Hemagglutination (Fimbriae) and Hydrophobicity in Adherence of *Serratia marcescens* to Urinary Tract Epithelium and Contact Lenses


Abstract. The capacity of 59 isolates of *Serratia marcescens*, obtained from urinary tract infections, wounds, and contact lenses or their paraphernalia, to agglutinate erythrocytes from different animal species was tested. Three main patterns were found: mannose-sensitive agglutination of guinea-pig, fowl or horse erythrocyte; mannose-resistant agglutination of chicken or pigeon erythrocytes alone or in combination with mannose-sensitive agglutination; and no agglutination. Hemagglutination capacity was associated with isolates from urinary tract infection, but not with isolates associated with contact lenses. Adherence to human urinary tract epithelium did not correlate with the hemagglutination patterns nor with the origin of the isolates. Some strains of different hemagglutination pattern were selected for the study of hydrophobicity and adherence to contact lens polymers. Hydrophobicity, as determined by degree of partition in hexadecane and water (BATH-values), correlated neither with degree of adherence to contact lens polymers nor with the hemagglutination pattern. For a representative strain there was an excellent correlation ($r^2 = 0.98$) between adherence and the water content (hydrophobicity) of the lens polymers. These results suggest that, as with tissues, other factors interact with hydrophobicity in causing adherence to plastics.

Bacterial adhesins can be classified according to the agglutination patterns that result in the binding of bacteria to erythrocytes from various species. Hemagglutination of guinea-pig erythrocytes, which is inhibited by $\alpha$-mannose (MS), is caused by type 1 pili or fimbriae, and is a common feature of *Enterobacteriaceae*, including *Serratia*, grown in a liquid medium [4, 17]. Type 1 fimbriae also mediated binding of bacteria to colonic epithelium, secretory IgA, and urinary slime, but have not been shown to play a major role as virulence factors in infectious diseases [4]. Hemagglutination of erythrocytes that is not inhibited by $\alpha$-mannose is called mannose-resistant (MR). Old et al. [17] found four types of hemagglutinins (HAs) for 56 isolates of *Serratia*, representing nine species. All 11 isolates of *S. marcescens* examined produced three types of HAs MR/K (Klebsiella-like) associated with type 3 fimbriae that agglutinated tannic acid-treated erythrocytes of species of different animals, MS (mannose-sensitive) associated with type 1 fimbriae, and MR/P (Proteus-like) that agglutinated untanned erythrocytes of species of different animals. The so-called P-fimbriae, which mediate binding of *Escherichia coli* to uroepithelial cells, are adhesins that cause a blood-group-dependent, mannose-resistant binding of erythrocytes [7]. Several adhesins may be co-expressed in a single bacterial isolate.

*Serratia marcescens* does not normally colonize the intestine of healthy humans [18], but hospitalized patients may harbor the bacterium, which can cause extra-intestinal infections, especially in immunocompromised individuals [3, 23]. As well as other *Enterobacteriaceae*, *S. marcescens* also expresses pili [1], and Yamamoto et al. [26] have shown that adherence of a strain of...
S. marcescens to urinary tract epithelium was mediated by these structures.

In recent years there have been several reports of keratitis due to Serratia marcescens in soft-contact-lens wearers [2, 10–12, 19]. The bacterium has been isolated from the lenses, the lens cases, and the solutions for wetting and soaking of the lenses, even when the solutions should have contained appropriate amount of disinfectants [2, 14, 20]. Adherence phenomena have been implicated in the failure of disinfectant solutions to eradicate S. marcescens [13, 25]. As the presence of fimbriae has been reported to decrease the hydrophobicity of cells of Serratia [9], fimbriae-mediated adherence may interact with hydrophobicity in different ways when S. marcescens is involved in urinary tract infections as compared with its association with contact lenses. This study was performed to compare the role of hemagglutination (fimbriae) and hydrophobicity in adherence of S. marcescens to urinary tract epithelium and contact lenses.

Materials and Methods

Bacterial strains. Twenty-two isolates of Serratia marcescens from urinary tract infections, 19 isolates from wounds, and one isolate from an eye with conjunctivitis were obtained from the Department of Bacteriology, University of Goteborg (Goteborg, Sweden). Seventeen isolates from contact lenses and contact lens paraphernalia were obtained from the collection at Georgia State University (Atlanta, Georgia). All isolates except GSU-81-024 were nonpigmented. Bacteria were transferred from deep agar to a concentration of about 2 x 10^8 bacteria/ml. Suspended in phosphate-buffered saline (PBS) (300 μM/ml pH 7.2) were nonpigmented. Bacteria were transferred from deep agar to a concentration of about 2 x 10^8 bacteria/ml.

Hemagglutination test. Fresh erythrocytes from human (blood group A Rh+), ox, horse, sheep, guinea-pig, rat, chicken, and pigeon were washed in PBS and diluted to 3–5% suspension. The erythrocytes from each species were tested separately. Twenty microliters of the bacterial suspension and 20 μl of the erythrocyte suspension were mixed on a glass tile. Strains agglutinating the erythrocytes in the absence, but not in the presence, of a 2.5% D-mannose solution were considered to have mannose-sensitive hemagglutinins (MS). Strains agglutinating erythrocytes in the presence of D-mannose were considered to have mannose-resistant hemagglutinins (MR) [5].

Adherence to urinary epithelium cells. Urinary epithelium cells were collected from fresh urine (non-secretory, blood group A Rh+). The urine was centrifuged for 10 min at 700 rpm and washed twice in PBS. The cells were counted in a Burker chamber and diluted to a suitable cell concentration. The urinary epithelial cell suspension was mixed in PBS with the bacterial suspension to a final concentration of 10^5 cells/ml and 10^6 bacteria/ml. One ml of that mixture was incubated at 37°C and rotated for 45 min. The cells were washed twice in PBS and centrifuged at 700 rpm for 10 min. Forty cells from each sample were counted under the microscope.

Cell hydrophobicity tests. Agar for determination of Congo red binding contained 0.003% Congo red in TSA. Bacteria streaked out to form single colonies, displayed either intensely colored (CR+) colonies that bound Congo red or colonies that remained nonpigmented (CR-) [24]. Bacteria grown overnight in shaken culture at 37°C were harvested by centrifugation and washed twice in and suspended in PBS, pH 7.0. The optical density (OD) at 600 nm was adjusted to 0.4. To 3.0 ml of this bacterial suspension in cuvettes, 1.0 ml of hexadecane was added, and the mixture was vortexed vigorously for 30 s. The OD[H2] of the water phase was read after oil and water phase separation (minimum 5 min) [22]. Results are expressed as the percentage decrease in the turbidity of the original suspension. The bacterial adherence to hexadecane (BATH) test was done at least in triplicate. An isolate GSU 86-828, isolated from a contact lens, was used as a control and tested 10 times.

Contact lenses. The contact lenses used for the tests were siloxane acrylate (itafocon A) rigid gas-permeable lenses, 38% water-content phemfilcon A lenses, 55% water-content uvifilcon A lenses, and 70% water-content lidofilcon A lenses. The caps of X-cel Optacryl 60 deliver cases (polypropylene) were also tested for their affinity for adherence of bacteria. The caps were removed from the cases and cut into uniform circular discs. The discs were swabbed with alcohol and permitted to air dry.

Adherence to contact lenses. Adherence was determined by a modification of the procedure of Pringle and Fletcher [21] and Miller and Ahearn [15]. Washed bacteria suspended in minimal broth (Difco) were incubated for 1 h at 25°C. The bacteria were radiolabeled by addition of L-[4,5-3H] leucine (NEN Research Products, DuPont Co., Wilmington, Delaware) with additional incubation for 20 min. The bacteria were washed four times and suspended in normal saline to a concentration of approx. 10^9 CFU/ml (CFU = colony-forming unit). Individual lenses and discs from the lens cases were incubated with 3 ml of the radiolabeled bacterial suspension at room temperature for 2 h. The tests were done in triplicate. The lenses and discs were removed with sterile forceps and dipped five times in three 100-ml changes of PBS. Each lens and disc were placed in separate 20-ml glass vials. A 10-ml portion of Opti-Fluor scintillation cocktail (Packard Instruments Co. Inc., Downers Grove, Illinois) was added and mixed thoroughly. During this process all labeled material was released from the surface of the lens or the polypropylene disc. Vials were placed in a liquid scintillation counter (LS-7500, Beckman Instruments Inc., Fullerton, California). The level of quenching was determined by the H-numbering system. Scintillation counts were converted to cell numbers with a calibration curve relating counts/min to viable cell number. Quantified radiolabeled cell suspensions were serially diluted, and 100 μl of each dilution was distributed into 20-ml glass scintillation vials and counted as described above. Calibration curves for each strain were constructed. Viable bacterial counts of dilutions were obtained from pour plates of 100-μl samples in trypticase soy agar.

Quantitation of adherence. The adherence of bacteria to contact lenses was quantified as CFU/mm^2 from the formula: Total lens surface area (LSA) = outer lens area (OLA) + inner lens area (ILA) + outer lens perimeter area (OLPA). Here, OLA = 2