenzae* and *S.pnuemoniae* were poor.

DISCUSSION

The adherence capability of the isolates from human conjunctiva to liquid hydrocarbon was quite low. This is in contrast to previous report (Ahamioje ,1989) where it was shown that the maximum percentage adherence of clinical strains of *S. aureus* to liquid benzene and that of non-clinical isolates were 13% and 10.7% respectively, while its percentage adherence to liquid pentane were 30.8% and 16.5% for clinical and non-clinical isolates respectively. For *P. aeruginosa* its maximum percentage adherence values in liquid benzene and pentane were 24.07% and 49% respectively. These differences may be explained by the fact that the degree of attachment to various substrates is dependent on an interaction to multiple factors including strain, nature of the substrate, pH, electrolyte concentration, time and ionic charge of the hydrogen polymer as previously reported (Miller and Ahearn,1987). Adherence of *S aureus, S. pneumoniae and H influenzae* to pentane was higher than that of benzene and octane like-wise for that obtained for *P. aeruginosa*. This indicated that the organisms have greater affinity for liquid pentane than for benzene and octane.

Previous reports showed that while *P.aeruginosa* adhered well to polystyrene it exhibited very low adherence to liquid hydrocarbons (Miller and Ahearn, 1987;Enabulele *et al* 1998). These reports are in line with the findings obtained in this study in which *P.aeruginosa* adhered well to polystyrene but poorly to hydrocarbon and is consistent with the fact that attachment of organisms to various substrata is dependent on the interactions of multiple factors including strains and nature of substratum (Miller and Ahearn, 1987).

We conclude that cell surface hydrophobicity may not play a significant role in the adherence of bacteria to the conjunctiva. This is consistent with the observation that adherence of microbes to host surfaces is highly specific (Conway and Ronald, 1988). We therefore recommend that further studies be carried out to know the relevance of adherence in the pathogenicity of these organisms in conjunctivitis.

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