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Attachment of *Proteus mirabilis* to Human Urinary Sediment Epithelial Cells In Vitro Is Different from That of *Escherichia coli*

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The in vitro attachment of 335 *Proteus mirabilis* strains from various human sources to human urinary tract epithelial cells was measured. No significant difference in adhesive capacity was found between *P. mirabilis* strains isolated from the blood of 89 patients with bacteremia, the stools of 36 healthy subjects and 56 patients with diarrhea, and the urine of 62 adults and 92 children with bacteriuria. High mean adhesion values were observed in all groups. The *P. mirabilis* strains attached only to squamous cells and not to transitional epithelial cells, whereas most of the *Escherichia coli* strains tested attached to both cell types; strains from patients with acute pyelonephritis attached more often than those from patients with acute cystitis or asymptomatic bacteriuria. The attachment of *P. mirabilis* to squamous epithelial cells was high about day 15 of the menstrual cycle of the epithelial cell donor, but low at the beginning and the end of the cycle. In contrast, the attachment of *E. coli* to squamous and transitional epithelial cells did not vary significantly with the menstrual cycle of the cell donor. Differences in adhesion characteristics of *E. coli* and *P. mirabilis* may relate to the differences in clinical appearance of urinary tract infections produced by the two organisms.

Proteus are rarely a cause of nonobstructive urinary tract infections (UTI). *Proteus* UTI is more common in young boys and in patients with repeated infections or abnormalities in the urinary tract (1). Differences in bacterial virulence factors which explain the lower efficiency of *Proteus* in causing UTI in patients without obstruction of the urinary tract have been looked for (7). When *Escherichia coli* causes primary UTI in children, the severity of infection relates to certain O and K antigens on the bacteria and to resistance to the bactericidal effect of normal human serum (3). Although *P. mirabilis* possess O antigens, no special association has been found between the type of O antigen and UTI (2). Furthermore, *P. mirabilis* strains lack a polysaccharide capsule (7, 8).

The capacity of *E. coli* isolated from the urine of children with UTI to attach to human urinary sediment epithelial cells in vitro relates to the severity of infection produced by the strains in vivo (12, 13). Adhesive capacity has been suggested to be a virulence factor for a strain of *P. mirabilis* causing experimental pyelonephritis in rats (10). No relationship between virulence and capacity to attach to human vaginal epithelial cells was found for a small number of *Proteus* strains from UTI patients (6; T. Stamey, personal communication).

The aim of the present study was to measure the attachment of *P. mirabilis* to human urinary sediment epithelial cells in relation to the clinical origin of the strains and to compare the adhesion characteristics with those of *E. coli* strains.

MATERIALS AND METHODS

Bacteria. A total of 335 *P. mirabilis* strains were included in the study (see Table 1). For isolation criteria see Larsson and Olling (7). The strains were collected from the blood of patients with bacteremia, stools of healthy controls and patients with diarrhea, and from the urine of bacteriuric adults and from children with or without neurogenic bladder disorders. All the strains had previously been typed for *Proteus* O antigen and for sensitivity to the bactericidal effect of normal human serum (7). For comparison, 90 *E. coli* strains, isolated from the urine of 30 children with acute pyelonephritis, 30 with acute cystitis, and 30 with asymptomatic bacteriuria (ABU) (13), were used.

Attachment to human urinary sediment epithelial cells in vitro. The capacity of each strain to attach to human urinary sediment epithelial cells in vitro was tested, using epithelial cells from the sediment of urine freshly voided by either of two nonbacteriuric adult female donors as earlier described (11). The epithelial cells were suspended in phosphate-buffered saline (pH 7.2, 300 mosmol/liter) and quantitated by direct light microscopy (11). The bacteria were cultured overnight in brain heart infusion broth, spun down, suspended in phosphate-buffered saline,

and quantitated by direct light microscopy. To 10^5 epithelial cells were added 10^8 bacteria and phosphate-buffered saline to a volume of 1 ml. After incubation for 60 min at 37°C, unattached bacteria were eliminated by repeated washing with 10 ml of phosphate-buffered saline. The number of bacteria attached to 40 epithelial cells (10 transitional and 30 squamous) was counted by direct light microscopy. A drop of trypan blue was added to allow exclusion of stained dead cells. The adhesion of each strain was given as the mean number of bacteria per cell determined from a count of 40 cells. The sediment epithelial cells were classified as squamous or transitional on the basis of morphology by light microscopy (2, 5).

Experimental design. First, all 335 *P. mirabilis* strains from the six diagnosis groups were tested for adhesive capacity. Equal numbers of strains from each of the groups of *P. mirabilis* strains were tested each day to avoid systematic day-to-day variation inherent in the method (11).

Second, the attachment to either squamous or transitional epithelial cells was compared for *P. mirabilis* and *E. coli* strains. A sample of 30 *P. mirabilis* strains was randomly selected from each diagnosis group and compared with 30 *E. coli* strains from each of the three UTI groups earlier described. Three strains from each of the six groups of *P. mirabilis* strains and two strains from each of the three groups of *E. coli* strains were tested daily. The sediment epithelial cells for this part of the study were obtained from one person. The adhesion for each strain is given as the mean number of bacteria attached to 10 transitional epithelial cells and 30 squamous epithelial cells.

Third, preliminary experiments had indicated a variation of *P. mirabilis* attachment in relation to the menstrual cycle of the cell donor. Thus, the day in the menstrual cycle of the cell donor was registered in the experiments mentioned above. Furthermore, three groups of strains were tested about day 15 and retested either at the beginning or end of the cycle.

Statistical significance was tested by the χ^2 test.

RESULTS

The adhesive capacity of the *P. mirabilis* strains was high, regardless of origin (Table 1). No consistent difference in mean adhesion was found between the *P. mirabilis* strains isolated from UTI patients and those from patients with other types of infections (Table 1). No correlation was found between adhesive capacity and presence or absence of O antigen or resistance or sensitivity to the bactericidal effect of normal human serum (data not shown).

The *P. mirabilis* strains attached only to squamous and not to transitional epithelial cells (Table 2). Many of the *E. coli* strains attached to both epithelial cell types: 100% of the acute pyelonephritis strains, 60% of the acute cystitis strains, and 30% of the ABU strains.

The attachment of *P. mirabilis* to squamous epithelial cells varied with the menstrual cycle of the cell donor, with a maximum around day 15 (Fig. 1). This was found both by using the mean adhesion values of the randomly chosen strains tested each day and by comparing duplicate experiments with the same strains on different cycle days (Table 3). No relationship to the menstrual cycle was found for the attachment of the *E. coli* strains to transitional epithelial cells or to the total of 40 cells counted in each sample. A weak but not significant tendency of higher *E. coli* attachment to squamous epithelial cells about day 15 than that found early or late in the cycle is indicated in Table 3.

TABLE 1. *Attachment of P. mirabilis strains from various sources to human sediment epithelial cells*

Diagnosis	Origin of *P. mirabilis* strains	No. of strains	Mean adhesion (bacteria/epithelial cell)[a]	Proportion of adhering strains[b]
Bacteremia	Blood	89	20.5	55
Diarrhea	Stools	56	24.9	68
Healthy	Stools	36	32.4	83
Adult bacteriuria	Urine	62	20.7	48
Childhood bacteriuria	Urine	46	32.6	70
Childhood NBD[c]	Urine	46	41.3	85

[a] Each value represents the mean of the individual adhesion values of all the strains in each diagnosis group.

[b] Each value represents the proportion of strains in each group with a mean adherence of ≥10 bacteria per epithelial cell (percentage of the total number of strains in each group).

[c] NBD, Neurogenic bladder disorders.

DISCUSSION

The capacity to attach to human urinary sediment epithelial cells was high for most of the 335 *P. mirabilis* strains tested in the present study. A subsample of 180 *P. mirabilis* strains, 30 from each patient group, was studied more closely, and the adhesion characteristics were compared with those of 90 *E. coli* strains. All the *P. mirabilis* strains attached only to squamous and not to transitional epithelial cells. The attachment was higher on about day 15 of the menstrual cycle of the epithelial cell donor than early or late in the cycle.

No consistent relationship was found between the adhesive capacity and the clinical origin of the *P. mirabilis* strains. Isolates from severely ill patients, from children in which *P. mirabilis* colonized in the bladder but who had no symptoms, and those from the stools of healthy persons attached to about the same extent. This is in contrast to *E. coli*, in which isolates from the urine of patients with symptomatic UTI attached significantly more than, for example, iso-

TABLE 2. *Attachment of E. coli and P. mirabilis bacteria to squamous or transitional epithelial cells from the sediment of human urine*

Species	Diagnosis[a]	Origin	No. of strains	Mean adhesion (bacteria/epithelial cell)	
				Transitional	Squamous
E. coli	Acute pyelonephritis	Urine	30	16	17
	Acute cystitis	Urine	30	8	8
	ABU	Urine	30	2	5
P. mirabilis	Adult	Urine	30	0	17
	Childhood NBD	Urine	30	0	25
	Childhood non-NBD	Urine	30	0	24
	Bacteremia	Blood	30	0	19
	Diarrhea	Stools	30	0	18
	Healthy	Stools	30	0	26

[a] ABU, Asymptomatic bacteriuria; NBD, neurogenic bladder disorders.

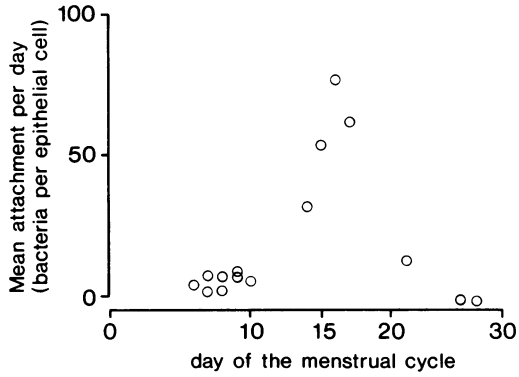

FIG. 1. *Attachment of P. mirabilis to human urinary tract epithelial cells, harvested from one cell donor throughout the menstrual cycle. Each day, 18 strains were tested, and the values represent the mean adhesion of the 18 strains as tested during two cycles.*

TABLE 3. *Attachment to squamous epithelial cells of P. mirabilis and E. coli strains: repeated testing of the same groups of strains on different days of the menstrual cycle*

Strain	No. of strains	Day of cycle	Attachment		Day of cycle	Attachment	
			Mean (bacteria/cell)	Proportion (%)[a]		Mean (bacteria/cell)	Proportion (%)[a]
P. mirabilis	12	16	76	83	28	5	17
	12	17	60	83	8	7	25
	22	15	65	86	27	4	28
E. coli	8	16	10	37	28	2	0
	8	17	16	50	8	1	0
	14	15	7	14	27	7	29

[a] Each value represents the proportion of strains with a mean adhesion of ≥10 bacteria per cell.

lates from the stools of healthy children (12, 13). The capacity to attach to a surface coated with squamous epithelium may still be a factor promoting colonization by *P. mirabilis* preceding the onset of UTI and other infections in relation to mucous membranes. Other bacterial properties may then determine the virulence and initiate disease.

In *E. coli* causing UTI, lipopolysaccharide (O antigens) and capsular polysaccharide (K antigens) are associated with virulence (3). *E. coli* strains belonging to the O groups that are most often associated with acute pyelonephritis attached more to human urinary sediment epithelial cells than did strains of other O groups (12). In the present study, however, the adhesive capacity of the *P. mirabilis* strains was not related to the O antigen specificity. Since *P. mirabilis* mostly infect hosts compromised by underlying disease or abnormalities, the number of virulence factors required to produce disease may be smaller than that for *E. coli*.

The patient groups contracting UTI due to *P. mirabilis* differ from those who get *E. coli* UTI. In children without obstructions of the urinary flow, 80% of first UTIs are caused by *E. coli* (3), and *E. coli* are isolated from the majority of patients with bacteriuria both among hospital patients and outpatients. In patients with recurrent UTI, obstructions, stones, residual urine, etc., *P. mirabilis* become increasingly common (14). The deficient attachment to bladder epithelial cells of the *P. mirabilis* strains tested in the present study may indicate that *Proteus* colonize the vaginal and periurethral area as efficiently as *E. coli*, but are eliminated more easily from the bladder at voiding unless there is residual urine, obstructions, or other predisposing factors.

The attachment of the *E. coli* strains tested was much less affected by the hormonal status

of the cell donor than was the attachment of *P. mirabilis*. Higher attachment of *E. coli* to exfoliated urinary sediment epithelial cells during the early phase of the menstrual cycle has been suggested (9) but has been confirmed neither by our system, nor by using periurethral cells (G. Källenius, personal communication). About day 15 of the menstrual cycle, the estrogen levels are maximal and the vaginal epithelium is in the proliferative phase. A hormone-induced change in the epithelium may explain the higher receptivity for attaching *P. mirabilis* bacteria. It is known that epithelial maturation involves a change in the surface glycolipid pattern of the cells (4). Glycolipids from human sediment epithelial cells are shown to be receptor structures for some *E. coli* bacteria attaching to those cells (H. Leffler and C. Svanborg Edén, submitted for publication). If a similar receptor structure exists for *P. mirabilis* bacteria attaching to squamous epithelial cells, it might vary in density and be maximal on around day 15 of the menstrual cycle. The hormonal status of the patients attracting *Proteus* UTI and the possibility of a link to hormone levels among young boys contracting UTI need investigation.

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