Detection of Extraintestinal Pathogenic *Escherichia coli* among Normal Stool Flora of Young, Healthy, Unmarried Males & Females as Predisposing Factor to Extraintestinal Infections: A Comparison Study

Sareaa Maseer Gatya Al-Mayahie*  
Ahmed Edan Dhamad Al-Jafary*  
Alaa Ali Matrood Al-Kafajy*  
Zaynab Abaas Jassim Al-Maliky*  
Hanaa Hani Mohammed Ali Al-Swadi*  
Jenan Tuama Sabah Al-Qurbawi*

Received 13, October, 2009  
Accepted 22, March, 2010

Abstract:  
In this study we surveyed the dominant normal stool flora of randomly selected healthy, young (18-23 years old), unmarried (doctrinal) Iraqi college students (males and females) for the carriage of extraintestinal pathogenic *E. coli* (ExPEC). ExPEC virulence was detected phenotypically by mannose resistant hemagglutination of human red blood cells (MRHA) and mannose sensitive (MS) agglutination of Bakers' yeast (*Saccharomyces cerevisiae*). From 88 college students, 264 *E. coli* isolates were obtained (3 isolates per person): 123 from 41 females and 141 from 47 males. Of these isolates, 56% (149/264) caused MS agglutination of yeast cells and 4.16% (11/264) showed MRHA. Eighty two percent (9/11) of the isolates with MRHA also caused MS agglutination of yeast cells. Statistically the difference is not significant (*P* < 0.05) among males and females regarding the MS agglutination of yeast cells: 59% (72/123) of females' isolates vs. 55% (77/141) of males' isolates. Conversely, the difference is clear regarding the carriage of isolates with MRHA. All the isolates with MRHA were distributed among females' dominant stool flora (11/123: 8.94%) whereas none of the males' dominant stool flora showed MRHA (0/141: 0%). Five females out of 41 (12.19%) had isolates with MRHA. All the three isolates in 2 of these 5 females showed MRHA, 2 isolates in another 2 showed MRHA, and only one isolate in 1 female caused MRHA. Therefore we can say that the difference among males and females in fecal carriage of *E. coli*, with characteristics of ExPEC, can be a predisposing factor of females to ExPEC infections more than males.

Key words: fecal *E. coli*, Young males and females, Fimbriae

Introduction:  
*Escherichia coli* (*E. coli*) is a member of the normal intestinal microflora of humans and animals. It is also a common cause of extraintestinal infections such as urinary tract infections (UTI) and septicemia [1, 2], neonatal meningitis and septicemia [3, 4]. Vaginal *E. coli* has also been reported to be sexually transmissible to a male partner [5]. Recently *E. coli* has been implicated as a cause of a case known as aerobic vaginitis [3, 5]. The fecal flora is a reservoir for more virulent *E. coli* strains [6], although only a small fraction are uropathogenic [7]. Uropathogenicity may be a side effect of *E. coli*'s adaptation to the intestinal milieu [8].

*Microbiology/Department of Biology/College of Science/University of Wassit*
It is widely accepted that the reservoir for UTI is the human bowel flora and that most infections result from uropathogens moving into the bladder via the urethra [10, 11]. It is generally thought that E. coli strains involved in neonatal infections originate from the vagina, which in turn colonized from a rectal source [12].

Uropathogenic E. coli possess an array of virulence properties that act at different stages of the infectious process [13]. E. coli isolated from female reproductive tract infection and neonatal sepsis possess unique properties that may enhance their virulence. These properties are similar to those associated with other E. coli extraintestinal infections [14]. Among the most important of uropathogenic E. coli virulence characteristics are surface glycoprotein projections called fimbriae or pili which serve as ligands for glycoprotein or glycolipid receptors on uroepithelial cells. Fimbriated E. coli can be categorized as either mannose sensitive (MS) or mannose resistant (MR), based on their ability to agglutinate erythrocytes in the presence of mannose [15, 16]. The most well studied are type 1, P, and S fimbriae [11, 17]. Type 1 fimbriae are common among E. coli strains from all clinical categories of UTI and among fecal strains [15, 18, 19]. P-fimbriated strains (MR) are strongly associated with acute uncomplicated pyelonephritis [16]. Also P pili are important for ascending the UT [5] and enhancing the colonization of the UT [20]. S pili are often associated with E. coli strains that cause sepsis, meningitis and ascending UTIs [11]. Additionally Dulawa [21] reviewed that E. coli persistence in the colonic flora is facilitated by the same bacterial adhesins that promote attachment to the uroepithelium.

The incidence of UTI varies with sex, age, and predisposing conditions. UTI more frequently afflicts women than men at all ages, except in the first year of life, and it is very uncommon in healthy, young, and middle-aged men [13, 22, 23, 24]. Women who experience acute UTI are characterized by both genetic predisposition and behavioral factors. The most exposed genetic factors are non-secretors of blood substance. The most important behavioral risk factors are: recent sexual activity, use of spermicidal agents and diaphragm [16, 21]. The large difference in UTI prevalence between men and women is thought to result from a variety of factors: the greater distance between the anus and the urethral meatus, the drier environment surrounding the male urethra; the greater length of the male urethra and the antibacterial activity of prostatic fluid [16]. Since intestinal flora are considered the natural reservoir for pathogenic strains of E. coli in extraintestinal infections and as the females are more susceptible to these infections, we carried out this study to compare the carriage of extraintestinal pathogenic E. coli (ExPEC) among normal stool flora isolated from healthy, young, unmarried males and females as predisposing factor to ExPEC infections in addition to the above mentioned factors.

**Materials and Methods:**

**Study Individuals**

A total of 88 adult volunteers who were not aware of any illness at the time of sampling were included in this study. These individuals were young, unmarried males and females (aged 18-23 years), who have no sexual contact before (doctrinal), and did not recently administered antibiotics. All of them are students of
Specimen Collection and Transport

The specimens were collected during the period December 2008 through March 2009. A single fecal specimen was collected per person according to Vandepite et al. [25]. The specimens were transported to the laboratory within 1-2 hours after collection.

Specimen Processing

It is carried out according to Plos et al. [26]. The fecal material was dilution streaked onto eosine methylene blue agar (EMB) (Himedia). After incubation, from each plate the last three colonies (with the appropriate color and morphology that is characteristics of E. coli) at the end of the streak area were selected and subcultured onto EMB plate again, incubated, and then subcultured onto tryptic soy agar plates (TSA) (Himedia) which were then kept in the refrigerator for further work.

Identification of the Isolates

All isolates were identified morphologically and biochemically according to Forbes et al. [27] and MacFaddin [28].

Detection of E. coli' Fimbrial Adhesins

E. coli' fimbrial adhesins were determined by using MRHA of human RBCs (blood group O) according to Cook et al. [14]. Briefly a small amount of bacteria was suspended with a sterile toothpick into a drop of 5% (vol/vol) fresh human erythrocytes suspended in buffered saline containing 50 mM D-mannose. After the bacteria were thoroughly suspended on a glass plate, the plate was gently rocked on ice and the erythrocytes were observed for agglutination.

The expression of type 1 fimbriae was carried out according to Schembri et al. [29] by using MS agglutination of Bakers' yeast (Saccharomyces cerevisiae) obtained from local market. Bacterial cells were mixed with yeast cells (5%) on glass slide and observed for agglutination.

Statistical Analysis

To establish the significance of the results, $\chi^2$ was used as appropriate according to [30]. The level of significance was set at P value of < 0.05.

Results and Discussion:

Fecal E. coli Dominant Strains

From 88 persons, 264 E. coli isolates (3 isolates per person) were obtained (Table 1). These isolates represented the dominant strains from each individual.

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of individuals</th>
<th>No. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>41</td>
<td>123</td>
</tr>
<tr>
<td>Males</td>
<td>47</td>
<td>141</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>264</td>
</tr>
</tbody>
</table>

The choice of the dominant isolates in this study is in accordance with others. Gordon et al. [31] and Schlager et al. [32] showed that the E. coli clonal community of a person is numerically dominated by one strain, or at most a few strains. Schlager et al. [32] and Johnson et al. [33] showed that the selection of the last three colonies at the end of the streak area provided $\geq 97\%$ probability of capturing the quantitively predominant clone in the sample. Schlager et al. [32] found that dominant clones were more likely than minor clones to
spread to the periurethra. In young girls (6 years old) Schlager et al. [19] showed that when a dominant clone positive for P adhesin was present in the stool, 44% of the time the same clone was detected in the urine; when a dominant clone negative for P adhesin was present in the stool, 24% of the time the same clone was detected in the urine. They also showed that during the weeks of sampling, dominant clones were more commonly positive for P adhesin (16/37) than were nondominant clones (9/54).

**Fimbrial Adhesins as Indicators of \textit{E. coli} Pathogenicity**

Among 264 \textit{E. coli} stool isolates, 11 (4.16 %) showed MRHA of human RBCs and 149 (56%) caused MS agglutination of yeast cells. Of the females' isolates, 8.94% caused MRHA. Gander et al. [34] found that 11% of normal fecal control \textit{E. coli} isolated from adults demonstrated MRHA. Cook et al. [14] found that 16% of stool \textit{E. coli} isolated from healthy women showed MRHA of human RBCs. In healthy school children, Hagberg et al. [35] found that 16% of normal fecal \textit{E. coli} showed MRHA. Mulvey [11] reviewed that about 80% of commensal \textit{E. coli} fecal isolates encode FimH adhesins (type 1 fimbriae adhesin) while about 70% of UPEC isolates express Fim H variants.

Our dependence on MRHA and MS agglutination of yeast cells to detect \textit{E. coli} fimbrial adhesins is in agreement with others. Naveen and Mathi [36] showed that MRHA can be used for presumptive identification of virulence factors in \textit{E. coli}. Cook et al. [14] demonstrated that MRHA is a consequence of expression of one or more types of fimbrial adhesins on the bacterial cell surface and is a proxy for specific adherence to epithelial tissue and has been linked to bacterial virulence. A significantly greater proportion of \textit{E. coli} isolated from infections expressed MRHA phenotype compared with fecal isolates. Phenotypic expression of P fimbriae can be detected by MRHA of human erythrocytes [37]. Blanco et al. [38] found that \textit{pap} (P fimbriae gene) and/\textit{sfa} (S fimbriae gene) were found in 90% of strains expressing MRHA versus 37% of MRHA-negative strains. Hagberg et al. [35] showed that the bacterial surface antigen(s) mediating MRHA of human erythrocytes and attachment to human UT epithelial cells may be one factor selecting for \textit{E. coli} from among the fecal flora which infect the UT. The highest proportion of strains with this property was found among acute pyelonephritis isolates (77%), and the lowest proportion of strains with this property was found among normal fecal \textit{E. coli} (16%). Also it is well known that type 1 fimbriae of \textit{E. coli} cause MS agglutination of yeast cells [11, 29].

This study was based on fimbrial adhesins of \textit{E. coli} (which were detected by MRHA and MS yeast agglutination) as indicators of pathogenicity because these adhesins represent a critical extraintestinal \textit{E. coli} virulence factors. This is consistent with others. Mulvey [11] reviewed that the presentation of adhesive molecules (adhesins) by uropathogenic \textit{E. coli}, more so than the expression of toxins or other virulence factors, is arguably the most important determinant of pathogenicity. Dulawa [21] reported that the initial event leading to community-acquired UTI is intestinal colonization with a uropathogenic strain of \textit{E. coli}. Also Foxman et al. [5]; Nowrouzian et al. [8]; and Nowrouzian et al. [39] showed that adhesins and other virulence factors may contribute to the persistence of \textit{E. coli} strains in the human intestine. Adlerberth et al. [40] suggested that adhesins mediating
adherence to intestinal epithelial cells, especially P fimbriae, enhance the persistence of E. coli in the large intestine of infants. Plos et al. [26] considered that children with at least one p-fimbriated E. coli strain in their fecal flora were defined as carriers and that children who develop UTI have an increased tendency to carry p-fimbriated E. coli in their stools. Schlager et al. [19] reported that the presence of P adhesin was a marker for the persistence of the dominant clone in the stool of healthy newborns. Type 1 fimbriae are common among E. coli strains from all clinical categories of UTI and among fecal strains [11, 15, 41].

Eighty two percent (9/11) of the isolates with MRHA also caused MS agglutination of yeast cells. Hagberg et al. [35] found that most strains with MRHA, however, simultaneously induced MSHA. As well as Ishikawa [42] showed that almost all of the P-fimbriated E. coli had also type 1 fimbriae and that simultaneous presence of P- and type-1 fimbriae was the most significant virulence factor in UTIs. Gander et al. [34] found that E. coli expressing MR and/or P agglutinins with MS agglutinins predominated in all clinical categories. Hull et al. [43] concluded that it is possible that P pili, type 1 pili, or both are required for persistence of E. coli in the human bladder.

Distribution of Isolates with MS Agglutination of Yeast Cells and MRHA among Males and Females
The distribution of isolates with MS agglutination of yeast cells among males and females is shown in Table 2.

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of individuals</th>
<th>No. of isolates</th>
<th>No. of isolates with MRHA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>41</td>
<td>123</td>
<td>72 (59)</td>
</tr>
<tr>
<td>Males</td>
<td>47</td>
<td>141</td>
<td>77 (55)</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>264</td>
<td>149 (56)</td>
</tr>
</tbody>
</table>

There is no significant difference (P < 0.05) among males and females regarding the carriage of type 1-fimbriated E. coli. Orndorff and Bloch [44] and Bloch et al. [45] have concluded that the intestine is a primary niche for type 1 piliated E. coli. Zhang et al. [1] detected FimH (type 1 fimbriae adhesin) in 58% of intestinal flora while Mulvey [11] reviewed that about 80% of commensal E. coli fecal isolates encode FimH adhesins while about 70% of UPEC isolates express Fim H variants. In Japan Obata-Yasuka et al. [46] found that 98% of stool E. coli isolates from healthy people have Fim H.

All the isolates with MRHA are distributed among females' dominant stool flora whereas none of the males' dominant stool flora showed MRHA (Table 3).

Table 3: Distribution of normal stool E. coli isolates with MRHA among males and females.

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. of individuals</th>
<th>No. of isolates</th>
<th>No. of isolates with MRHA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>41</td>
<td>123</td>
<td>72 (59)</td>
</tr>
<tr>
<td>Males</td>
<td>47</td>
<td>141</td>
<td>77 (55)</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>264</td>
<td>149 (56)</td>
</tr>
</tbody>
</table>

Five females out of 41 (12.19%) had isolates with MRHA. All the three isolates in 2 of these 5 females showed MRHA, 2 isolates in
another 2 showed MRHA, and only one isolate in 1 female caused MRHA. These results confirm our thought that the females' intestinal carriage of ExPEC is more than the males and this can be considered as a possible predisposing factor of females to ExPEC infections especially UTI in addition to other predisposing factors.

To our knowledge this is the first study in this respect (Comparison between young, healthy, doctrinal males and females at the normal stool flora level) so we did not find figures in literatures to compare with but there is some evidence from several studies that are in agreement with our thought. Gordon et al. [47] demonstrated that the genotype of the dominant E. coli strain present in a host is, in part, determined by the sex and age of that host. They also demonstrated that the intestinal tract of males and females appears to represent different environments for E. coli. So that They suggested that the morphological, physiological, and dietary differences that occur among human individuals of different sex or age may influence the distribution of E. coli genotypes. In comparison with previous studies Johnson et al. [48], in their study of characteristics differentiating E. coli strains that cause cystitis or pyelonephritis from fecal E. coli using phylotyping, virulence genotyping, and O typing, found that the present fecal isolates appear more virulent than other reported fecal isolates and they demonstrated that the incidence of UTI is higher in the present control population than in similar populations with a less-virulent fecal flora. Ruiz et al. [49] concluded that the physiology of men makes it more difficult for E. coli to cause UTI in men than in women. Dalet et al. [50] suggested that males are significantly more resistant than the females to UTI both parenchymal and UT. They deduced that underlying factors are more predisposing to UTI the smaller the adherence rate of isolated strains is. The strongest confirmation of our results is that obtained by Gordon et al. [47]: It is well known that fecal isolates of E. coli are divided into four main genetic groups (ECOR groups) designated A, B1, B2, and D [51], extraintestinal pathogenic E. coli strains are mostly derived from phylogenetic group B2 [1, 40], Gordon et al. [47] found that the probability of recovering a B2 strain in a host increases with host age in females but declines with host age in males. In men Johnson et al. [33] found that the urine clone actually might have been present as a minor compartment of the fecal population but was missed because of the incomplete sampling. They supported the "special pathogenicity" hypothesis over the "prevalence" hypothesis as an explanation for why certain E. coli strains cause fertile UTI (FUTI) in men; the urine clone clearly was not highly prevalent within the fecal flora for most subjects. Fecal predominance does not enable clones that lack "special pathogenicity" to cause FUTI in men. They suggested that there is a possibility of alternate infection routes in some men and the "special pathogenicity" hypothesis.

Conclusion:

According to the results of this study, we can conclude that the differences in carriage of dominant normal stool flora with characteristics of ExPEC among males and females, can be a possible predisposing factor of women to ExPEC infections more than men.

References:


المستوى خارج الامعاء Escherichia coli

النبت الطبيعي لخروج ذكور واناث شباب إصابة و غير متزوجين كعامل

معرض للاصابات خارج الامعاء:دراسة مقارنة

الخلاصة:

تضمنت هذه الدراسة إجراء مسح للنبت الطبيعي السائد في الخروج المأخوذ من طلاب كليات إيران Escherichia coli (Extrakistolic Pathogenic Escherichia coli) لتحديد ما إذا كان النبات الطبيعي السائد قد تسبب في علاجات متزوجين. تم الرد على النبات الطبيعي السائد (Escherichia coli) مع استمراره في اكتساب الشكل. في مراقبة MRHA، لم تظهر أي عرق محسوبي (P<0.05) بين الذكور والإناث من ناحية الشكل.

التحليل:

الخلاصة: الدراسة مسح النبات الطبيعي السائد في الخروج المأخوذ من طلاب كليات إيران Escherichia coli (Extrakistolic Pathogenic Escherichia coli) لتحديد ما إذا كان النبات الطبيعي السائد قد تسبب في علاجات متزوجين. تم الرد على النبات الطبيعي السائد (Escherichia coli) مع استمراره في اكتساب الشكل. في مراقبة MRHA، لم تظهر أي عرق محسوبي (P<0.05) بين الذكور والإناث من ناحية الشكل.

المراجع:
