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In vitro adherence of *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus aureus* to human ureter

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Summary. *Staphylococcus saprophyticus* adhered to human ureteral epithelium in vitro. The levels of adherence, which were determined quantitatively with the scanning electron microscope, correlated well with bacterial hemagglutinating activities with sheep erythrocytes ($r = 0.9459$, $P < 0.01$). Transmission electron microscopy revealed that the adhering bacteria and the hemagglutinating bacteria possessed similar pili-like structures on their cell surfaces. *Staphylococcus epidermidis, Staphylococcus haemolyticus,* and *Staphylococcus aureus* did not adhere to the epithelium. Only *S. aureus* adhered markedly to the connective tissue of the ureter, and adhesion of this organism was direct via its cell wall. This adherence test system clearly showed up differences in the abilities of these staphylococcal species to adhere to the urinary tract.

Key words: Bacterial adherence – Urinary tract – *Staphylococcus saprophyticus* – *Staphylococcus epidermidis* – *Staphylococcus haemolyticus* – *Staphylococcus aureus*

Bacterial adherence to the urinary mucosa is an important initial event in the establishment of urinary tract infections (UTI) [2]. Most uropathogenic *Escherichia coli* possess P or type 1 pili, which mediate the bacterial adherence [19]. After *E. coli, Staphylococcus saprophyticus* is the second most frequent urinary isolate from uncomplicated UTI [10, 11, 20]. *S. saprophyticus* agglutinates sheep erythrocytes [9], adheres to urinary exfoliated cells [3, 12] and cultured human cancer cells [1], and causes UTI in animal models [6, 16]. However, the manner of adherence of *S. saprophyticus* to the human urinary mucosa is unclear. The surface of the human urinary mucosa consists mainly of mature epithelium (umbrella cells), which has microfolds. On the other hand, some parts of the surface consist of young epithelium, which has microvilli [13]. Previously, we used human ureter excised from patients with renal cell carcinoma and demonstrated that P-piliated *E. coli* do not adhere to the mature epithelium but do adhere to the young epithelium, and that type 1-piliated *E. coli* adhere to both types of epithelium in vitro [4, 5]. In this study, the adherence of *S. saprophyticus* to the ureter was demonstrated in the same model system. The correlation between the hemagglutinating activities and the levels of adherence to the ureteral epithelium was analyzed quantitatively. The adherence of *Staphylococcus epidermidis, Staphylococcus haemolyticus* and *Staphylococcus aureus* were compared with that of *S. saprophyticus.*

Materials and methods

Thirteen strains of *S. saprophyticus* were isolated from cases of uncomplicated UTI. Six strains of *S. epidermidis,* three strains of *S. haemolyticus* and five strains of *S. aureus* were isolated from cases of complicated UTI. The species were identified by the method of Kloos and Schleifer [14]. The reference strains used were American Type Culture Collection (ATCC) 15305 *(S. saprophyticus),* ATCC 12228 *(S. epidermidis),* ATCC 29970 *(S. haemolyticus)* and ATCC 12598 *(S. aureus).* The bacteria were cultured in nutrient broth (Eiken, Tokyo, Japan) for 48 h at 37°C without agitation.

Hemagglutination (HA) and adherence tests were performed as described previously [5]. Briefly, cultured bacteria were suspended in phosphate-buffered saline (PBS) at pH 7.4. The turbidity of the bacterial suspension was adjusted to 300 Klett units (measured in a Klett-Summerson colorimeter with a red filter) for HA tests, and to 800 Klett units for adherence tests.

To determine the HA titer, two-fold serial dilutions of the bacterial suspension were made, and 100-μl aliquots were each mixed with 100 μl of 3% (vol./vol.) sheep erythrocytes in 24-well tissue culture plates (A/S Nunc, Roskilde, Denmark) for 20 min at room temperature (approx. 22°C). The HA titer was expressed as the highest dilution giving visible HA.

For adherence tests, human ureters were obtained from ten patients with renal cell carcinoma. They were cut into pieces (1 cm^2) and immersed in 1.5 ml bacterial suspension. After incubation for 10 min at 28°C, the specimens were washed in PBS (pH 7.4) and fixed in 2.5% (vol./vol.) glutaraldehyde in PBS (pH 7.4) for 2 h at 4°C.

For scanning electron microscopy (SEM), the ureteral specimens were treated by the same procedure as described previously [5] and analyzed using a Hitachi S-800 scanning electron microscope. To determine the levels of bacterial adherence, 30 electron microscopic fields obtained at ×4000 (23×28 μm) were randomly chosen and photographed. The average number of adherent bacteria per electron microscopic field was used as the adherence index [5, 21].

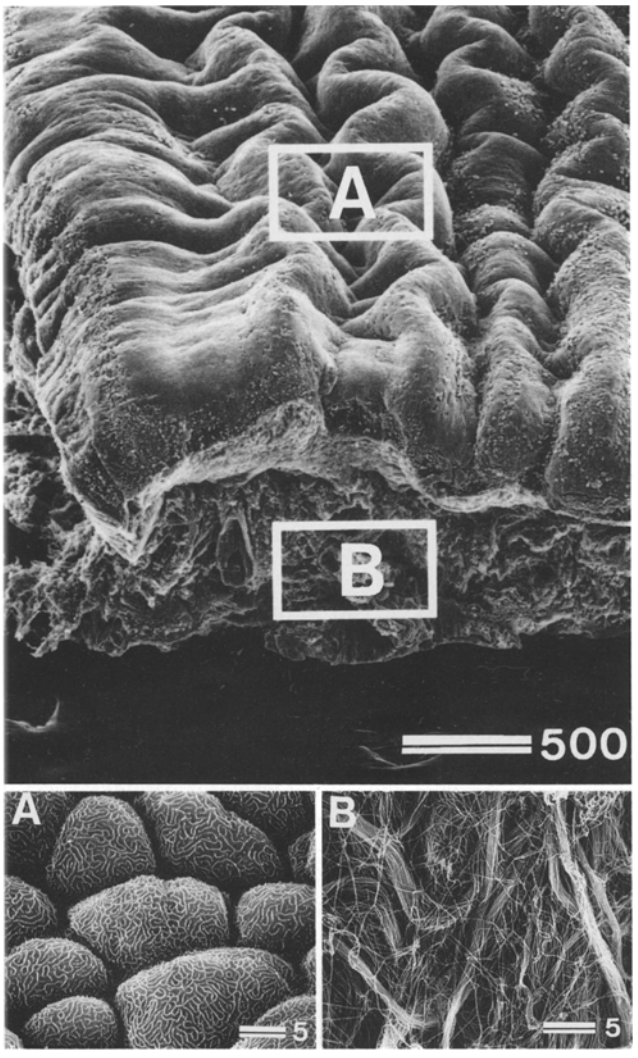

Fig. 1A, B. Scanning electron micrograph of control human ureter. **A** epithelium; **B** connective tissue. *Numbers* indicate length of *scale bars* (μm)

For transmission electron microscopy (TEM), the ureteral specimens fixed in glutaraldehyde were further cut into smaller pieces ($1 \times 2\,mm^2$). The pieces were subsequently fixed in 1% osmium tetroxide for 2 h at 4°C, dehydrated with graded concentrations of ethanol, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate, and analyzed with a JEM-1200 transmission electron microscope. The HA products of *S. saprophyticus* were fixed in 2.5% (vol./vol.) glutaraldehyde in PBS (pH 7.4) for 2 h at 4°C and treated for TEM by the same procedure.

Results

SEM of the control human ureter is shown in Fig. 1. *S. saprophyticus* adhered to the epithelium of the human ureter (Fig. 2A), whereas it did not adhere markedly to the connective tissue. The HA titers and adherence indices of the strains are compiled in Table 1. The indices for adherence to the mature epithelium possessing surface microfolds were not significantly different from those for

Table 1. Hemagglutination (HA) titers[a] and adherence indices[b] of *S. saprophyticus*[c]

Strains	HA titer using 3% sheep erythrocytes	Adherence index to	
		Epithelium[d]	Connective tissue
SS18	1:128	139.6 ± 64.8 (144.6 ± 78.8*)	1.4 ± 1.2
ATCC 15305	1: 64	98.2 ± 84.5 (81.2 ± 38.7*)	2.4 ± 2.1
SS 9	1: 64	78.6 ± 57.8 (74.7 ± 33.5*)	1.9 ± 1.7
SS13	1: 64	54.3 ± 38.7 (66.8 ± 28.8*)	0.8 ± 0.7
SS 7	1: 64	39.1 ± 37.1	1.6 ± 1.9
SS12	1: 32	34.2 ± 35.2	1.5 ± 1.1
SS14	1: 32	25.4 ± 22.3	2.7 ± 3.3
SS11	1: 32	17.4 ± 15.8	2.2 ± 2.0
SS23	1: 32	7.6 ± 5.3	0.8 ± 0.7
SS 5	1: 16	6.3 ± 4.8	1.2 ± 1.4
SS22	1: 16	1.2 ± 1.6	2.2 ± 2.9
SS19	1: 8	1.5 ± 1.8	1.5 ± 2.1
SS24	1: 8	1.4 ± 1.3	1.4 ± 2.0
SS 6	1: 4	0.8 ± 1.0	1.6 ± 2.5

The numbers in parentheses are the adherence indices to epithelium possessing surface microvilli.
[a] HA titers indicate the highest dilution that still yielded positive results
[b] Mean ± standard deviations for 30 determinations
[c] *S. saprophyticus* was incubated in nutrient broth for 48 h at 37°C
[d] Epithelium possessing surface microfolds
* Not significant

Table 2. HA titers[a] and adherence indices[b] of *S. aureus*[c]

Strains	HA titer using 3% sheep erythrocytes	Adherence index to	
		Epithelium[d]	Connective tissue
SA 10	< 1:1	0.1 ± 0.3	627.4 ± 289.1
SA 16	< 1:1	0.1 ± 0.3	846.8 ± 174.5
SA 21	< 1:1	0.1 ± 0.3	301.0 ± 156.5
SA 25	< 1:1	0.1 ± 0.3	111.5 ± 34.3
SA 26	< 1:1	0.0 ± 0.0	342.1 ± 145.5
ATCC 12598	< 1:1	0.1 ± 0.3	214.8 ± 108.6

[a] HA titers indicate the highest dilution to yield positive results
[b] Means ± standard deviations for 30 determinations
[c] *S. aureus* was incubated in nutrient broth for 48 h at 37°C
[d] Epithelium possessing surface microfolds

adherence to the young epithelium possessing surface microvilli. Good correlation between the HA titers and the adherence indices to the ureteral epithelium is seen in Fig. 3 ($r = 0.9459$, $P < 0.01$). TEM revealed that the tested *S. saprophyticus* strains (SS18, 9, 13 and 7) adhered to the ureteral surface at a distance and showed pili-like structures between the bacteria and the epithelium (Fig. 2B). Similar pili-like structures were observed between the

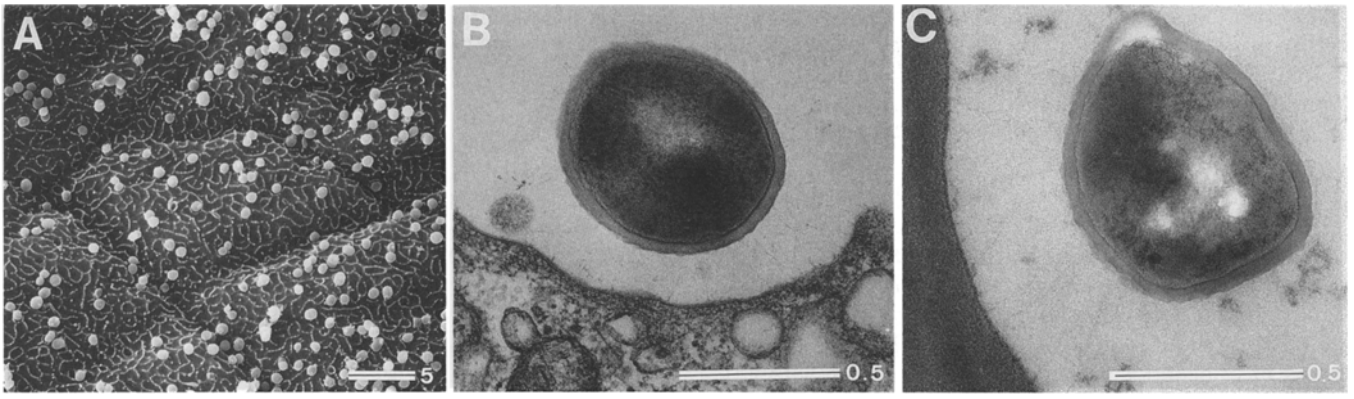

Fig. 2. **A** Scanning and **B** transmission electron micrographs of *S. saprophyticus* SS18 adhering to the ureteral epithelium possessing surface microfolds. **C** Transmission electron micrograph of SS18 that hemagglutinated sheep erythrocytes. *Numbers* indicate length of *scale bars* (μm)

hemagglutinating bacteria and sheep erythrocytes (Fig. 2C). *S. epidermidis, S. haemolyticus* and *S. aureus* did not show HA (< 1:1) or adherence to the epithelium of the human ureter (adherence index: < 1.0). *S. epidermidis* and *S. haemolyicus* did not adhere to the connective tissue (adherence index: < 1.0). Only *S. aureus* showed marked adherence to the connective tissue (Table 2, Fig. 4A). *S. aureus* (SA16) adhered directly to the collagen of the connective tissue via its cell wall (Fig. 4B).

Discussion

S. saprophyticus is isolated mainly from uncomplicated UTI [10, 11, 20], whereas *S. epidermidis, S. haemolyticus* and *S. aureus* are isolated from complicated UTI [17; 18]. This adherence study clearly showed the differences in the abilities of these staphylococcal species to adhere to the human urinary tract. Adhesion to the epithelium is considered to be a unique characteristic of *S. saprophyticus,* allowing the agent to establish uncomplicated UTI. Moreover, it is likely that adhesins to the epithelium are not necessary for *S. epidermidis, S. haemolyticus* and *S. aureus* to cause complicated UTI. This resembles the events of P-piliated *E. coli.* P-piliation is not necessary for

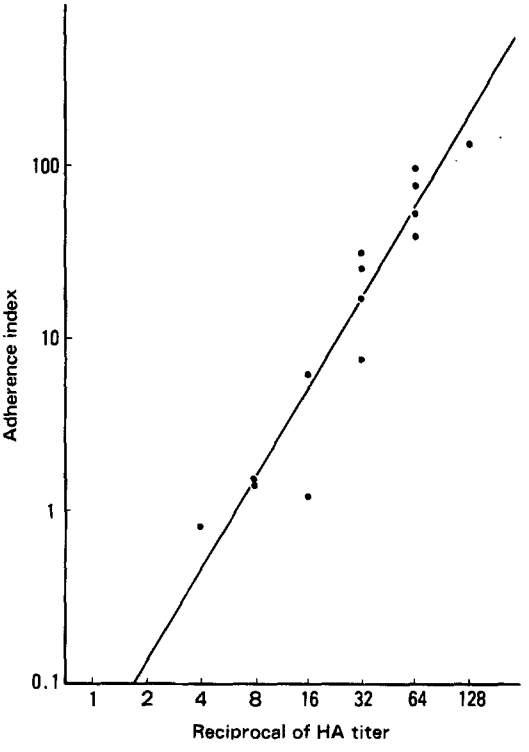

Fig. 3. Correlation between HA activity and adherence index of *S. saprophyticus* to the ureteral epithelium possessing surface microfolds. Data from Table 1

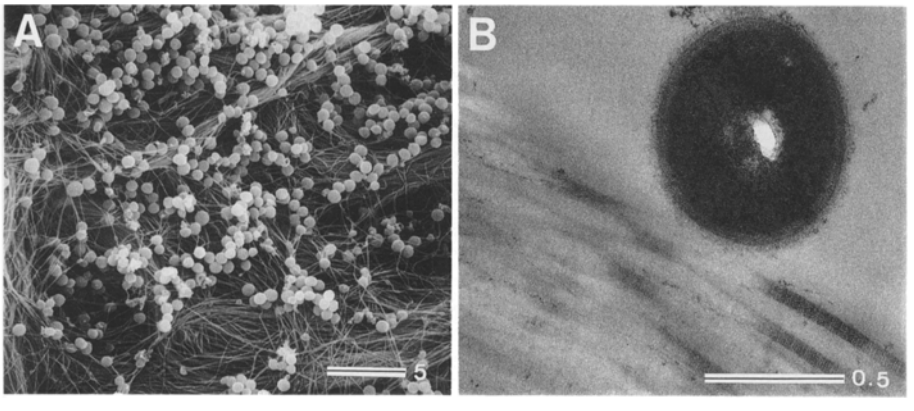

Fig. 4. **A** Scanning and **B** transmission electron micrographs of *S. aureus* SA16 adhering to the connective tissue of human ureter. *Numbers* indicate length of *scale bars* (μm)

E. coli to cause complicated UTI with vesico-ureteral reflux or obstructive uropathy [15].

The adhesins and HAs of *S. saprophyticus* have not been clearly identified. Some researchers have suggested that they are located on the cell wall [7, 9]. However, in this study, *S. saprophyticus* was never seen to adhere directly to the human ureteral epithelium via its cell wall. The bacteria adhered to the epithelium indirectly by way of pili-like structures. The pili-like structures seemed to play an important role in the bacterial adherence and HA of *S. saprophyticus.* The pili-like structures were so slender that they were difficult to identify by SEM. The structures might have been lost in the process of coating with gold-palladium [5, 21].

Only *S. aureus* showed striking adherence to the connective tissue of the human ureter. The surface of the urinary tract is usually covered with mucosa, but if the mucosa is damaged, exposing the underlying connective tissue in the urinary tract, it will provide a good adherence target for *S. aureus.* Most of our urinary isolates of *S. aureus* were from patients who had undergone urinary tract surgery or percutaneous nephrostomy, which would have resulted in exposure of connective tissue. Collagen binding by *S. aureus* has been reported previously [8]. TEM observations revealed that *S. aureus* adhered directly to the collagen of the connective tissue via its cell wall, which contrasts to the pili-mediated adherence of *S. saprophyticus* to the epithelium. It is likely that adhesins to the collagen are located on the cell wall of *S. aureus.*

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