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Original Article

The comparison between virulence factors of *Escherichia coli* isolated from urinary tract infections and feacal flora

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Abstract

Urinary tract infections (UTIs) are significant health problem, with *Escherichia coli* as the primary pathogen in proximally 80% of cases. The adhesion of *E coli* to the host cell can be influenced by cell surface hydrophobicity (CSH) and is an important factor for the development of infections. This study was conducted to find the relation between CSH and one of the adhesions (mannose resistant haemagglutin, MRHA) and a virulence factor (haemolysin), in the bacterial isolates from UTIs and comparison of the UTI isolates with normal faecal flora. The results showed a significant difference in the expression of MRHA in the UTIs compared to that of faecal flora (48% and 12%, respectively, P = 0.012). CSH was determined by two methods of salt aggregation test and bacterial adhesion to hydrocarbons. The results of these tests were correlative and UTIs isolates were found to be more hydrophobic than the normal faecal flora, while the standard strains of enterohaemorrhagic *E coli* were more hydrophobic than the normal faecal flora. Hemolysin production was higher in the isolates from UTI (28% in UTIs compared to 6% in faecal flora P = 0.0035). In conclusion, we found that the pathogenic *E. coli* express more MRHA, are more hemolytic and have a higher cell surface hydrophobicity which may help them to start an infection.

Keywords: Urinary tract infections; Cell Surface Hydrophobicity; Mannose resistant; Haemagglutin; Haemolysin; *Escherichia coli*

INTRODUCTION

Escherichia coli is the most common cause of urinary tract infections (UTI) world wide. Pyelonephritic strains of *E. coli* are able to bind to the human kidney and start the infection process (1). Certain O: K: H serotypes and virulence factors occur more frequently in urinary than faecal isolates, suggesting that uropathogenic isolates are different from normal bowel inhabitants (2,3). These markers are usually chromosomally encoded, at different frequencecies causing disease states ranging from asymptomatic bacterimia to chronic pyelonephritis (2). Attachment to host tissue is the key event in the early stages of most infectious (1). The initial adhesion of microbes to tissue can be aid in the attachment of the organism to almost all types of cells including neutrophils, epithelial cells, and also to medical devises such as urines catheters, prosthetic devices, vascular grafts and suture material (4,5). Production of hemolysins are also important in bacterial pathogenicity, and the hemolytic strains of many bacteria including *E. coli* were reported to be more common in the UTI isolates (6,7). However despite

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extensive work on the pathogenicity of UTI isolates, the cause and effect relationship between bacterial adhesion, and other virulence factors on the initiation of UTI has not been proven. There are controversies concerning the origin of *E. coli* strains which cause UTIs in men. It is unknown whether in human, especially the men with UTI the causative *E. coli* strains usually derive immediately from host's own intestinal flora (faecal-urethral hypothesis), or the particular strains do so merely because of their high prevalence with the host's intestinal flora (prevalence hypothesis), or the special pathogenicity hypothesis, that of UTI isolates upon faecal flora unable them to cause UTI. This study was performed in order to compare the pathogenicity of faecal and UTI isolates. Three proposed virulence factors namely hemolysin, haemagglutinin and cell surface hydrophobicity was determined and compared in the *E. coli* isolates from the UTIs with that of normal feacal flora.

MATERIALS AND METHODS

Bacterial isolates

Samples from Urinary tract or stool of patients admitted to clinical laboratories were included in this study. From these samples 100 *E. coli* isolates were identified by common biochemical reactions (8), these were comprised of 50 isolates from symptomatic urinary tract infections and 50 isolates from faecal flora. Reference strains of *E. coli* ATCC 25922 (non-pathogenic) and two strains of verotoxogenic (VT1 and VT2) strains of enterohaemorrhagic *E. coli (*obtained from Pasteur Institute in Iran), were included in this study.

Mannose resistant fimbrial haemagglutination (MRHA)

For this experiment the bacteria were grown on blood agar plates. After overnight incubation at 37 ºC, 1 to 2 single colonies were picked and suspended in saline (0.85% NaCl) to give a turbid suspension [2.4 x 10^9 CFU/ml equaling to the tube 8 of McFarland standards (8)]. Red blood cells suspension (3%) in PBS was prepared by washing fresh citrated blood group O^+ (2000 × g, 10 min, 4 ºC). The test was performed in presence of 0.5% (w/v) D-mannose according to Duguid et al. (9). Haemagglutination was considered to be mannose resistance when it occurred in presence of D-mannose. All slides were placed at 4 ºC, and agglutination was observed after 2, 5, 10 and 15 min, the weak reactions after 5 min incubations were considered positive if it was enhanced during the incubation.

Cell surface hydrophobicity (CSH)

CSH was determined by the method of salt aggregation (SAT) and adherence to hydrocarbons (AHT).

SAT: A selected number of bacteria (Table 2) including the standard strains were grown on nutrient broth culture to the end of logarithmic phase of growth. Fifty ml of broth cultures were incubated under standstill conditions in 250 ml flasks. The bacterial cells were harvested (2000 x g,10 min, 4 ºC), washed three times with PBS (0.02 M, pH 6.8) and the suspension was diluted with the same buffer to match with McFarland tube 10 to get colony counts of approximately 5x10^9 (8).

Standard procedure of salt aggregation test was performed according to Lindahl et al. (10). Sodium phosphate buffer was used to dilute the solution of 4 M Ammonium sulfate ranged from 0.02 to 4 M. The pH of the mixture was adjusted to 6.8 with NaOH when necessary. On a glass depression slide, 25 µl of bacterial suspension was mixed with an equal volume of salt solutions. The mixture was gently rocked for 2 min at room temperature and observed for aggregation. The highest dilution of salt (final concentration) giving visible aggregation was considered as a numerical value for bacterial surface hydrophobicity. All reactions were compared to the reaction at the highest molarity of the salt (positive control), and bacterial suspension mixed with equal volume of 0.002 M phosphate buffer (pH 6.8) was regarded as the negative control.

Bacterial adhesion to hydrocarbon (BATH) BATH was assessed with xylene (Merck) according to Rosenberg (11). The bacterial suspension was prepared as was mentioned by SAT. One ml of the bacterial suspension was mixed with 1 ml phosphate buffer (0.02 M, pH 6.8), and the absorbency of the sample was measured with a Nova spectrophotometer (LKB, Germany) at 400 nm. After that, to 1 ml of this bacterial suspension 0.6 ml of xylene was added. The mixture was vigorously mixed for 2 min, and allowed to settle for 20 min at room temperature. Following phase separation, the bottom aqueous phase was removed and carefully transferred to a disposable 1 ml cuvette, and the light absorbance at 400 nm was recorded. Turbidity of the aqueous phase (A), and initial sample (A^{o}) was used to determine the percentage of the cells that adhered to hydrocarbons using the following formula:

Percent adhesion= $1-(A/A_{\circ})$ x100 (12).

The hemolytic activity of the isolates was observed after 24 h of incubation of the bacteria grown on defibrinated washed sheep blood agar (WSBA) plates (13).

RESULTS

From 100 *E. coli* isolates tested 24 (48%) from UTI and 12 (24%) from normal faecal flora showed MRHA. Presence of MRHA in UTI isolates was significantly higher than faecal flora (P = 0.012). Hemolysin production on WSBA was higher in the isolates from UTI compared to faecal flora (28% and 6%, respectively, P = 0.0035).

The data for adherence to xylene, SAT, MRHA and hemolysin production for selected isolates are presented in Table 1. All the isolates from UTI showed higher absorbance to hydrocarbon tested, were aggregated at lower salt concentrations, and showed complete hemolysis on WSBA.The enterohemorrhagic strains were similar to UTI isolates in respect to adherence to hydrocarbon and SAT, but were not hemolytic.

DISCUSSION

It has been suggested that special pathogenicity is the main casual factor in febrile UTIs in men (14). However the mechanism of pathogenesis of these organ-

Table 1. Percent absorbance to hydrocarbon xylene (BATH), salt aggregation (SAT), mannose resistance haemagglutination (MRHA) and hemolysin production by selected isolates of *E. coli* from UTI or faecal flora.

Code	Source	%BATH	SAT[a]	MRHA[b]	Hemolysis
526	Faecal	5	≤4	+	-
263	Faecal	27	≤4	-	-
440	Faecal	5	≤4	-	+
480	Faecal	23	3.8	+	-
523	UTI	55	012	+	+
530	UTI	84	0.08	-	+
434	UTI	40	0.12	-	+
172	UTI	46	0.08	-	+
VT1	PI*	40	0.08	-	±
VT2	PI*	45	0.012	-	-
E. coli 25922	ATCC	5	4	+	-

[a]The value represents the lowest ammonium sulfate concentration causing bacteria to aggregate in the standard procedure.

[b]Haemagglutinin resistance to 0.5% (W/V) d-mannose (MRHA) is noted.

PI*: Pasteur Institute of Iran. *Enterohemorrhagic E. coli* producing VT1 and VT2 type toxins.

-isms is yet to be well understood and several virulence factors have been postulated. These include a hemolysin protein and the mannose resistant P fimbriae (7). Fimbraei and pilli when present, can contribute to the hydrophobic character of the cell, and for some strains the presence of pilli appear to be required for adhesion (15). In pyelonephritic strains of *E. coli,* the adhesion binds to the specific receptor containing glycolipid on the surface of human epithelial cells. This interaction allows the bacteria to gain a foot hold on the tissue and resist being displaced by the mechanical and physiological forces in the kidney (1).

MRHA are adhesive factors, which are important in the establishment of pathogenic strains of *E. coli* to various host tissue, and the genetic information for a number of them is closely associated with other virulence factors (7). MRHA are also important adherence factors in intestinal infections caused by *E. coli* (9,16). In this study, expression of MRHA was detected to be higher in the isolates from UTI (50%) compared with feacal flora (26%), P = 0.012. This is in accordance with Soleimani Rahbar et al. (17) who also reported a higher rate of isolation of MRHA from UTI compared to faecal flora.

Hemolysin production is a property largely associated with *E. coli* strains which causes extra-intestinal infections in human, whereas it is rarely found in faecal isolates from healthy persons (6). We also found a significantly higher rate of hemolysin production in the isolates from UTI (28%) compared with that of faecal isolates (6%), P = 0.0035. Two isolates from faecal flora and 10 isolates from UTI expressed MRHA and hemolysin simultaneously (P = 0.01). This is not surprising, since in *E. coli* hemolysin and the P-fimbriae are often associated on the same pathogenic island (7).

Cell surface hydrophobicity (CSH) has been suggested to be important in cell adhesion and pathogenicity in *E. coli.* Pathogenic intestinal *E. coli* such as interoadherent affecting and introinvasive strains is reported to express MRHA adhesines which confers increased CSH to them (18). Enterotoxogenic *E. coli* is also reported to be more hydrophobic than the commensal isolates (19). Pvzova et al. found significat difference in the CSH, and presence of fimbria in the *E. coli* strains isolated from lower urinary tract infection compared to those strains isolated from pyelonephritic patients, which were more hydrophobic and pilated (20). In this study CSH was tested by the SAT and BATH methods. The results for these tests were correlative and showed that pathogenic isolates either causing UTI or intestinal diseases (verotoxogenic strains) were more hydrophobic than the normal faecal flora. The overall results showed that the isolates from UTI were more hemolytic, express more MRHA, and had a higher level of CSH. Further work with more isolates, is necessary to understand the effect of bacterial adherence and CSH as the starting point for the production of either UTI or intestinal infections by *E. coli.* Since the antibacterial agents can effect the CSH and alter the bacterial adhesion to different surfaces (4,7) measurement of these parameters under the sub-inhibitory concentration of the antibacterial agents, which the bacteria will grow under clinical conditions, can be an attractive target for the development of new effective antimicrobial treatment for the urinary tract infections.

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