Urol Res (1993) 21:313–317

Urological Research

Antibacterial activity of the bladder mucosa

Theresa A. Schlager, Jacob A. Lohr, J. Owen Hendley

University of Virginia, Department of Pediatrics, Division of Infectious Disease, Box 386, University of Virginia School of Medicine, Charlottesville, VA 22908, USA

Received: 1 February 1993 / Accepted: 25 June 1993

Summary. Antibacterial activity of bladder mucosa is believed to be a host defense against infection. In this study we examined the antibacterial activity of the bladder mucosa without the effect of voiding. In addition we examined whether the property of adherence was advantageous for the organism in contact with the bladder mucosa. For this, three adhering and three nonadhering strains of *E. coli* were placed in contact with the bladder mucosa for 4 h in an in vivo rabbit model. *E. coli* grown in broth and applied to 32 bladders increased in titer by 1 log. *E. coli* grown in rabbit urine and applied to seven bladders increased in titer by 1.2 log. In contrast *E. coli* inoculated into control vials containing broth increased in titer by 2.3 log ($P = 0.01$). There was no significant difference in the titer between adhering and nonadhering strains of *E. coli* after 4 h of contact with the bladder mucosa. Bladder mucosa may have an inhibitory effect on bacterial growth (regardless of adherence characteristics) and with urine flow serves to prevent infection of the bladder urine.

Key words: Adherence – Bladder – Urinary tract infection

Although the pathogenesis of bacterial infections of the urinary tract is poorly understood, occurrence of infection must depend on the interaction between the urinary tract defenses of the human host and the virulence factors of the bacteria. The effectiveness of the bladder defenses was demonstrated in 1961 by Cox and Hinman [2]. They inoculated 10 million *E. coli* into the bladders of four healthy adult males, and found that bladder urine became sterile by 72 h after inoculation. Cox and Hinman hypothesized that sterilization was accomplished by two defenses: (1) bacterial clearance by urine flow and voiding, and (2) an undefined antibacterial mechanism of the bladder. Following this work, bactericidal activity which kills bacteria in contact with the bladder mucosa has been thought to be an important adjunct to the mechanical clearance by urine flow for maintaining sterility of bladder urine [2, 6].

Adherence to uroepithelium is currently thought to be a virulence factor important for bacteria infecting the urinary bladder [3–5, 11, 12, 14, 16]. *E. coli* isolated from the urine of infected patients attach to human uroepithelial cells in vitro in larger numbers than do *E. coli* isolated from the urine of patients with asymptomatic bacteriuria or from the stool of patients without urinary tract infection (UTI). Adherence to uroepithelial cells could be advantageous to organisms in producing ascending infection of the bladder by facilitating colonization of the periurethra. Once bacteria gain entry to the bladder, however, adherence to uroepithelial cells may not be advantageous. Although it might aid the bacteria in resisting removal by urine flow, attachment to bladder epithelial cells might lead to death of the bacteria if they activate the putative bactericidal activity of the bladder mucosa.

The purpose of this study was to examine the bactericidal activity of the bladder without the effect of urine flow. In addition we wanted to determine whether the property of adherence was advantageous to an organism placed in contact with intact bladder mucosa.

Materials and methods

Bacteria

Six strains of *E. coli* isolated from the urine of individuals with UTI were utilized. Four strains were from children at the University of Virginia Medical Center and two were from adults (kindly provided by Dr. Walter Stamm at the University of Washington). One of the strains from adults was known to have type 1 fimbriae (F+) and one did not (F−). Two of the strains from children were known to have P fimbriae (pap+) by agglutination of α-D-gal-(1,4)-β-D-gal-O-$(CH_2)8$-$COOCH_3$ coated latex beads (2% w/v suspension; Chembiomed, Edmonton, Canada). Organisms were stored at −70 °C in Trypticase soy broth (TSB)

Correspondence to: Theresa A. Schlager, M.D.; FAX: 1(804)924-5244

and 50% glycerol. For use bacteria were grown overnight on 5% sheep blood agar then lifted into broth for a second overnight passage. The F+ strain was grown on Trypticase soy agar (TSA) containing 25 μg/ml chloramphenicol to maintain expression of type 1 fimbriae.

For the in vitro adherence assay bacteria were grown overnight at 37 °C in brain heart infusion broth, sedimented at 300 xg and resuspended in phosphate-buffered saline (PBS) at pH 7.2 to the density of McFarland standard no. 8, which corresponded to a viability titer of 10^8 cfu/ml. For the in vivo bladder bactericidal assay bacteria were grown overnight (37 °C) in TSB and diluted in TSB to obtain the desired concentration of organisms in stationary phase. As noted below, one adhering strain was also grown and diluted in rabbit urine obtained by bladder aspiration instead of TSB.

Viability titers were done by standard methods using 5% sheep blood agar plates and pour plates containing TSA.

Adherence assay

The adherence assay developed by Svanborg-Eden et al. [15] was modified to utilize rabbit bladder mucosal cells. Mucosal cells were harvested from an excised bladder from a New Zealand white (NZW) rabbit weighing 2–3 kg by brushing the mucosa lightly with a cytology brush. Cells were eluted from the brush into PBS by mixing on a vortex mixer and counted with a hemocytometer.

The incubation mixture containing 105 mucosal cells and 108 bacteria in 1 ml PBS was shaken (20 rpm, Orbital Shaker, Bellco Glass, Vineland, N.J.) for 60 min at 37 °C. Unattached bacteria were removed by repeated washing in PBS with differential centrifugation at 300 xg. Forty epithelial cells, which were alive as determined by trypan blue exclusion, were examined under ×400 magnification in a light microscope. Attached bacteria were counted, and the mean number of bacteria per cell was calculated. A strain was designated as "nonadhering" if <10 bacteria per cell were attached (Fig. 1 a) or "adhering" if >50 bacteria per cell were attached (Fig. 1 b).

Bladder bactericidal assay

The bactericidal activity of the bladder mucosa was examined using a rabbit model developed by Mulholland and colleagues [8]. The bladder of a living NZW male rabbit, anesthetized with ketamine–xylazine, was isolated under sterile conditions. The bladder urine was removed by needle aspiration and both ureters ligated. Just prior to closure of the abdominal incision, 10 μl TSB containing organisms were inoculated into the bladder lumen through a 25-gauge needle attached to a 50-μl syringe (Hamilton, Reno, Nev.). The sterility of the bladder urine prior to inoculation of the broth was confirmed using standard methods. The volume of 10 μl was used in order to ensure that the bacteria were in contact with the bladder mucosa [8]. To confirm this, we injected volumes of India ink ranging from 10 to 50 μl into the lumens of excised rabbit bladders and then opened the bladders to examine the distribution of ink. The bladder wall was coated after injection of 10 μl, whereas ≥20 μl produced a visible puddle of ink.

After 4 h the rabbit was killed. The bladder was opened to check for residual urine, then excised and homogenized with 2 ml PBS in a tissue grinder (Wheaton, Millville, N.J.). The liquid portion of the homogenate was decanted, the volume measured and a viability titer performed. The number of organisms inoculated onto the bladder mucosa at time zero was calculated (viability titer of the organism suspension inoculated×0.01 ml, the volume instilled into the bladder). The number of organisms present on the bladder mucosa after 4 h was determined by ascertaining the total number of organisms in the homogenate (viability titer of the homogenate×volume recovered).

The reliability of the homogenization technique for detecting all the bacteria on the bladder mucosa was established by inoculating each of five excised rabbit bladders with organisms followed immediately by homogenization of the bladder and titration of the homogenates. The number of organisms inoculated was essentially the same as the number of organisms detected in the bladder homogenate (differences ranged from 0.1 to 0.4 log 10; the average difference was 0.2 log 10).

As a control, an identical inoculum (10 μl) to that placed on the bladder was inoculated into sterile round-bottom glass vials containing 1 ml TSB. To simulate the bladder, vials were sealed and incubated *without shaking* at 37 °C for 4 h. Viability titers were done by standard methods using 5% sheep blood agar plates and pour plates containing TSA.

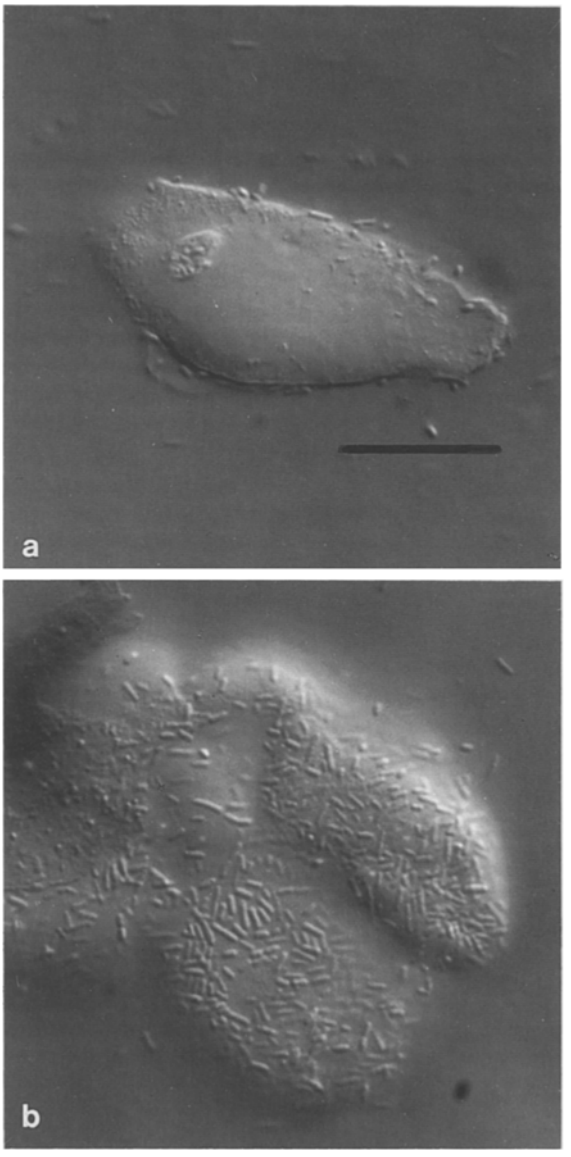

Fig. 1 a, b. Adherence of *E. coli* to rabbit bladder mucosal cells. **a** Nonadhering strain; **b** adhering strain. Scale bar represents 10 μm

Statistics

The change in the number of viable organisms during 4 h of contact with the bladder mucosa or in a vial was calculated and expressed in log 10. The "log change" of *E. coli* on mucosa was compared with the "log change" in a vial using the paired and unpaired Student's *t*-test [13].

Results

Survival of E. coli on bladder mucosa

Ten strains of *E. coli* isolated from the urine of patients with UTI were screened for adherence to rabbit bladder mucosal cells. Three of the ten adhered and seven did not. The adherence characteristics of the three adhering

strains and three of the nonadhering strains were confirmed by two additional runs prior to their use in the in vivo model. The three adhering strains included the F+ strain and a pap+ strain. The three nonadhering strains included the F− and a pap+ strain.

The bactericidal activity of the rabbit bladder mucosa was examined in 38 experiments utilizing the six strains of *E. coli*. In the initial six experiments, an *E. coli* strain grown overnight in TSB was inoculated undiluted (10^4 to 10^6 organisms) into the bladder lumen. The organisms did not decrease in number as expected after 4 h of contact with the bladder mucosa. In fact, the number of organisms increased in three bladders, with an average titer rise of 0.56 log. In subsequent experiments organisms grown overnight were diluted to approximate an inoculum of 100 organism.

Adhering *E. coli* were diluted and applied to 20 rabbit bladders (Fig. 2). The titer declined in two bladders (av. −0.8 log 10) by 4 h and the titer was stable in one bladder. The number of organisms increased in 17 bladders, with an average titer rise of 1.5 log. The behavior of the pap+ strain applied to 12 bladders was indistinguishable from that of the F+ strain applied to four bladders or the other adhering strain. Nonadhering strains of *E. coli* were applied to a total 12 rabbit bladders (Fig. 2), each strain being applied to four bladders. The titer declined (av. −0.9 log) by 4 h in four bladders, was stable in two, and the organisms increased in titer (av. 1.8 log) in six bladders. The behavior of the pap+ strain was indistinguishable from that of F− or the third nonadhering strain. The results of these experiments may also be expressed as the change in the number of viable organisms during 4 h of contact with bladder mucosa (Fig. 3). The mean change for adhering *E. coli* applied to 20 bladders was +1.2 log and the mean change for nonadhering *E. coli* on 12 bladders was +0.6 log ($P = 0.2$).

The possibility that the observed growth of *E. coli* in contact with rabbit bladder mucosa might have been due

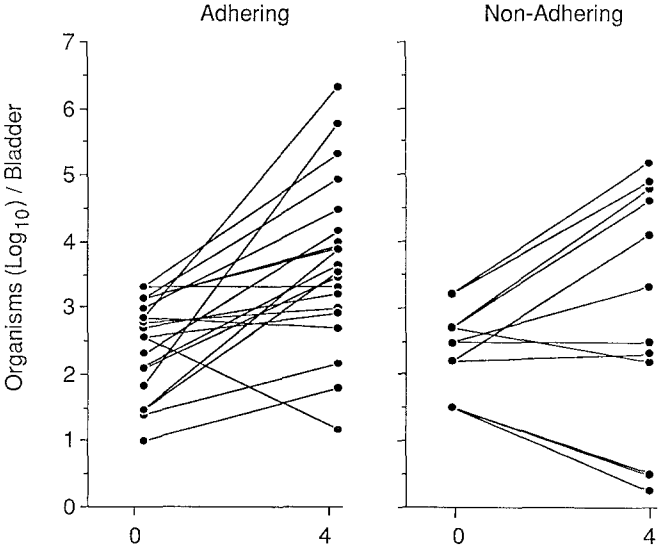

Fig. 2. Effect of contact with rabbit bladder mucosa on viability of *E. coli*

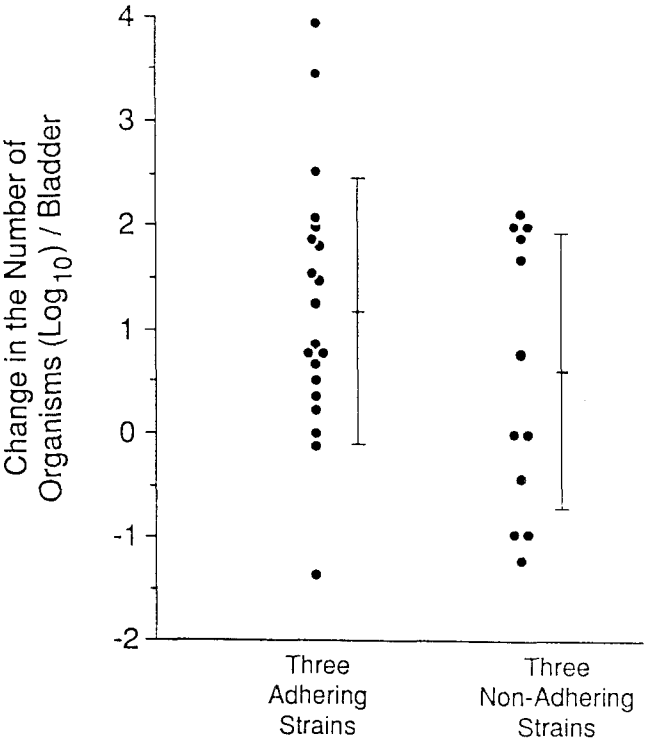

Fig. 3. Change in viability of *E. coli* during 4 h on rabbit bladder mucosa. *Verticle bars* indicate the SD

to a protective or stimulatory effect of broth was explored. An adhering pap+ strain of *E. coli* was grown in rabbit urine overnight and diluted in rabbit urine. The number of organisms increased in all seven bladders challenged, with an average titer rise of 1.26 log.

In 22 experiments utilizing all six strains of *E. coli* an identical inoculum (about 100 organisms) to that placed on the bladder mucosa was inoculated into stationary vials containing 1 ml TSB (37 °C, without shaking, for 4 h). The number of organisms increased in all 22 vials, with an average titer rise of 2.3 log.

The results of these experiments are summarized in Fig. 4. The results are expressed as the change in the number of viable organisms during 4 h of contact with the bladder mucosa or in a vial. The mean change for *E. coli* grown in TSB and applied to 32 bladders was +1.0 log (filled circles in Fig. 4), the mean change for *E. coli* grown in rabbit urine and applied to seven bladders was +1.2 log (open stars), and the mean change for *E. coli* inoculated into 22 vials containing TSB was +2.3 log (squares) ($P = 0.01$).

Discussion

Cox and Hinman [2] compared the survival of 10^7 *E. coli* inoculated into the urinary bladder of four adult human males with the survival of 10^7 *E. coli* placed into a sterile flask which was filled and emptied with human urine at regular intervals. The number of bacteria in urine in the flask which was being filled and emptied was maintained at a constant level over 72 h. In contrast while the

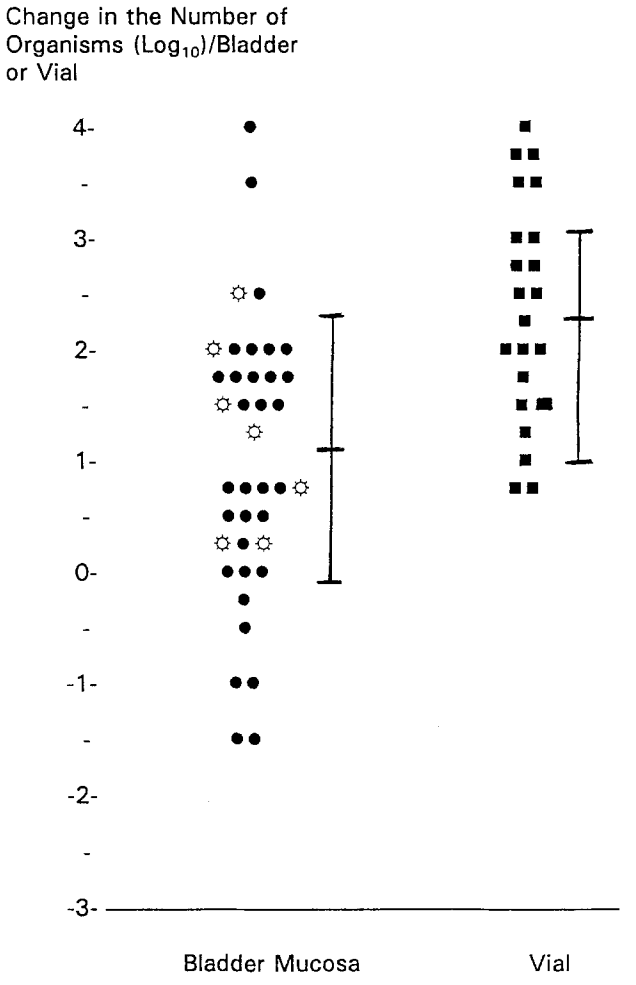

Fig. 4. Change in viability of *E. coli* during 4 h on rabbit bladder mucosa and in vial. *Vertical bars, brackets*, SD; *circles*, change in number of organisms grown in broth and applied to bladder (log 10); *stars*, change in number of organisms grown in rabbit urine and applied to bladder; *squares*, change in number of organisms inoculated into vials

number of *E. coli* in voided urine obtained from human bladders increased by 0.5 log or remained the same at 4 h after inoculation, after 6 h bacterial counts declined steadily and by 72 h bladder urine was sterile. They concluded that voiding alone could not explain the inherent sterility of the bladder urine and suggested that an additional "antibacterial action" of the bladder was necessary to maintain sterility of bladder urine.

Few studies have examined this "antibacterial activity" of the bladder mucosa without the effect of urine flow [1, 8]. Cobbs and Kaye [1] inoculated 10^3 to 10^7 organisms into the bladders of anesthetized rats after ureteral ligation and aspiration of bladder urine. The number of viable organisms decreased after 4 h of contact with the bladder mucosa, but this decrease did not occur consistently. Over 24 h bacterial growth was observed when 10^5 of one of three strains of *E. coli* or *P. roteus mirabilis* was placed in contact with bladder mucosa. Mullholland et al. [8] inoculated 10–100 µl of 10^4 *E. coli* into the bladders of anesthetized rabbits after ureteral ligation and aspiration of bladder urine. At 4 h six of ten animal bladders showed a tenfold increase over the inoculum.

In our experiments we examined the antibacterial activity of the bladder when *E. coli* were placed in contact with the bladder mucosa. In addition we examined the property of adherence. We hypothesized that adhering *E. coli* would enhance this antibacterial activity of the bladder. We found that *E. coli* in contact with the bladder mucosa grew 1 log less than the same number of organisms in a stationary vial, regardless of adherence characteristics.

Bacterial adherence to suspended uroepithelial cells is thought to be an important step in ascending infection of the urinary tract [3, 16]. This adherence has been shown to be mediated by type 1 (F+) adhesin in cystitis [9] and by P (pap+) adhesin in pyelonephritis [7, 10]. However, adherence of bacteria to suspended uroepithelial cells in vitro may not predict adherence to the intact bladder mucosa. One of the P (pap+) strains of *E. coli* adhered to suspended uroepithelial cells and one P (pap+) strain did not, though both strains behaved similarly when placed on intact bladder mucosa. Virkola et al. [17] studied the presence of binding sites on uroepithelia in the human bladder and found that none of the UTI-associated adhesins bound efficiently to the bladder epithelium. They proposed that adhesion to uroepithelium may not be important for *E. coli* colonization of the bladder. In our in vivo model we demonstrated that the titer of adhering *E. coli* was similar to the titer of nonadhering *E. coli* after 4 h of contact with the bladder mucosa. Thus adherence may facilitate colonization of the periurethra and ascension of the organism *to* the bladder. However, once bacteria gain entry to the bladder adherence may not play a role in infection of the bladder mucosa.

Our results demonstrate that contact with the bladder mucosa has an inhibitory effect on multiplication of bacteria regardless of adherence characteristics. This mild inhibitory effect of the mucosa along with bacterial dilution by urine and voiding may be sufficient to prevent infection of the bladder urine. Further elucidation of this inhibitory effect is required, including the role of cytokines and other defense regulators.

Acknowledgements. This work was supported in part by the National Institutes of Health training grant no. T32AI07046 and in part by the Pendleton Laboratory Research Fund in Pediatric Infectious Disease.

References

1. Cobbs G, Kaye C (1967) Antibacterial mechanisms in the urinary bladder. Yale J Biol Med 40:93
2. Cox CE, Hinman F (1961) Experiments with induced bacteriuria, vesical emptying and bacterial growth on the mechanism of bladder defense to infection. J Urol 86:739
3. Eden CS, Hanson LA, Jodal U, Lindberg U, Akerlund AS (1976) Variable adherence to normal human urinary tract epithelial cells of *Escherichia coli* strains associated with various forms or urinary tract infection. Lancet I:490
4. Kallenius G, Winberg J (1978) Bacterial adherence to periurethral epithelial cells in girls prone to urinary tract infections. Lancet II:540

5. Kallenius G, Mollby R, Winberg J (1980) In vitro adhesion of uropathogenic *Escherichia coli* to human periurethral cells. Infect Immun 28:972
6. Kunin C (1987) Detection, prevention, and management of urinary tract infections, 4th edn. Lea & Febiger, Philadelphia, p 313
7. Latham RH, Stamm WE (1984) Role of fimbriated *Escherichia coli* in urinary tract infections in adult women: correlation with localization studies. J Infect Dis 149:835
8. Mulholland SG, Foster EA, Paquin AJ, Gillenwater JY (1969) The effect of rabbit vesical mucosa on bacterial growth. Invest Urol 6:593
9. Orndorff PE, Bloch CA (1990) The role of type 1 pili in the pathogenesis of *Escherichia coli* infections: a short review and some new ideas. Microb Pathog 9:75
10. Pere A, Nowicki B, Saxen H, Siitonen A, Korhonen TK (1987) Expression of P, type-1, and type 1C fimbriae of *Escherichia coli* in the urine of patients with acute urinary tract infection. J Infect Dis 156:567
11. Reid G, Sobel J (1987) Bacterial adherence in the pathogenesis of urinary tract infection: a review. Rev Infect Dis 9:470
12. Schaeffer A, Jones J, Dunn JK (1981) Association of in vitro adherence to vaginal and buccal epithelial cells with susceptibility of women to recurrent urinary tract infections. N Engl J Med 304:1062
13. Snedecor GW, Cochran WG (1967) Statistical methods. Iowa State University Press, p 59
14. Svanborg-Eden C, Jodal U (1979) Attachment of *Escherichia coli* to urinary sediment epithelial cells from urinary tract infection-prone and healthy children. Infect Immun 26:837
15. Svanborg-Eden C, Eriksson B, Hanson LA (1977) Adhesion of *Escherichia coli* to human uroepithelial cells in vitro. Infect Immun 18:767
16. Svanborg-Eden C, Eriksson B, Hanson LA, Jodal U, Kaijser B, Janson GL, Lindberg U, Olling S (1978) Adhesion to normal human uroepithelial cells of *Escherichia coli* from children with various forms of urinary tract infection. J Pediatr 93:398
17. Virkola R, Westerlund B, Holthofer H, Parkkinen J, Kekomaki M, Korhonen TK (1988) Binding characteristics of *Escherichia coli* adhesins in human urinary bladder. Infect Immun 56:2615