Attachment of Proteus mirabilis to human urinary sediment epithelial cells in vitro

AL-Araji K. Mohammed*

Received 5/3/2004 ; accepted 2/2/2005

الخلاصة

تم جمع 335 نموذج لعتر بكتريا بروتياس ميرابيليس من مختلف مصادر جسم الانسان حيث تم مشاهدة قابلية التصاقها بالخلايا المبطنة للجهاز البولي والموجودة في ترسبات الادرار, حيث تم عزل 30 بكتريا من نماذج الدم الملوث لتلك البكتريا وكذلك من مسحات لألتهابات حروق الجلد والبالغ عددها 59 مسحة ومن نماذج البراز والبالغ عددها 36 نموذج من اشخاص في صحة جيدة وكذلك من نماذج خروج لحالات الأسهال والبالغ عددها 56 نموذج ومن نماذج ادرار 62 نموذج من البالغين و92 نموذج من الصغار والتي كانت جميعها تحتوي هذه البكتريا.

من خلال الدراسة تم ملاحظة قابلية التصاق بكتريا البروتياس ميرابيليس بالخلايا المبطنة المترسبة والموجودة في الادرار حيث تكون نسبتها عالية للخلايا المبطنة الأعتيادية ولم تحدث عملية الألتصاق للخلايا المبطنة الموجودة في الجهاز البولي للأنسان من النوع المتدرج. وتم ملاحظة ايضاً قابلية تلك البكتريا بالألتصاق بنسبة عالية في اليوم الخامس عشر من الدورة الشهرية لدى النساء وتقل نسبتها بدرجة عالية في بداية الدورة ونهايتها.

ABSTRACT

In vitro attachment of 335 *Proteus mirabilis* strains from various human sources to human urinary tract epithelial in adhesive capacity was found between *Proteus mirabilis* strains were isolated from the blood of 30 patients with Bacteria, Burns swabs 59 infected skin, the stool of 36 healthy subjects and 56 patients with diarrhea and the urine of 62 adults and 92 children with bacteremia. High mean adhesion values were observed in all groups. The *Proteus mirabilis* strains attached only to sequamous cells and not to transitional epithelial cells.

The attachment of *Proteus mirabilis* to sequamous 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.

INTRODUCTION :

Proteus are rarely a cause of non obstructive 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)]. Difference in bacterial virulence factors which explain the lower efficiency of Proteus in causing UTI in patient without obstruction of the urinary tract have been looked for[(2)].

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.

The aim of the present study was to measure the attachment of *Proteus mirabilis* to human urinary sediment epithelial cells in relation to the clinical origin of the strains.

MATERIALS AND METHODS:

Bacteria :

A total of 335 *Proteus mirabilis* strains were included in the study (see table 1). The strains were collected from the blood of patients with bacteremia burns swabs from infected skin, stool of the healthy controls and patients with diarrhea and from the urine of bacteremic adults and from children with or without neurogenic bladder disorders.

*Pharmaceutical Biotechnology & Microbiology Department, College of Pharmacy, Almustansiriya University, Baghdad – Iraq.

All the strains had previously been typed for Proteus O-antigen and for sensitivity to the bactericidal effect of normal human serum.

Bactericidal Sensitivity of Normal Human Serum :

Bacteria were grown at 37° C in neutrient broth (Oxoid England) cells were sedimented by centrifugation at 8000 r.p.m. for 10 minutes and suspended in one-fifth the original volume of Veronal-buffered glucose mixture.
A 0.1ml sample of the bacterial cell suspension was added to 0.9ml of serum-veronal-buffered glucose mixture and incubated in a standing water bath at 37° C survival of 100% was equivalent to 108 cells/ml, in all cases. Duplicated 0.05ml samples were withdrawn after 5,10,20,30,60 and in some cases 120 minutes of incubation, diluted in buffered saline with gelatin solution and plated by the soft agar. Sera diluents and agar used with *Proteus mirabilis* strain were supplemented with 20µg of thymidine per milliliter. Agar plates were incubated for 24 or 48 hours at 37° C and colonies were enumerated.

Adherence to human Uroepithelial cells :

Throughout all experiments, human uroepithelial cells were collected from the freshly voided urine of one healthy woman without a history of urinary tract infection. To collect these cells 30ml of urine was filtered slowly under low volume on a 12µm sortorius membrane filter (diameter 5cm, Gattingen West Germany) until a small volume was left. This residue was washed three times with phosphate-buffered-saline (PBS pH 7.0). After the third washing the residue was filtered down completely allowing the epithelial cells to settle on the filter at random.
The epithelial cells were transferred to a glass cover slip with epithelial cells was placed in sterile small glass Petri dishes and dried for 15 minutes.
The adherence assays were performed in these sterile glass Petri dishes. Bacteria were grown over night in neutrient broth (Oxoid, London, England) with gentle agitation 37℃ cells were spun down and suspended carefully in phosphate buffered saline to a concentration of 108 cells/ml.

Adherence assay :

5ml of the bacterial suspension was added to sterile glass Petri dishes containing a cover slip with attached epithelial cells and incubated for one hour with gentle shaking at 37° C. The cover slip was removed from the glass Petri dishes and washed twice with PBS to remove bacteria that had not adhered. The epithelial cells were fixed for 15 minutes in methanol, washed twice again with PBS and stained for 20 minutes with 30% filtered Giemsa stains. After two washings with distilled water, the cover slip was dried in the air and mounted upside down on a glass slide.
The number of bacteria attached to 40 epithelial cells (10 transitional and 30 sequamous) was counted by directed light microscopy. 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 a sequamous or transitional on the basis of morphology by light microscopy.

Experimental design :

First: All 335 *Proteus mirabilis* strains from the serum diagnosis groups were tested for adhesive capacity, equal number of strains from each of the groups of *Proteus mirabilis* strains were tested each day to avoid systematic day-to-day variation.
Second: The attachment to either sequamous or transitional epithelial cells was compared for *Proteus mirabilis*. A sample of 30 *Proteus mirabilis* strains was randomly selected from each diagnosis group. Three strains from each of the seven groups of *Proteus mirabilis* strains were tested daily.

The sediment epithelial cells for this part of the study were obtained from one person. The adhesion for each strains is given as the mean number of bacteria attached to 10 transitional epithelial cells and 30 sequamous epithelial cells.

Third: Preliminary experiments had indicated a variation of *Proteus mirabilis* attachment in relation to the menstrual cycle of the cell donor. Thus, the day in the menstrual cycle of the 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.

RESULTS:

The adhesive capacity of the *Proteus mirabilis* strains was high, regardless of origin (table 1).

Table 1 . Attachment of Proteus mirabilis strains from various sources to human sediment epithelial cells

Diagnosis	Origin of Proteus mirabilis strains	No. of strains	Mean adhesion bacterial / epithelial cells	Proportion of adhering strains
Bacteremia	Blood	30	6.8	18
Burns swab	Swabs	59	13.6	37
Diarrhea	Stool	56	24.9	68
Healthy	Stools	36	32.4	83
Adult bacteriurea	Urine	62	20.7	48
Childhood bacteriurea	Urine	46	32.6	70
Childhood neurogenic Bladder disorders	Urine	46	41.3	85

No consistent difference in mean adhesion was found between the *Proteus mirabilis* strains isolated from (UTI) patients and those from patient with other types of infection (table 1). No correlation was found between adhesive capacity and presence of O-antigen or resistance or sensitivity to the bactericidal effect of normal human serum (data not shown).

The *Proteus mirabilis* strains attached only to sequamous and not transitional epithelial cells (table 2).

Table 2 . Attachment of P. mirabilis bacteria to squamous and transitional epithelial cells from the sediment of human urine

Bacteria	Diagnosis	Origin	No. of strains	Mean adhesion (bacteria / epithelial cell)	
				transitional	squamous
Proteus mirabilis	1- Adult	urine	30	-	17
	2-Childhood neurogenic Bladder disorder	urine	30	-	25
	3-Childhood non-neurogenic bladder disorder	urine	30	-	24
	4- Bacterimia	blood	30	-	19
	5- Diarrhea	stools	30	-	18
	6- Burn infection	swabs	30	-	17
	7- Healthy	stools	30	-	26

The attachment of *Proteus mirabilis* to sequamous epithelial cells varied with the menstrual cycle of the cell donor with a maximum around day 15. This was found both by using the mean adhesives values of the randomly chosen strains tested each day and by comparing duplicate experiments with the same strains on different cycle day (table 3).

Table 3 . Attachment to sqaumous epithelial cells P. mirabilis strains, repeated testing of the same groups of strains on different days of the menstrual cycle

Strains	No. of strains	Day of cycle	Attachment		Day of cycle	Attachment	
			Mean bacterial/cells	Proportion (%)		Mean (bacterial/cells	Proportion (%)
Proteus	12	16	76	83	28	5	17
mirabilis	12	17	60	83	8	7	25
	22	15	65	86	27	4	28

DISCUSSION:

The capacity to attach to human urinary sediment epithelial cells was high for most of the 335 *Proteus mirabilis* strains tested in the present study. A sub sample of 210 *Proteus mirabilis* strains 30 from each patient group was studied more closely. All *Proteus mirabilis* strains attached only to sequamous and not to transitional epithelial cells. The attachment was higher on about day 15 of the menstrual cycle of the epithelial cells donor than early or late in the cycle. No consistent relationship was found between the adhesive capacity and the clinical origin of the *Proteus mirabilis* strains. Isolates from severely ill patients, from children in which *Proteus mirabilis* colonized in the bladder but who no symptoms and those from the stool of healthy persons attached to about the same extent.

The capacity to attach to surface coated with sequamous epithelium may still be a factor promoting colonization by *Proteus mirabilis* preceding onset of UTI and other infections in relation to mucus membranes. Other bacterial properties may then determine the virulence and initiate disease. The deficient attachment to bladder epithelial cells of the *Proteus mirabilis* strains tested in the present study may indicate that Proteus colonize the vaginal and periurethral area as efficiently but are eliminated more easily from the bladder at voiding unless there is residual urine, obstructions, or other predisposing factors. 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 *Proteus mirabilis* bacteria. It is known that epithelial maturation involve a change in the surface glycolipid pattern of the cells (Karlsson 1996).

The normal status of the patient attracting Proteus UTI and the possibility of a link to hormone level among young boys contracting UTI need investigation.

REFERENCES:

1. Bergstram T. Sex differences in childhood urinary tract infection. Achieved Disease Children 1972, 47:227 – 232
2. Karlsson K.A. 1996, Aspects on structure and function of sphingolipid in cell surface membranes pages 245 – 274. In S. Abrahamson and I. Pascher (ed). Structure of biological membrane. Plenum publishing corporation, New York.
3. Larsson P. and S. Olling O-antigen distribution and sensitivity to the bacterial effect of normal human serum of Proteus strains from clinical specimens. Medical Microbiology and Immunology 1979, 163: 77 – 82.