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Cell-surface hydrophobicity of *Staphylococcus saprophyticus*

P. F. SCHNEIDER[1] AND T. V. RILEY[2*]

[1] *Western Diagnostic Pathology, Myaree, Western Australia 6154*
[2] *Department of Microbiology, University of Western Australia and Sir Charles Gairdner Hospital, The Queen Elizabeth II Medical Centre, Nedlands, Western Australia 6009*

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SUMMARY

The cell-surface hydrophobicity of 100 urinary isolates of *Staphylococcus saprophyticus*, cultured from symptomatic females in the general population, was assessed using a two-phase aqueous: hydrocarbon system. Relatively strong cell-surface hydrophobicity was exhibited by 79 isolates using the criteria employed, while only 2 of the remaining 21 isolates failed to demonstrate any detectable hydrophobicity. Cell-surface hydrophobicity may be a virulence factor of *S. saprophyticus*, important in adherence of the organism to uroepithelia. Additionally, the data support the concept that cell-surface hydrophobicity may be a useful predictor of clinical significance of coagulase-negative staphylococci isolated from clinical sources.

INTRODUCTION

Staphylococcus saprophyticus is recognized in most communities as the second most frequent cause of urinary tract infection (UTI) in non-hospitalized females of child-bearing age [1]. It is seldom implicated in UTI outside this group. The reason(s) for this apparent host specificity remain unclear, but it may be surmised that yet to be defined host factor(s) interact with virulence factors of *S. saprophyticus* to culminate in UTI.

Little is known about the virulence factors of *S. saprophyticus*. Unlike *S. aureus*, it does not appear to produce endonucleases, coagulase, deoxyribonuclease or phosphatases [2]. Direct heamagglutination of sheep erythrocytes occurs but its role in virulence is unclear [3]. Slime production, which may be important in the inhibition of T-cell-mediated defence function [4], adherence [5] and protection from antibiotic activity [6], has been observed in *S. saprophyticus*.

S. saprophyticus is associated with extra-urinary tract infection rarely, possibly because *S. saprophyticus* demonstrates a higher level of adherence to uroepithelia than other types of epithelia [7, 8], Consequently the adherence potential of *S. saprophyticus* may be one important factor in the pathogenesis of UTI caused by this organism.

Cell-surface hydrophobicity has been extensively studied in relation to bacterial adherence to surfaces [9, 10]. Hydrophobic interactions between bacterial cells

* Author for correspondence.

and mammalian cells promote adherence [11–13] and these forces may therefore play an important role in the attachment of *S. saprophyticus* to uroepithelium.

Furthermore, recent studies have suggested that cell-surface hydrophobicity, as determined by the degree of partitioning in a two-phase aqueous:hydrocarbon system, may be a marker for clinical significance of coagulase-negative staphylococci (CNS). It has been postulated that this test alone, or in conjunction with other tests (species identification and slime production) enables prediction of the clinical significance of CNS isolated from various clinical sources [14].

The purpose of the present study was to investigate cell-surface hydrophobicity in 100 urinary isolates of *S. saprophyticus*.

MATERIALS AND METHODS

Isolates

All strains of *S. saprophyticus* were isolated from urine specimens, from symptomatic female patients, submitted to a large private pathology laboratory serving the general community. All catalase-positive, coagulase-negative, Gram-positive cocci were initially screened for novobiocin (5 μg disk) susceptibility [15] and later identified using the classification scheme of Kloos and Schleifer [16], as incorporated in the API STAPH micromethod.

Isolates were stored frozen at −10 °C in tryptic soy broth (TSB) (Oxoid) with 10% glycerol and cultured on blood agar plates (Oxoid) overnight at 37 °C when required. A total of 100 strains of *S. saprophyticus* was available for testing.

Hydrophobicity test

The cell-surface hydrophobicity test was performed as reported by Martin and colleagues [14]. Briefly, 10 ml of TSB in glass tubes was inoculated with the test organism and incubated for 18–24 h. Following centrifugation of the broth culture to pellet the cells, the TSB was decanted and the cell pellet washed twice with sterile saline (0·85%). The pellets were finally resuspended in saline to an optical density (OD) of 0·3 at 600 nm.

Once the OD of each suspension was recorded ($OD_{initial}$), 0.25 ml of toluene was added. The tubes were shaken at 37 °C on a rotary mixer at 400 rpm for 15 min and allowed to equilibrate at room temperature for 10 min. The lower aqueous phase was aspirated and the OD determined (OD_{final}).

The hydrophobicity index (HPBI) was calculated as:

$$\text{HPBI} = \frac{OD_{initial} - OD_{final}}{OD_{initial}} \times 100\,\%.$$

Isolates with a hydrophobicity index greater than 70% were arbitrarily classified as hydrophobic. This is in accord with the cut-off chosen by Martin and colleagues [14], using a similar technique with CNS isolated from various sources.

Each strain was tested in duplicate and a control strain was included with each run. No significant variation of HPBI of the control was noted throughout testing.

RESULTS

The HPBIs of 100 clinical isolates of *S. saprophyticus* are shown graphically in Fig. 1. The HPBIs ranged from 0–99% and approximated a bimodal distribution.

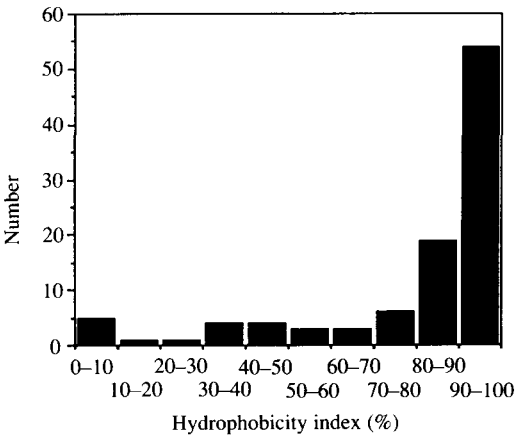

Fig. 1. Cell-surface hydrophobicity of 100 isolates of *S. saprophyticus* measured using a two-phase aqueous:hydrocarbon system.

Seventy-nine of the *S. saprophyticus* isolates gave a HPBI of 70–100% indicating relatively strong cell-surface hydrophobic forces. Six of the isolates tested were found to be relatively hydrophilic (HPBI 0–20%). Two strains only did not exhibit any detectable hydrophobic attractions.

DISCUSSION

Relatively little is known regarding the pathogenesis of UTI due to *S. saprophyticus*. Unlike *S. epidermidis*, an opportunistic urinary tract pathogen with a low pathogenic potential, *S. saprophyticus* is considered to be a primary urinary pathogen [1] and in this respect is comparable to *Escherichia coli*. In studies with *E. coli* (and other uropathogens), adherence to uroepithelium was a central factor in the pathogenesis of UTI [17, 18]. Strains of *E. coli* considered pathogenic displayed a higher adhesive ability to uroepithelium than to epithelia from other body sites [7, 8]. Furthermore, in recent studies utilizing cell culture monolayers, *S. saprophyticus* displayed greater cell-surface adherence that *S. epidermidis* [19, 20] perhaps, in part, accounting for the relatively low frequency of UTI due to *S. epidermidis* in patients not predisposed by implementation of a foreign device or a urinary tract lesion. Adherence therefore seems an important virulence factor in the pathogenesis of UTI due to *S. saprophyticus*.

Hydrophobic forces are believed to be important in the initial events leading to irreversible adherence of bacteria to a surface [21]. A recent study also suggested that hydrophobic groups, or cell wall constituents associated with these groups, may interfere with opsonic activity of fixed IgG molecules and thus afford a means of protection from the host's immune response [22]. The role of this phenomenon in recurrent infections is unclear.

The majority of *S. saprophyticus* strains tested in the present study displayed a high level of cell-surface hydrophobicity indicating that hydrophobic forces may indeed play an important role in adherence of *S. saprophyticus*. Of the 21 clinical isolates classified as non-hydrophobic, only 2 did not exhibit detectable hydrophobicity (HPBI = 0%). Five other strains exhibited a HPBI of less than

10%; the remainder of the 'non-hydrophobic' strains [7] exhibited HPBIs between 10–70%. The level of hydrophobicity required to sufficiently promote adherence is unknown however, and may vary depending on the nature of the surface.

Several binding mechanisms may be involved in the irreversible attachment of *S. saprophyticus* to uroepithelium. *S. saprophyticus* causes agglutination of sheep erythrocytes [3] but the significance of this is unclear and, recently, fimbriae-like structures were observed [23]. Due to the relatively narrow target group apparently susceptible to *S. saprophyticus* UTI, it is possible that specific host factors may also be important in the development of UTI. Cell-surface receptors on human epithelial cells are altered during differentiation [24]. Alteration of cell-surface receptors for *S. saprophyticus*, by physiological or other means, may be one possible consideration to explain this predilection.

Cell-surface hydrophobicity of bacteria is generally regarded as being an important virulence factor in urinary pathogens, and recently in CNS [14]. In one study, 71% of *E. coli* isolated from patients with UTI were found to be hydrophobic [25] as were 85% of proteus, 78% of klebsiella and 92% of enterobacter [26].

It is difficult to determine from our findings whether the majority of isolates were hydrophobic because they were urinary pathogens or because they were *S. saprophyticus*. Ideally, strains of *S. saprophyticus* from extra-urinary sites should have been tested also, however, there is no recognized reservoir for *S. saprophyticus*. Some reports suggested that *S. saprophyticus* can be found on the skin in the peri-urethral area although this is not a general finding [1]. The rare nature of extra-urinary tract infections with *S. saprophyticus* only adds to the difficulties of investigating the pathogenesis of *S. saprophyticus* UTI.

Martin and colleagues [14] explored the relationship between cell-surface hydrophobicity and significance in clinical isolates of CNS. They concluded that isolates of CNS displaying relatively high hydrophobicity (greater than 70%) could be considered clinically significant (predictive value 79%). With regard to *S. saprophyticus*, it would be reasonable to expect that cell-surface hydrophobicity was prominently expressed. In the present study, 79% of *S. saprophyticus* displayed strong hydrophobic tendencies when tested by the two-phase aqueous:hydrocarbon system. Hydrophobic interactions may therefore play an important role in the adherence of *S. saprophyticus* to uroepithelium and may contribute to its virulence. Finally, the results obtained here support the view that cell-surface hydrophobicity may be a useful predictor of clinical significance in CNS.

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