Detection of Fimbrial Adhesins of *Escherichia coli* Isolated from Pregnant and Non-Pregnant Women with Symptomatic Genital Tract Infection as a Risk Factor for Urinary Tract, and Neonatal Infections: a Comparison Study.

التحري عن اللواصق المخملية ((Fimbrial Adhesins ضمن الايشيرشية القولونية المعزولة من نساء حوامل وغير حوامل مصابات بالتهاب المسلك التناسلي المصحوب بالأعراض كعامل معرض للإصابة بالتهاب المسلك البولي وإصابات الأطفال حديثي الولادة: دراسة مقارنة

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الخلاصة

تم الحصول على 52 (17.3%) عزلة *E. coli* كمسبب لالتهاب المسلك التناسلي، من 299 امرأة (156حوامل و143 غير حوامل) بعمر18-45 سنة. ليس هناك فرق معنوي بين تلك المجموعتين من المرضى من ناحية انتشار بكتريا *E. coli* والتي عزلت من 14.1% من الحوامل و 20.9% من غير الحوامل . أظهرت 26.9% من تلك العزلات تلازن مقاوم للمانوز لكريات الدم الحمر للإنسان، كما سببت 88.4 % تلازن حساس للمانوز لخميرة الخبز. لا يوجد فرق معنوي بين عزلات الحوامل وغير الحوامل من ناحية التعبير عن Type 1 fimbriae (86.3% و 90% من عزلات الحوامل وغير الحوامل، على التوالي) و P fimbriae (27.2% و 26.6% من عزلات الحوامل وغير الحوامل، على التوالي) و Dr fimbriae(0% في كلا المجموعتين من المرضى). بينما كان الفرق واضحا بالنسبة للتعبير عن S fimbriae والتي تم التعبير عنها ضمن عزلات غير الحوامل (6.6%) فقط. كانت كل العزلات الحاوية علىS fimbriae حاوية أيضا على P fimbriae (2/2: 100%) وعلى Type 1 fimbriae (2/2: 100%). في الحوامل كانت كل العزلات الحاوية على P fimbriae حاوية أيضا علىType 1 fimbriae(6/6: 100 %)، بينما في غير الحوامل كانت سبع عزلات من العزلات الحاوية علىP fimbriae حاوية أيضا على Type 1 fimbriae(7/8: 87.5%). من نتائج الدراسة الحالية نستطيع الاستنتاج بما إن *E. coli* المهبلية ممكن أن تكون واحدة من المسببات المؤدية لإصابات المسلك البولي وإصابات الأطفال حديثي الولاة ، فأن كلا المجموعتين من المرضى (حوامل وغير حوامل) المصابات بالتهاب المسلك التناسلي المسبب عن *E. coli* ، تمتلك نفس الفرصة للإصابة بالتهاب المسلك البولي، كذلك هناك تردد قليل لإصابة الأطفال حديثي الولادة ببكتريا *E. coli* المهبلية.

Abstract

Fifty two (17.3%) *Escherichia coli* isolates were obtained as a causative agent of symptomatic genital tract infection, from 299 (pregnant 156 and non-pregnant 143) patients aged 18-45 years. There is no significant difference between the two patient groups regarding the prevalence of *E. coli* which was isolated from 14.1% of pregnant and 20.9% of non-pregnant. As a whole 26.9% of this study isolates showed MRHA of human RBCs and 88.4% caused MS agglutination of Baker's yeast. The difference is not significant between pregnant and non-pregnant women's isolates regarding the expression of Type 1 fimbriae (86.3% and 90% of pregnant and non-pregnant women's isolates, respectively); P fimbriae (27.2% and 26.6% of pregnant and non-pregnant women's isolates, respectively) and Dr fimbriae (0% in both patient groups). While the difference is clear for the expression of S fimbriae. S fimbriae were expressed only by non-pregnant women's isolates (6.6%) whereas none of the pregnant women's isolates expressed this type of fimbriae. All the S fimbriated isolates were P- (2/2: 100%) and Type 1-fimbriated (2/2: 100%). In pregnant, all of the P-fimbriated isolates were also Type 1-fimbriated (6/6: 100%) while in non-pregnant, seven P-fimbriated isolates were also Type 1-fimbriated (7/8: 87.5%). From the present results it can be concluded that as vaginal *E.*

coli may be one of the possible causes leading to UTI and neonatal infections, both pregnant and non-pregnant patients with genital tract infection caused by *E. coli*, have the same chance to contact UTI and there is a low frequency of neonatal infections caused by these patients' vaginal *E. coli*.

Key words: Vaginal *E. coli*, fimbrial adhesins, UTI, neonatal infections.

Introduction

Vaginal *Escherichia coli* (VEC) is a reservoir along the fecal-vaginal-urinary/neonatal course of transmission in extraintestinal *E. coli* infections (1). Vaginal *E. coli* may also cause symptomatic infections such as vaginitis or tubo-ovarian abscess and is associated with life threatening neonatal sepsis and meningitis (2, 3, 4, 5). *Escherichia coli* strains involved in neonatal infections are thought to originate from the natural flora of pregnant women (6). Neonates are presumably exposed to *E . coli* during passage through the birth canal (7, 8). One critical aspect leading to urinary tract infection (UTI) is the ability of uropathogenic *E. coli* (UPEC) strains to move from the intestinal tract and establish themselves in the urinary tract (UT). In some cases this movement may be facilitated by UPEC strains establishing themselves first in the vagina (9). These colonizers of the female introitus predisposes the women to recurrent UTI (10). Women often suffer from an enhanced susceptibility to recurrent urinary and genital tract infections in association with uropathogenic *E. coli* strains (11).

The vagina and/or the cervix favors colonization by strains that possess features different from those of fecal flora strains. The human anatomical sites may then be considered as barriers that select for strains with a greater capacity to cause amniotic and invasive diseases in neonates (6). *Escherichia coli* isolated from females reproductive tract infection (RTI) and neonatal sepsis possess unique properties that may enhance their virulence. These properties are similar to those associated with other *E. coli* extra-intestinal infections (12). Capsular antigen (K1), alpha-hemolysin, the iron uptake aerobactin system, adhesins (FIC fimbriae, P pili, and S-pili) and the IbeA protein are considered to be the major virulence factors related to neonatal pathogenicity due to *E. coli* (13, 14, 15). Uropathogenic *E. coli* strains are more likely to have P pili, S pili, afimbrial adhesin, and toxins such as hemolysin and cytotoxic necrotizing factor 1 (4, 5, 16).

Adherence is a critical step in the pathogenesis of *E. coli* meningitis. Factors involved in the binding of *E. coli* to brain microvascular endothelial cells (BMECs) include S fimbriae which are also important in UPEC pathogenesis (5, 16). Adhesive organelles, including type 1, P, and S pili along with Dr adhesins, promote both bacterial attachment to and invasion of host tissues within the UT (2, 16, 17).Vaginal *E. coli* share common virulence factor profiles, phylogenetic groups and serotypes with *E. coli* strains from urinary and neonatal (blood and CSF) origins (1). In the following study, virulence-associated adherence characteristics of *E. coli* isolated from females with genital tract infection (Pregnant and non-pregnant), were examined. Adherence characteristics were detected phenotypically by mannose resistant hemagglutination for P-, S-, and Dr fimbriae and mannose sensitive agglutination of Baker's yeast for type 1 fimbriae. Expression of these fimbrial adhesins was compared between *E. coli* isolated from pregnant and those isolated from non-pregnant women as a risk factor for urinary tract and neonatal infections.

Materials and Methods

Patients

A total of 299 high vaginal swabs (one swab per patient) were collected from pregnant and non-pregnant women (aged 18 to 45 years) with symptomatic genital tract infection who visited Obstetrics and Gynecology Clinics in Al-Kut/Wassit Province/Iraq.

Specimen Collection and Processing

Specimens were collected during May 2008 to June 2010. High vaginal swabs were collected by the Gynecologist (18) and streaked immediately after collection on eosine methylene blue agar (EMB) (Himedia) and blood agar plates. The plates were incubated at 37°C for 24-48 hours at ambient air. Only those samples that gave significant growth were considered as infection.

Identification of the Isolates

All isolates were identified biochemically (19, 20).

Adhesins determination

The expression of adhesins was defined by hemagglutination and inhibition of hemagglutination, using microscope slide assays (12). Briefly, hemagglutination (HA) was performed using human and cow erythrocytes. Inhibition of HA was performed with P1antigen-containing pigeon egg white (PEW) , and with D-mannose (21). Isolates were considered to express P-fimbriae if HA was positive with human erythrocytes and inhibition of HA was positive with pigeon egg white. Mannose resistant hemagglutination (MRHA) inhibition was defined as a two-level decrease in the intensity of MRHA in the presence of inhibitor. S fimbriae were detected by MRHA of human and cow erythrocytes (22, 23). Dr fimbriae expression was detected by MRHA of human erythrocytes and its inhibition by 10μM chloramphenicol (24). The expression of type 1 fimbriae was carried out using MS agglutination of bakers' yeast cells (*Saccharomyces cerevisceae*) obtained from local market (25). D-mannose always inhibited agglutination of yeast cells (mannose sensitive, MS agglutination), but it never inhibited HA of human and cow erythrocytes (mannose resistant, MRHA).

Statistical Analysis

Existence of a difference in the distribution of the studied determinants among the different groups of strains was tested by the χ^2 test. A P value below 0.05 was considered to indicate statistical significance (26).

Results and Discussion

Prevalence of *E. coli* among Pregnant and non-pregnant patients

In order to evaluate the risk of vaginal *E. coli* (VEC) to the patients and their neonates, frequency of *E. coli* isolation from females' genital tract infections (GTIs) must first be estimated. Fifty two (17.3%) *E. coli* isolates were obtained as a causative agent of symptomatic genital tract infection, from 299 pregnant and non-pregnant patients (Table-1). Since *E. coli* is one of the normal vaginal flora, so that only those samples that gave significant growth were considered as infection while samples with scanty growth were neglected.

Table-1: Frequency of *E. coli* isolation from pregnant and non-pregnant women with symptomatic genital tract infection.

Patient group	No. of patients	No. (%) of *E. coli* isolates
Pregnant	156	22 (14.1)
Non-pregnant	143	30 (20.9)
Total	299	52 (17.3)

The percent distribution of *E. coli* isolates among pregnant (14.1%) and non-pregnant women (20.9%) observed in this study is consistent with others. *Escherichia coli* have been reportedly identified in 24-31% of pregnant women (31). Vaginal colonization was observed in 3-20% of pregnant women (6). Mumtaz *et al*. (32) isolated *E. coli* from 13.7% of non-pregnant women with vaginitis. There is no significant difference between the two patient groups regarding the prevalence of *E. coli*. Obata-Yasuka *et al.* (1) found that *E. coli* is one of the common organisms in the microflora of pregnant as well as non-pregnant women. Cook *et al*. (12) and Lawson (27) reported that vaginitis-associated isolates represented the predominant vaginal flora present concurrent with symptoms. French *et al*. (28), Donders *et al.* (29), and Donders *et al.* (30) demonstrated that the *E. coli* is one of the predominant microorganisms in cases of aerobic vaginitis.

MRHA and MS agglutination phenotypes of the isolates

Escherichia coli' adherence factors were detected phenotypically by MRHA of human and cow RBCs and by MS agglutination of Baker's yeast (Table-2). As a whole 26.9% of this study isolates demonstrated MRHA of human RBCs and 88.4% caused MS agglutination of yeast cells. Few studies have studied the phenotypic adherence factors of vaginal *E. coli.* Most studies characterized these factors at the genotype level by PCR technique. The only prevalent study was that carried out by Cook *et al.* (12) who found that 48% of vaginitis isolates caused MRHA and that genes associated with one or more D-mannose resistant fimbriae types were detected in 60% of vaginitis and neonatal sepsis isolates.

Mannose resistant hemagglutination (MRHA) is strongly associated with extraintestinal *E. coli* virulence (12). This *in vitro* phenotype is a proxy for specific adherence to epithelial tissue and has been linked to bacterial virulence (33). This adherence phenotype was associated with the presence of P fimbriae (pap) genes (12). For UPEC hemagglutination is mediated by P fimbriae (34) and MRHA can be mediated by P fimbriae and also X, F1C, Dr fimbriae. Thus MRHA positive strains can be considered as UPEC most likely having P fimbriae (33).

Table-2: MRHA of human and cow RBCs and MS agglutination of Baker's yeast by pregnant and non-pregnant patients' genital tract infection *E. coli* isolates.

Patient group	No. of *E. coli* isolates	No. (%) of *E. coli* isolates with:				
		MS agglutination of yeast cells	MRHA of human RBCs	MRHA of human RBCs in the presence of:		MRHA of human and cow RBCs
				PEW	10μM chloramphenicol	
Pregnant	22	19 (86.3)	6 (27.2)	0	6 (27.2)	0
Non- Pregnant	30	27 (90)	8 (26.6)	0	8 (26.6)	2(6.6)
Total	52	46 (88.4)	14 (26.9)	0	14 (26.9)	2 (3.8)

MS: mannose-sensitive; MRHA: mannose-resistant hemagglutination; PEW: pigeon egg white**,** 0: absence of the property.

Distribution of fimbriated *E. coli* among patients

Escherichia coli' fimbrial adhesins were predicted from the results of MRHA and MS agglutination as presented in Table-2. The predicted fimbrial types were summarized in Table-3.

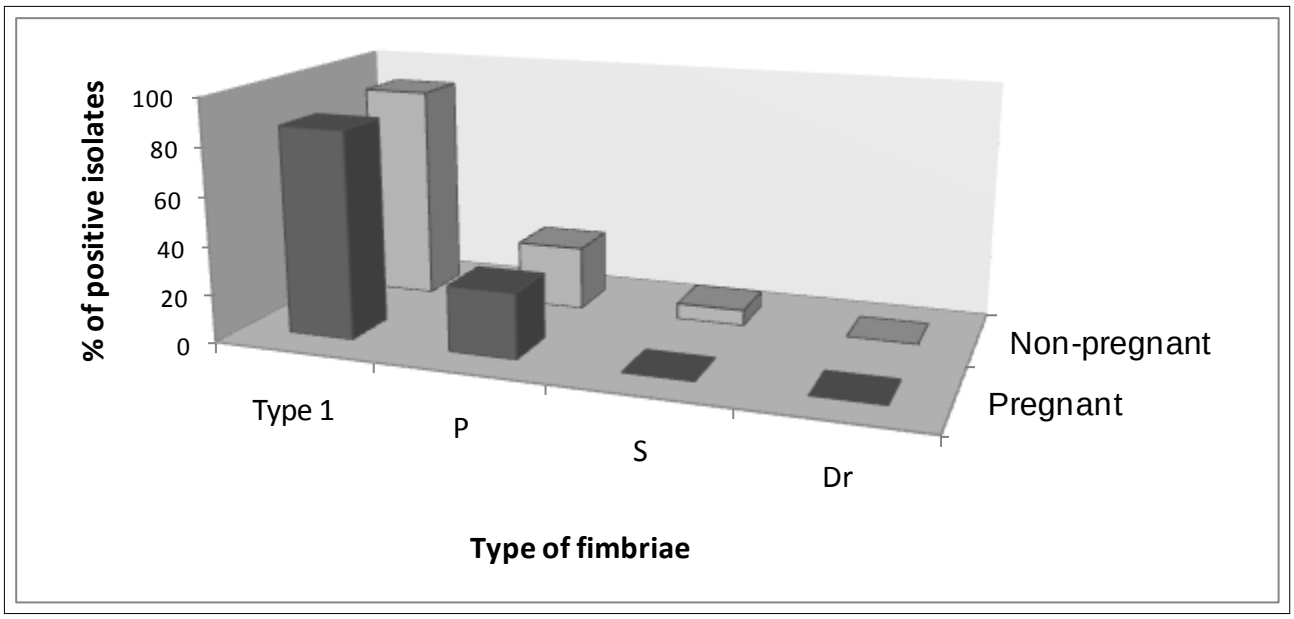
Table-3: Fimbrial adhesins of *E. coli* isolated from pregnant and non-pregnant patients with symptomatic genital tract infection.

Patient group	No. of *E. coli* isolates	No. (%) of *E. coli* isolates expressing:			
		Type 1 fimbriae	P fimbriae	S fimbriae	Dr fimbriae
Pregnant	22	19 (86.3)	6 (27.2)	0	0
Non-pregnant	30	27 (90)	8 (26.6)	2 (6.6)	0
Total	52	46 (88.4)	14 (26.9)	2 (3.8)	0

0: absence of the property.

The difference is not significant between pregnant and non-pregnant patients regarding the expression of Type 1, P and Dr fimbriae. While the difference is clear for the expression of S fimbriae (Fig. 1). This result is consistent with Obata-Yasuka *et al*. (1) who found that the prevalence of virulence factors among the vaginal *E. coli* (VEC) isolates showed that there were no significant differences between the non-pregnant women and the pregnant women or between the asymptomatic women and the symptomatic women.

Fig.1: Distribution of fimbriated *E. coli* among pregnant and non-pregnant patients with symptomatic genital tract infection.



Type 1 fimbriae were expressed by 86.3% and 90% of pregnant and non-pregnant women's isolates, respectively. This result is expected as type 1 fimbriae represent the universal *E. coli*' fimbrial adhesin in all cases (5, 16, 35). Obata-Yasuka *et al*. (1) detected fimH (Type 1 fimbriae adhesin gene) in 100% of both pregnant and non-pregnant women's isolates. Type 1 fimbriae contribute significantly to colonization of the bladder (36, 37) and may contribute to reproductive tract colonization as well.

P fimbriae were detected in 27.2% and 26.6% of pregnant and non-pregnant women's isolates, respectively. Obata-Yasuka *et al*. (1) found *papC* (P fimbriae gene) in 47% and 50% of non-pregnant and pregnant symptomatic patients' isolates, respectively. Cook *et al*. (12) showed that 46% of vaginitis isolates had *papC*. The reason for this difference with these researchers may

be explained by that these researchers depended on genotype while this study was depended on phenotype which may not predicted by genotype as demonstrated by Johnson *et al.* (38) who concluded that phenotype presumably is more closely related to pathogenesis than is genotype but cannot be predicted reliably based on genotype. P fimbriae clearly- contribute to *E. coli'* extraintestinal virulence (33, 39, 40). P fimbriae, and possibly Type 1C fimbriae, contribute to *E. coli* GTI (12).

S fimbriae were expressed only by non-pregnant women's isolates (6.6%) whereas none of the pregnant women's isolates expressed this type of fimbriae. Cook *et al*. (12) demonstrated *sfa* (S fimbriae gene) in 14% of isolates. Whereas Obata-Yasuka *et al*. (1) found that 19% of non-pregnant and 30% of pregnant women's *E. coli* isolates had *sfaDE* (S fimbriae gene). Birosoval *et al.* (11) reported that statistical analysis revealed an increased occurrence of *cnf*1 and *sfa/foc* (S fimbriae gene) in *E. coli* isolates from vaginal swabs (11). The present study results are consistent with Cook *et al*. (12) who found no significant differences in frequency of *fim* (Type 1 fimbriae adhesin gene), *sfa*, (S fimbriae gene) or *dra (*Dr fimbriae gene) genes in infection isolates compared with fecal isolates. In this study the low prevalence of S fimbriae among VEC isolates may indicate that this type of fimbriae may has no role in genital tract infections but is important in infections for which the genital tract is the source, so that the bacteria may have genes coding for this type of fimbriae but are not expressed. Mulvey (16) reviewed that S pili may facilitate bacterial dissemination within host tissues and are often associated with *E. coli* strains that cause sepsis, meningitis, and ascending UTIs, including pyelonephritis.

None of this study isolates expressed Dr fimbriae. Cook *et al*. (12) reported that 14% of vaginitis isolates had *dra* (Dr fimbriae gene) whereas Obata-Yasuka *et al*. (1) found that 7% of symptomatic non-pregnant women's and 5% of symptomatic pregnant women's isolates had *afa/dra* gene. This low prevalence of this fimbrial type indicates that it is not general and is associated with specific disease state. It is associated with upper urinary tract infections, especially recurrent UTI. Mulvey (16) reviewed that Dr adhesin family members are proposed to facilitate ascending colonization and chronic interstitial infection of the urinary tract. In addition, infection with *E. coli* strains expressing Dr fimbriae results in a twofold increase in the risk for a recurrent UTI.

All the S fimbriated isolates were P- (2/2: 100%) and Type 1-fimbriated (2/2: 100%). In pregnant women, all of the P-fimbriated isolates were also Type 1-fimbriated (6/6: 100%) while in non-pregnant women, seven P-fimbriated isolates were also type 1-fimbriated (7/8: 87.5%). Kasper *et al.* (3) demonstrated that a given pathogen usually possesses multiple adhesins for binding to a variety of host cells (e.g., in *E. coli*: type 1 fimbriae, Sfa/Foc, P pili). In UPEC, Mulvey (16) reviewed that variable expression of different adhesive organelles may allow UPEC to alter its binding characteristics in response to environmental changes encountered within a host during the course of an infection. Potentially, this can greatly expand the number of host receptors with which UPEC can interact during a UTI and may facilitate bacterial dissemination within the urinary tract. Furthermore, modulation of adhesive organelles may enable UPEC to escape rapid detection by the host immune system. This explanation can be applied to VEC with multiple adhesive organelles.

Adhesive factors of vaginal *E. coli* as a risk factor for UTI

Both this study patient groups' isolates had P fimbriae (27.2% in pregnant vs. 26.6% in non-pregnant women) and Type 1 fimbriae (86.3% in pregnant vs. 90% in non-pregnant women). Whereas only non-pregnant women's isolates expressed S fimbriae (6.6%). These fimbrial adhesins are important virulence factors in UTI. This means that both patient groups have the same chance to contact UTI especially cystitis, as most isolates of both patient groups are Type-1 fimbriated. Kaper *et al.* (41) reported that both type 1 and P fimbriae help in adhering to uroepithelial cells in the lower urinary tract. In UPEC, the presence of type 1 fimbriae may increase their virulence for the urinary tract by promoting bacterial persistence and by enhancing the inflammatory response to infection (42). Type 1 fimbriae contribute significantly to colonization of the bladder (36, 37). Gillespie and Hawkey (5) demonstrated that UPEC strains initiate infection by binding to the

superficial bladder epithelial cells that line the luminal surface of the bladder. In the majority of cases, this is achieved by type 1 pili.

For P fimbriae, although the difference is not significant, a larger proportion of pregnant women's isolates expressed this fimbrial adhesin and this means that these patients may be exposed to the danger of pyelonephritis, as this infection is more common among pregnant than non-pregnant women. Gillespie and Hawkey (5) explained that there exists a strong relationship between the presence of P fimbriae and severity of infection, especially pyelonephritis. In UTI, P fimbriae mediate specific attachment of uropathogenic *E. coli* to kidney tissue and elicit a cytokine response in those cells (33, 43). Acute pyelonephritis is more common in pregnant women than in non-pregnant women and is probably due to stasis of urine and bacteriurea in the UT caused by relative obstruction (1). Ovalle and Levancini (44) found that a good proportion of *E. coli* causing UTI in pregnancy are P fimbriated. Hence there is an increased chance for pregnant women to develop pyelonephritis.

Adhesive factors of vaginal *E. coli* as a risk factor for neonatal infections

In this study none of the pregnant women's isolates had S fimbriae and only 6.6% of the non-pregnant women's isolates expressed this type of fimbriae. S-fimbriae are known to comprise a key virulence factor in the pathogenesis of neonatal meningitis caused by *E. coli* (11, 45). Birosoval *et al*. (11) found that persistence of S-fimbriated α-hemolytic *E. coli* strains in the vagina of pregnant women may expose neonates to a higher risk of infection. All the S-fimbriated isolates were also P-fimbriated (2/2: 100%). The role of P fimbriae in neonatal infections is not known. Bingen *et al.* (46) reported that the coexistence of *pap* and *sfa/foc* adhesin-mediating operons seem critical in the pathogenesis of neonatal meningitis, whereas Gillespie and. Hawkey (5) demonstrated that whilst possession of P fimbriae is important in *E. coli* causing pyelonephritis, it is not thought to be relevant in strains responsible for neonatal meningitis. According to this study results, there is a low danger of neonatal infections. Other researchers reported that the incidence of neonatal meningitis is low. In the United States, the incidence of neonatal meningitis was 0.1 per 1,000 live births (2). Watt *et al.* (6) showed that only a few *E. coli* strains can complicate pregnancy or cause neonatal infections as few specific pathogenic determinants have been described for *E. coli* causing neonatal meningitis.

From this study results it can be concluded that as vaginal *E. coli* may be one of the possible causes leading to UTI and neonatal infection, both pregnant and non-pregnant patients with genital tract infection caused by *E. coli*, have the same chance to contact UTI and there is a low frequency of neonatal infections caused by vaginal *E. coli*.

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