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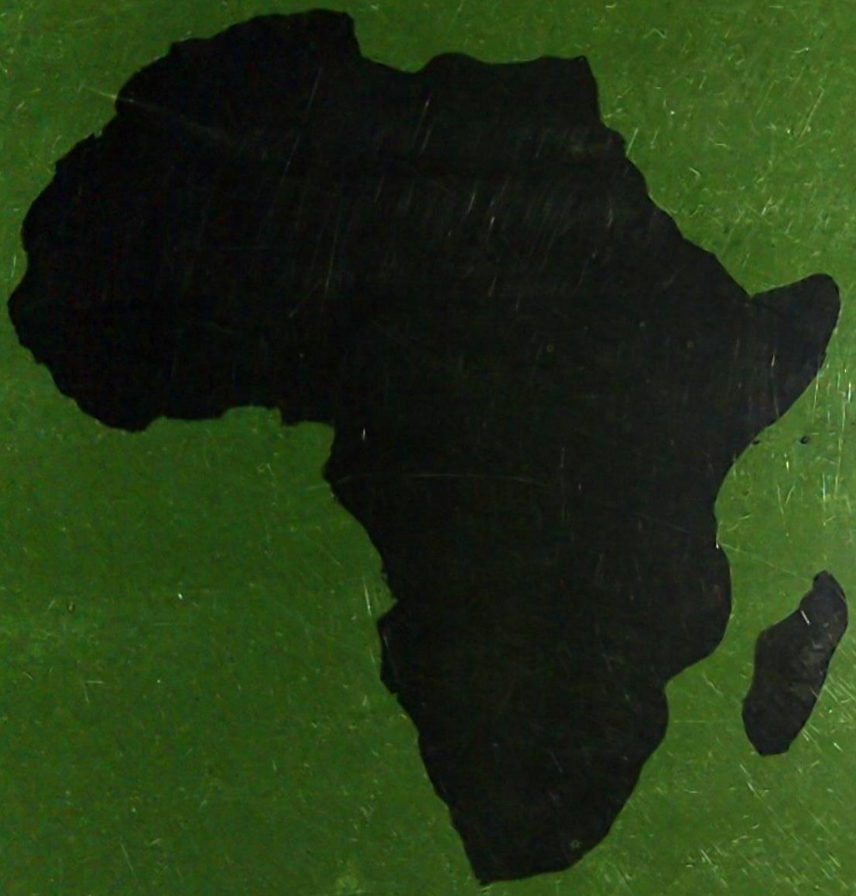

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Adherence of *Staphylococcus aureus* isolated from urine to medical prostheses and glass

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Summary

The physicochemical surface property of two *Staphylococcus aureus* isolates obtained from urine (isolates B and C) and a standard strain ATCC 13709 (isolate A) were compared. Isolate B was the most hydrophobic while isolate A was least hydrophobic as determined by bacterial adherence to hydrocarbon (BATH). Isolate A was most adherent to silicone urinary catheter and least adherent to both glass and intravenous catheter placement unit. Isolate B was most adherent to glass while isolate C was most adherent to the intravenous catheter. The clinical isolates B and C were generally more adherent and more hydrophobic than the standard strain ATCC 13709. There was no direct correlation between hydrophobicity of isolates and their adherence to biomedical materials and glass in this work.

Keywords: Staphylococcus aureus, *adherence, medical prostheses.*

Résumé

La propriètè physicochimique superficielle de deux isolates de *Staphyloccoque aureus* obtenu des urines (isolates B et C) et un isolate standard ATCC 13709 (isolate A) etaient compares. L'adhèrence bactèrienne a l'hydrocarbon(BATH) montrait que l'isolate B ètait le plus hydrophobique alorsque l'isolate A ètait le moins hydrophobique . L isolate A le plus adhèrent a la sonde urinaire en silicon et moins adherent au verre et l'unitè de sonde placèe intraveneux. L'isolate B ètait plus adhèrent au verre qu'a l isolate C qui ètait plus adhèrent a la sonde intraveneux. Les isolates cliniques B et C ètaient genèrallement plus aadhèrent et plus hyddrophobique que le standard ATCC 13709. Cette ètude montrait qu'il n' y avait pas de correlation entre l'hyddrophobicitè des isolates et leur adhèrence aux matières biomèdicaux et au verre.

Introduction

Adhesion of bacteria to surfaces may be an important initial event in the pathogenesis of infectious diseases [1, 2] and adherent bacteria are often less sensitive to natural host defenses [3] and to antibiotics [4] than are bacteria suspended in body fluids. Bacteria that adhere to prostheses may serve as foci of infection [5]. Cell surface proteins in *Staphylococcus aureus* act as receptors for adhesion proteins of eukaryotes such as fibronectin, fibrinogen, laminin and collagen [6]. This binding to adhesion proteins represents a mechanical bacterial attachment to tissues [7]. Infecting bacteria are often surface-associated and the cell surface proteins expressed under specific conditions can therefore be expected to be more similar to those of bacteria grown on a solid surface than to those found in organisms grown in a liquid medium. The various surface proteins expressed are responsible for the hydrophobicity of the cell-surface and thus an important factor in the adhesion and proliferation of microorganisms on solid surfaces [8,9], including non-wettable plastics [10] and hydrocarbons [11]. *Staphylococcus aureus* strains isolated from human septicaemia, wound and UTI have been known to show very high cell-surface hydrophobicity [12], and do not have an enhanced ability to produce hydrophilic cell-surface via capsule production [13]. The hydrophobic interaction between a hydrophobic cell-surface and specific host target has been utilized in demonstrating that hydrophobised wound dressings bind staphylococci and speed up wound healing in experimental skin infections in young pigs injected with *Staphylococcus aureus* [14]. Studies on bacterial cell-hydrophobicity and adhesion are of importance from many different angles. In this study, the cell-surface hydrophobicity of two *Staphylococcus aureus* isolates from urine and a standard culture ATCC 13709, was compared with their adhesion to medical prostheses and glass.

Materials and methods

Bacteriology

Two *Staphylococcus aureus* isolates from urine of suspected UTI patients were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria. They were a methicillin-resistant *Staphylococcus aureus* (MRSA), isolate C; a methicillin-sensitive *Staphylococcus aureus* (MSSA) isolate B and ATCC 13709, isolate A obtained from the National Institute of Pharmaceutical Research and Development, Abuja, Nigeria.

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Bacterial adherence to hydrocarbon

The cell-surface hydrophobicity of the isolates was determined by bacterial adherence to hydrocarbon (BATH) as described by Rosenberg *et al.* and Rosenberg [15, 16]. The isolates were grown in 30 ml of Nutrient broth (NB) in a shaker bath at 37 °C and 120 rpm. Cells were harvested in the early stationary phase. Harvested cells were washed twice in phosphate-urea-magnesium buffer (PUM) at pH 7.1 (22.8 g $K_2HPO_4.3H_2O$, 7.26 g KH_2PO_4, 1.8 g urea, and 0.2 g $MgSO_4.7H_2O$, and distilled water to 100 ml). The washed, harvested cells were re-suspended in PUM buffer to an optical density (OD_{470}) of 1.0, and then vortexed with various volumes (0.2, 0.4 and 0.6ml) of hydrocarbon. The OD_{470} of the aqueous phase expressed as a percentage of

$$H.I. = \frac{O.D_{aq.}}{O.D_{cul.}} \times 100\%$$

the initial population in the aqueous phase before mixing was determined as the hydrophobicity index (H.I).

$O.D_{aq}$ = Optical density of the aqueous phase after mixing with organic solvent.

$O.D_{cul.}$ = Optical density of the culture before contact with organic solvent.

Measurement of adhesion

The biomaterials, urinary catheter (silicone, 10 x 8 x 4 mm), intravenous catheter placement unit (Angiocath®, 27 x 10 mm) and glass slide (10 x 20 mm) were sterilized together with the nutrient broth. The isolates were then inoculated into the flask and grown in a chemostated shaker bath at 37 °C and 120 rpm for 18 hr. The biomaterials and glass were then transferred aseptically into sterile normal saline and rinsed to remove loosely attached cells.

The pieces of the biomaterials and glass were then placed individually in universal bottles containing 9.9 ml normal saline. Each piece was then shaken vigorously on a vortex mixer for 2 min. to dislodge adhering cells. The dislodged cells were then counted on Nutrient agar (NA) plates after appropriate dilution had been incubated at 37°C for 18 hr. The percentage of cells adhering to the biosurfaces were determined for each biomaterial and glass as that fraction of the population in the medium without inserted biomaterial or glass.

Results and discussion

Isolate B was the most hydrophobic while isolate A was least hydrophobic (Fig.1). The differences in the hydrophobicity of the isolates may be due to the quantitative difference in the various surface components that affect the cell-surface hydrophobicity [17].

Capsular and lipopolysaccharide antigens also influence hydrophobicity [18]. The quantity and type of proteins expressed may have contributed to the observed differences in the degree of hydrophobicity in the three isolates [19].

There were marked differences in the adhesion of the three isolates as demonstrated in Fig. 2, which shows isolate A to be most adherent to silicone urinary catheter and least adherent to both glass and intravenous catheter compared with the other two isolates. Isolate B was most adherent to glass but showed considerably high adherence to both the urinary and intravenous catheters. Isolate C was most adherent to the intravenous catheter placement unit, but showed considerably low adherence to both glass and the silicone urinary catheter (lowest).

The ability of a bacterium to adhere to epithelial cells is commonly considered the first important step in the development of mucosal infections. [1] Adhesion to an inert surface has been used as a model for adherence to tissues [20]. This result is thus indicative of what is likely to take place when the various isolates come in contact with such materials in a clinical setting. The accumulation of bacteria on a surface is the net result of several factors [21], of which hydrophobic interaction has often been regarded as being of prime importance [17, 22].

Data from the work of Ofek et al. [23], on the hydrophobic interaction of Group A *Streptococci* with hexadecane droplets suggest that adherence to hydrocarbon measures the availability on the surface of the bacterial cells of lipophilic residues that are either hydrophobic regions of surface protein structures or more likely glycolipids complexed with and oriented by surface proteins. Binding of bacteria to increasingly hydrophobic surfaces has been demonstrated [24]. The general tendency of proteins to bind to increasingly hydrophobic surfaces has been reported [25] and this raises the possibility of bacterial surface proteins being responsible for hydrophobic attachment. The possible implications of the higher adhesion and hydrophobicity of the clinical isolates than the standard strain on the possible outcome of treatment of infections caused by the clinical stains is grave. Bacterial strains adhering to PVC catheters have been shown to survive exposure to cidal concentrations of drugs to which they were ordinarily sensitive when not attached [26].

Bacterial strains with high adhesion to surfaces of indwelling devices like catheters, intravascular lines, venticuloperitoneal shunts and nasogastric tubes are more likely to cause persistent infections due either to the fact that they are not readily dislodged and are therefore not accessible to antimicrobial agents. They thereby act as foci for re-infection and reservoir of resistant strains [27, 28]. This situation often lead to higher treatment cost and longer stays in the hospital [27].

The generally high adhesion of clinical isolates B and C and their higher hydrophobicity than the standard isolate

A suggests that there are surface structures in the clinical isolates that promoted adherence. There was however no direct correlation between hydrophobicity and adhesion in this present work. This might be related to the observations made when the adhesive role of specific *Staphylococcus aureus* surface proteins (protein A and clumping factor) to the silicone polymer used for manufacture of cerebrospinal fluid shunting systems were investigated [29]. The two proteins were judged to contribute non-specifically to adhesion. *Staphylococcus aureus* was also shown to be capable of hydrophobic binding, but this was found to be distinct from the demonstrated protein-mediated adhesion.

The overall results in this work show a significant difference in the surface hydrophobicity of the three isolates that is not substantially correlated by observed differences in their adhesion to medical biomaterials and glass.

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