

ADHESION OF UROPATHOGENIC AND ENTEROPATHOGENIC *Escherichia coli* TO HUMAN BUCCAL EPITHELIAL CELLS

ABDUL RAHMAN AL-FAHDAWI,

COLLEGE OF MEDICINE, AL-ANBAR UNIVERSITY



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ABSTRACT

Fifty urine samples were collected from patients with urinary tract infections and a 50 stool specimens from diarrhoeal patients . These samples were investigated bacteriologically. The invitro adherence test of Enteropathogenic and Uropathogenic *E. coli* to healthy human buccal epithelial cells was carried out. The results show no significant differences in the mean adhesion of these bacteria (two sources) on the epithelial cells as compared with the control . but there is a little variation among EPEC isolates and among UPEC isolates too as compared with the control.

INTRODUCTION:

The ability of bacteria to adhere to mucous membrane is regarded the first step in the pathogenesis of certain infections. Such attachment to epithelial cells initially results in colonization with or without subsequent invasion and inflammation^(1,2). The adherence of bacteria to the epithelial cells is the net result of a complex process which often involve “lock and key” type interaction between lectin-like bacterial attachment protein called “adhesin” and specific complex carbohydrate structures of the host cell membrane (receptors). However, this adherence would appear to depend on both host and bacterial factors^(3,4,5). However, it is found later that some bacteria may exhibit adhesive properties without expressing pili. These bacteria express soluble adhesins that surrounded the bacterial cells like a capsule and was called non-fimbrial adhesins (NFA). Studies show that fimbrial adhesins have an independent genetic determination than those of (NFA)^(6,7).

Furthermore, it has been observed that organisms freshly cultured from UTIs are frequently piliated. However, piliated bacteria not only to receptors of the UECs but pili also tend to aggregate at pH 4 and produce pili-pili aggregates that attach to mucous layer. Both mechanisms could be important in allowing greater number of bacteria to attach to mucosal surface and so it is very important in the pathogenesis of certain infections^(8,9).

METHODS:

Specimens' collection

A- Stool: Stool specimens were collected from a fifty patient complaining from diarrhea. The age of those patients ranging from (1-6) years . 33 male and the remainder are females in Al-Ramadi pediatrics hospital.

B- Rectal swabs: Rectal swabs were collected from 10 healthy infants to isolate *E. coli* bacteria as a control group.

C- Urine:Midstream urine samples were collected in

* Corresponding author at: College of Medicine, Al-Anbar University, Iraq.E-mail address: abdurahmanfahdawi@yahoo.com

clean and sterile screw capped bottles. In catheterized patients and patients having nephrostomy tubes, urine samples were collected from the catheter or the tube directly ⁽¹²⁾.

Cultivation and examination of samples

Properly collected samples were cultured in measured amount on solid media. Most of the specimens were dealt with within about one hour after collection ⁽¹²⁾. Each specimen was inoculated on nutrient, blood and MacConkey agar plates by direct streak plate method. The plates were then incubated at 37° C for (18-24) hours, thereafter, they were examined for bacterial growth ⁽¹²⁻¹⁴⁾.

3- Identification of bacterial growth

The colonies of bacteria may have different morphological characteristics on solid media as described by Parker ⁽¹⁵⁾.

Agglutination test: The agglutination test may be performed microscopically by mixing a loopful of antisera with a suspension of microorganisms on a slide and inspecting the result through the low power objective. This is commonly done for the identification of unknown culture ⁽¹¹⁾. This test was used to identify *E. coli* strains by treating them with specific antisera (*E. coli* specific antisera 1 and 2).

Human Buccal Epithelial Cells (HBECs)

Cells were collected by scraping the oral mucosal membranes of the healthy female with wooden

applicator and were suspended in phosphate buffer saline (PBS), then the cells were centrifuged at 3000 rpm washed 3 times and resuspended in PBS at a concentration of 2×10^6 / ml as determined by haemocytometer, then the cells was used in the adhesion test with the test bacteria.

In vitro adherence test

The technical details were described in a methodological study ⁽¹⁸⁾. Bacteria were transferred from deep agar subculture, where they had kept after isolation, to nutrient agar plates. After growth for 16-48 hrs at 37° C in brain heart infusion (BHI) broth, the bacteria were centrifugated and resuspended in PBS pH 7.4. Epithelial cells were washed and resuspended in PBS. 0.5 ml of an overnight culture of bacteria in broth was gently added to 0.5 ml of HBECs suspension. The mixture was mixed by inversion and then was incubated for 1 hr at 37° C with shaking every 10 minutes. The HBECs were then washed four times with PBS thus unattached bacteria were eliminated by repeated cycle of centrifugation and resuspension ^(17, 19,20,21). A drop of the final ECs suspension was deposited on a glass slide, air-dried, heat fixed and Gram stained. A control slide with no attached test bacteria was stained at the same time ⁽⁷⁾. Examination of the slide was done by light microscopy under oil immersion to demonstrate the attachment bacteria to

HBECs^(20,21).

6-Statistical methods: Chi square test was used in the present study.

RESULTS:

Table 1 and figure 1 show the percentages of adhering bacteria to HBECs from healthy females. It is obvious that there was no association between source of bacteria and adhering ability of bacterial isolates. No significant difference in the mean adhesion of bacteria was found to HBECs. The table also shows variation in the mean adhesion of different bacterial isolates to HBECs of the same source, compared with the control group. That means the difference in the adherence capacity of bacteria is related to the properties of the infecting microorganisms other than in HBECs.

Figure 2 shows the adhesion of Uropathogenic *E. coli* to HBECs under the microscope (oil immersion lense).

DISCUSSION:

Table 1 and figure 1 show the percentages of adherent bacteria to HBECs compared with the control. From these results it is obvious that most bacterial strains express or have the genetic potential to express several adhesins. Most strains show decrease in the number of attached bacteria (poorly attached to HBECs) that means strains carrying only type-1 pili attached poorly to HBECs, whereas strains carrying mannose resistant

agglutinin attached in high number⁽²²⁾. Also mannose-resistant adhesins on urinary tract have not been shown to cross-react⁽¹⁹⁾.

The ability of bacteria to adhere to HBECs does not depend on the amount of Gal α 1 \rightarrow 4 Gal β containing receptors on the HBECs. No significant differences in the mean adhesion of bacteria was found HBECs, this confirms the hypothesis that the expression of Gal α 1 \rightarrow 4 Gal β containing glycolipids in HBECs does not differ in other words, Gal α 1 \rightarrow 4 Gal β containing receptors were equally distributed on HBECs. thus the isolated pili and whole piliated bacteria showed identical haemagglutination and adhesion patterns⁽¹⁹⁾.

The attachment of bacterial strains with their adhesins was independent on source of infection, this was shown by similar levels of adherence to HBECs.

Tables 1 also shows a variation in the mean adhesion of different bacterial isolates to HBECs in the same source compared with control group. Within most HBECs a great variability in the number of adhering bacteria per cell was observed. A possible explanation of this observation could be differences in the size of HBECs. Cell-age could be another factor of importance for adhesion, also day-to-day variations of bacteria is an important factor⁽²²⁾.

The ability of bacteria to associate with

mammalian cells varies between cells from different species, tissues, and even individuals. Each bacterial strain has a special range of cells to which it can bind. The recognition of receptors by the bacteria is very necessary for attachment of bacteria to HBECs, other factor explaining the decrease number of attached bacteria to HBECs is decreased viability of bacteria or cells. The bacteria may carry more than one type of adhesive abilities ^(16,18).

The pathogenesis of UTIs and is poorly understood. Many factors may explain the variability of bacterial adherence to the cells, some properties of invading bacteria seem to play an important role, by surface characteristics of HBECs, by secretions normally bathing the cells, repeated washing may have reduced the number of attached bacteria. The individual spreads in number of adhering bacteria suggest real differences between cells within individual population perhaps related to cell-age. Cell-age differences which favour adhesion, in which some pathogens attach more readily to young epithelial cells other than more mature cells. The increased adherence appears to be non specific with respect to bacterial species but depends upon the HBECs characteristics such as surface charge and hydrophobic properties ⁽²³⁾. Bacterial surface characteristics are of importance for adherence of bacteria. This capacity

seems to be related to presence of pili ⁽²³⁾.

The ratio of the different cell types is known to vary between days and individuals ⁽²⁴⁾. The population of HBECs may contain both viable cells and cells in early stages of cell death seem much less sensitive to various treatments than do bacteria. Also the metabolic activity of the discharged cells may play an important role in the variation in receptivity for adhering bacteria between individual ECs ⁽¹⁸⁾.

The variation of adhesion properties of bacteria may be related to many factors like bacterial numbers, pH, and osmolarity, bacterial growth phase is important factor. Bacteria become more adherent in stationary phase and less in exponential phase, nutrient supply also necessary since the nutrients are very important in fimbriation of bacteria. There are several factors which play a role in decreasing the adhesive ability, like the bladder defence mechanism which induces several component such as the bladder mucin layer, Tamm-Horsfall protein, local immune response mainly SIgA (cellular and humoral immune response) and the wash out phenomena, also the growth limiting factors such as the pH of the urine and presence of metabolite which may participate in decreasing adherence of the bacteria to the HBECs. On the contrary there are several factors which favour the adhesion of bacteria to the

mucosa, like the production of the diffusible toxins affecting the epithelial cells, and subsequently enhance the adhesive ability, also if there is any immune deficiency that facilitates bacterial attachment as well as time of incubation of the bacteria in the urinary tract ⁽²⁵⁾.

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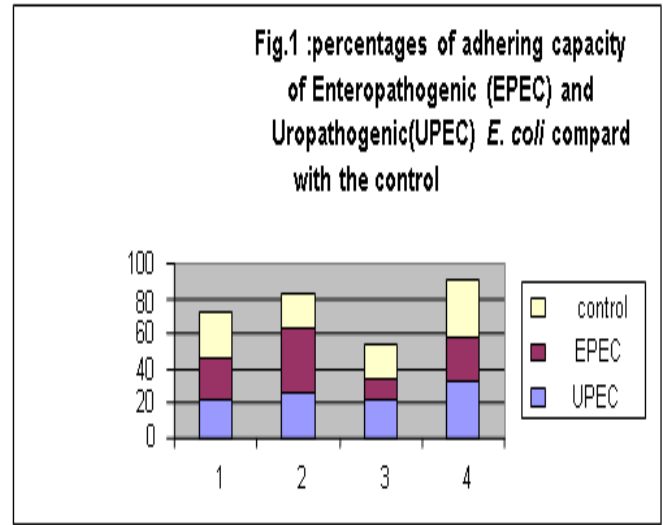
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Table 1: Percentages of adhering capacity of different Uropathogenic and Enteropathogenic *E. coli* isolates to HBECs (summarized data)

Bacterial isolates*	Group 1	Group 2	Group 3	Group 4
Uropathogenic <i>E. coli</i>	21.0	26.0	21.0	32.0
Enteropathogenic <i>E. coli</i>	25	37	13	25
Control	26.5	20.3	20.3	32.8

* Each group includes three isolates.



التصاق جراثيم الايشريكية القولونية المسببة لالتهابات المجاري البولية والإسهال على الخلايا الظهارية الوجدية للإنسان

عبد الرحمن محمد جبران الفهداوي

كلية الطب – جامعة الانبار

E.mail: abdurahmanfahdawi@yahoo.com

الخلاصة:

تم أخذ 50 عينة إدرار من المرضى المصابين بالتهابات المجاري البولية و 50 عينة براز من المرضى المصابين بالإسهال وتم تحليلها بكتيريولوجيا. تم اختبار اللصق على الخلايا الظهارية الوجدية للإنسان الطبيعيات أظهرت النتائج عدم وجود فرق معنوي في معدل التصاق الجراثيم على مختلف الخلايا الظهارية البولية. المستويات المتشابهة من التصاق الجراثيم على تلك الخلايا الظهارية تثبت تلك النتيجة، لكن وجود فروق بسيطة في معدل التصاق الجراثيم المعزولة من نفس المصدر بالمقارنة مع مجموعة السيطرة.