A ROLE OF SOME *ESCHERICHIA COLI* – VIRULENCE FACTORS IN CAUSING VAGINOSIS

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ABSTRACT

Bacterial vaginosis, also known as BV, is a condition that is frequently associated with vaginal inflammation and can be caused by changes in the bacterial composition of the vaginal microbiome. It is the most common vaginal infection among women of reproductive age. The goal of this study is to determine the antibiotic resistance and the role of some virulence genes of *E. coli* isolates in the vaginosis of non-pregnant women (16–45 years old) as well as the stool of the same patient. A total of 160 samples were collected (130 vaginal swab samples and 30 samples from the stool). All isolates have been identified by MacConkey and Eosin methylene blue media, biochemical tests, and PCR detection of the *UidA* gene. Just 50 isolates were diagnosed and confirmed as *E. coli* bacteria. Resistance to piperacillin was most frequently observed in 31 isolates (62%), followed by cefuroxime and cefixime in 28 isolates (56%). The polymerase chain reaction was used to detect virulence genes (*FimH* and *IutA*), and the results showed that both genes were present in 94% and 97% respectively of the isolates.

KEYWORDS: *UidA* gene, *FimH* gene, *IutA* gene, Antibiotic resistance, PCR.

دور بعض عوامل الضراوة لبكتيريا الأشريكية القولونية في حدوث التهاب المهبل

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المستخلص

التهاب المهبل البكتيري، هو حالة ترتبط بالتهاب المهبل ويمكن أن تحدث بسبب التغيرات في التركيب البكتيري للميكرو بيوم المهبلي. وهي العدوى المهبلية الأكثر شيوعًا بين النساء في سن الإنجاب. الهدف من هذه الدراسة هو تحديد مقاومة المضادات الحيوية ودور بعض جينات الفوعة للإشريكية القولونية في التهاب المهبل لدى النساء غير الحوامل (16–45 سنة)، وكذلك البراز لنفس المريض. تم جمع 160 عينة شملت (130 مسحة من المهبل و30 مسحة من البراز). تم التعرف على جميع العزلات باستخدام الاوساط الزرعيه Eosin Methylene Blue وMacConkey، اختبارات بايوكيميائية، والتشخيص باستخدام تفاعل البلمرة المتسلسل عن طريق كشف وجود جين (*UidA*). تم تشخيص وتأكيد 50 عزلة فقط على أنها بكتيريا الإشريكية القولونية. وجدت النتائج مقاومة للبيبراسيلين بنسبة كبيرة في 31 عزلة (62٪)، يليها سيفوروكسيم وسيفيكسيم في 28 عزلة (56٪). تم استخدام تفاعل البلمرة المتسلسل للكشف عن جينات الفوعة (*FimH*، *IutA*) وأظهرت النتيجة وجود كلا الجينين في العزلات بنسبة (94%) و (97٪).

كلمات مفتاحية: جينات *UidA*, *FimH*, *IutA*, مقاومة المضادات الحيوية، تفاعل البلمرة المتسلسل.

Received: 14/8/2022, Accepted:24/12/2022

INTRODUCTION

The colonization of Vaginal *Escherichia coli* (VEC) causes a variety of genitourinary diseases, including urinary tract infection (3, 25), newborn meningitis during pregnancy, and pelvic inflammatory disease (10), Due to the anatomical proximity of the "anorectal/vagina" region, which has been found to cause *E. coli* transmission to the vagina (9,23). The female vaginal tract has a variety of defense mechanisms that work together to avoid infection (2). the vast majority of the vaginal flora in healthy women belongs to the genus *Lactobacillus*, and it is crucial for the protection of the vaginal ecosystem as well as for immunity enhancement and opportunistic bacterial inhibition (6, 18). The acidic pH of a normal healthy vagina (typical range of 3.8–4.5) has been found to protect against urinary infections (UTI) and vaginitis, as well as to prevent the proliferation of pathogenic microbes (6), at which many other organisms cannot grow (17). The gram-negative, aerobic and facultative anaerobic *E. coli* bacteria are members of the *Enterobacteriaceae* family (14) and enhance their pathogenicity by a wide range of virulence factors, which add to the severity of the infection (24). VEC causes female urogenital tract infections by sharing a virulence factor profile with extra-intestinal pathogenic *E. coli* isolates (ExPEC), which differ from commensal flora and allow them to avoid defense mechanisms, colonize, and cause extra-intestinal infections (25). These virulence factors include the bacterial capsule, which helps the bacteria avoid the body's natural defenses; hemolysis that lysis host blood cells; adhesions like fimbriae that help the bacteria stick to the host and tissues; and multiple ways for the bacteria to get the iron it needs (7). The majority of virulence factors are encoded by mobile genetic elements that are referred to as pathogenicity islands (PAIs), which have been studied in pathogenic bacteria (13). In recent years, the prevalence of bacteria resistant to antibiotics has increased, probably because of improper antibiotic use (9). *E. coli* strains are the major cause of serious bacterial infections in the medical community, and treatment regimens vary depending on the source (25). Multi-drug resistant *Escherichia coli* infections are a significant public health problem (1). Numerous mechanisms exist in *E. coli* that confer resistance to antibiotics, including decreased antibiotic penetration, efflux pumps, target site modification, and β-lactamase enzymes. Bacterial diagnosis through the use of molecular methods has been shown to be beneficial in terms of overcoming some limitations of conventional biochemical and serological methods and improving sensitivity and bacterial rapidity (6). The *UidA* gene was used for identification in this study because 94-96% of *E. coli* strains produced the β-glucuronides enzyme (21). The goal of this research was to determine the role of some virulence factors of E. *coli* isolates in causing vaginosis.

MATERIALS AND METHODS

Isolation of *E. coli* bacteria: This study included 160 samples (130 from vagina and 30 from stool) from women (aged 16 to 45) with symptoms of vaginosis (inflammation, redness, itching, and discharge) from October 2021 to December 2021 figure 1. The participants in the study reported that they had not been given any antibiotics in the previous two weeks and completed a questionnaire to provide any additional information that was required for this investigation. Vaginal swabs were obtained from the vaginal wall or the posterior cervix of the female reproductive system using sterile swabs that contained transport media and disposable speculums. The swabs are cultured on MacConkey and EMB (Eosin methylene blue) media and incubated for 18–24 h at 37˚C. Standard IMViC biochemical tests (Indole, Methyl Red, Voges-Proskauer, and Citrate) and PCR amplification of the *UidA* gene were used to identify the *E. coli* bacteria.

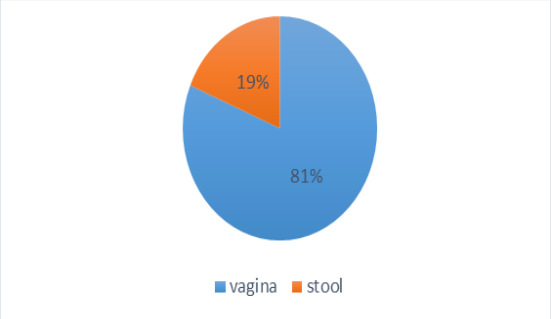

Figure 1. sample source from different site show that 81% of samples from vagina and 19% from stool

Antibiotic susceptibility method

The disc diffusion method was used to test antimicrobial susceptibility on Mueller Hinton agar using the following antimicrobial agents: piperacillin (100µg), ampicillin_sulbactm (10/10 µg), meropenem (10µg), amikacin (30µg), azithromycin (15µg), ofloxacin (5µg), chloramphenicol (30µg), cefixime (5µg), aztronam (30µg), cefuroxime (30µg), nalidix acid (30µg), trimethoprime (5µg). MDR strains were characterized as those that were resistant to three or more antimicrobial agents. The result was read by measuring the diameter of the inhibition zone in mm and comparing it with the National Community for Clinical Laboratory Standards (4).

Molecular study

1. DNA extraction : For DNA extraction, the FavorPrep™ mini kit (FAVORGEN/Taiwan) was used

2. Primers used in this study: Primers listed in the Table (1) were supplied by Alpha and Macrogen company for detection of *UidA* and virulence genes *FimH, IutA* of *E. coli.*

Table 1. primers used in this study

Gene	Primer sequence 5′→3′	Product size	Reference
UidA	F TGGTAATTACCGACGAAACGGC R ACGCGTGGTTACAGTCTTGCG	165 bp	This study
FimH	F GTGCCAATTCCTCTTACCGTT R TGGAATAATCGTACCGTTGCG	164 bp	This study
IutA	F CCATCAGTTGGCTGTTCAGA R GGAACTGGTCGGTCAGGTTT	155 bp	This study

Detection of *UidA*, *FimH* and *IutA* genes by Polymerase Chain Reaction: Polymerase chain reaction (PCR) was used to screen the isolates for the following virulence-associated genes: *FimH*, *IutA*, and *UidA*. Virulence genes predicted by the gene regions studied include the following: type 1 fimbriae (*FimH*), ferric aerobactin receptor (*IutA*), and beta-glucuronidase (*UidA*). DNA amplification from bacteria was accomplished in a total volume of 25 µl containing 12.5 µl of OneTaq® 2X Master Mix green, 5 µl (100) of DNA, 4.5 µl nuclease free water, and 1.5 µl of each primer. The PCR was done with a Gradient Thermocycler under the following conditions: denaturation for 1 min at 95 ˚C; 35 cycles of 30 s at 95 ˚C, 45 s at 56 ˚C, and 30 s at 72 ˚C; and a final extension step for 7 min at 72 ˚C. A 10 ml aliquot of the PCR product experienced gel electrophoresis on 2% agarose, followed by staining with red safe solution. The UV-induced fluorescence was used to detect amplified DNA fragments of specific sizes, and the size of the amplicons was assessed by comparing them to a DNA ladder consisting of 1000 bp that was also included on the same gel.

RESULTS AND DISSCUSION

Isolation and identification of *E .coli:* In this study, *E. coli* was isolated from infected women with the consent of the patient, and all information collected through the questionnaire was not shared with anyone. A total of 50 isolates were isolated from 160 samples collected from women's vagina with symptoms of inflammation and stool of the same patient under the supervision of the doctor. The isolates were identified by culture on selective and differential MacConkey and EMB media, which showed green with a metallic sheen (Figure 2), and via IMViC (indole-positive, methyl red-positive, voges-proskauer-negative, citrate utilization-negative) biochemical tests.

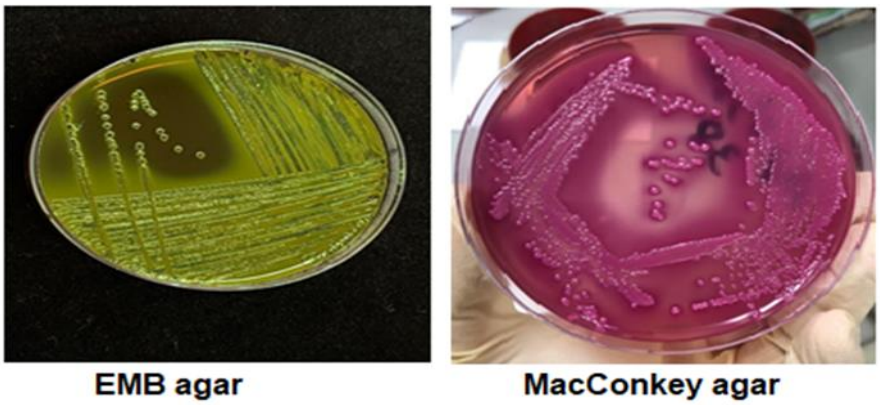

Figure 2. Growth of *Escherichia coli* on MacConKey and EMB media

Molecular detection of *UidA* gene

Following biochemical identification of all isolates, PCR confirmed the presence of *E. coli* in 100% of all 50 isolates with a PCR product size of 155bp of the *UidA* gene Figure 3. The *UidA* gene codes for β-D-glucuronides "is an enzyme that liberates glucuronic asid (GlcA) sugars from small-molecule conjugates and complex carbohydrates" (11), which is frequently used for the identification of *E. coli* (12). The findings are completely consistent with Hamazah *et al.* (7) claim that all *E. coli* isolates have the gene, whereas sina *et al.* (25) discovered that only 88.89% of isolates have the *UidA* gene. This variation may possibly be caused by the *UidA* structural gene mutation (15).

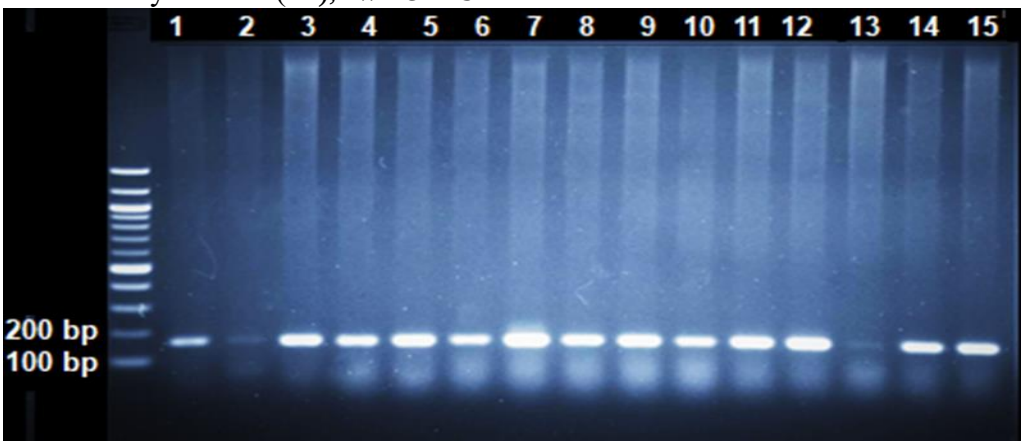

Figure 3. Gel electrophoreses of the *UidA* gene on 2% agarose gel for 70min at 80 volts with a DNA ladder of 1000bp

Antibiotic susceptibility test

The horizontal transfer of antimicrobial resistance genes between bacteria using plasmids, transposons, and integrins promotes the spread of multiresistant strains (6). As follows, *E. coli* isolates were found to have varying degrees of resistance to commonly tested antibiotics: piperacillin, 31 isolates (62%); cefuroxime, 28 isolates (56%); cefixime, 28 isolates (56%); trimethoprime 25 isolates (50%); ampicillin-sulbactm 24 isolates (48%); nalidixic acid 22 isolates (44%); azithromycin 14 isolates (28%); aztronam 10 isolates (20%); oflaxacin 10 isolates (20%); chloramphenicol 2 isoltes (4%); and other antibiotics such as amikacin and meropenem show no resistance. The results are seen in Figure 4. In China, the sensitivity of meropenem and amikacin was 100%, and this result entirely agreed with the total sensitivity that was found in this study and with the high rates of resistance to piperacillin and cefuroxime that were 73% and 50%, respectively (12), and disagrees with the low rate of ampicillin-sulbactm 35% resistance in the same study.

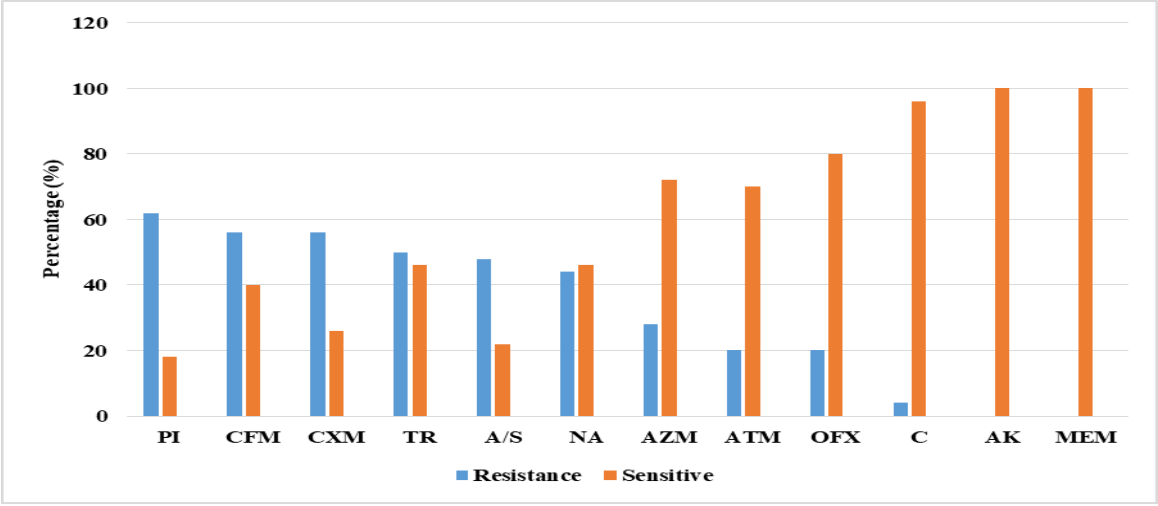

Figure 4. Antibiotic resistance in *E. coli* isolates, Piperacillin (PI), Cefixime (CFM), Cefuroxime (CXM), Trimethoprime (TR), Ampicillin-sulbactam (A/S), Nalidixacid (NA), Azithromycin (AZM), Aztronam (ATM), Ofloxacin (OFX), Chloramphenicol (C), Amikacin (AK), Meropenem (MEM).

Molecular amplification of virulence genes

All 50 isolates were used for the detection of the adhesion factor *FimH* gene and the ferric aerobactin receptor *IutA* gene. The results show that the prevalence of *FimH* is in 94% of all isolates and 97% of *IutA*. Gene size was detected after gel electrophoresis of the PCR amplification product (164,155bp) respectively. It is widely accepted that *E. coli's* adhesions are the most important pathogenicity factors. These molecules have the ability to activate pathways of communication between bacteria and host cells, thereby facilitating bacterial invasion and colonization of new tissues (22). The amplification of virulence genes in vaginal isolates was higher than that from stool (96%), while isolates from stool contained (92%). This rate was higher than those in Mexico and Iran (89.5%) for both of them (6,22). Iron is required for several different processes within cells, one of which is the transfer of electrons that occurs during the process of cellular respiration (22). The gene that codes for iron acquisition systems, also known as nutrition, was found most frequently in VEC strains *IutA*, and that was higher than that which was found by Sáez-López *et al*. (24). The rate of the *IutA* gene was higher in VEC (100%) isolates than that found in stool (92%). According to a questionnaire administered to patients, 52% of them have UTIs, a recurrent infection, burning, and itching.

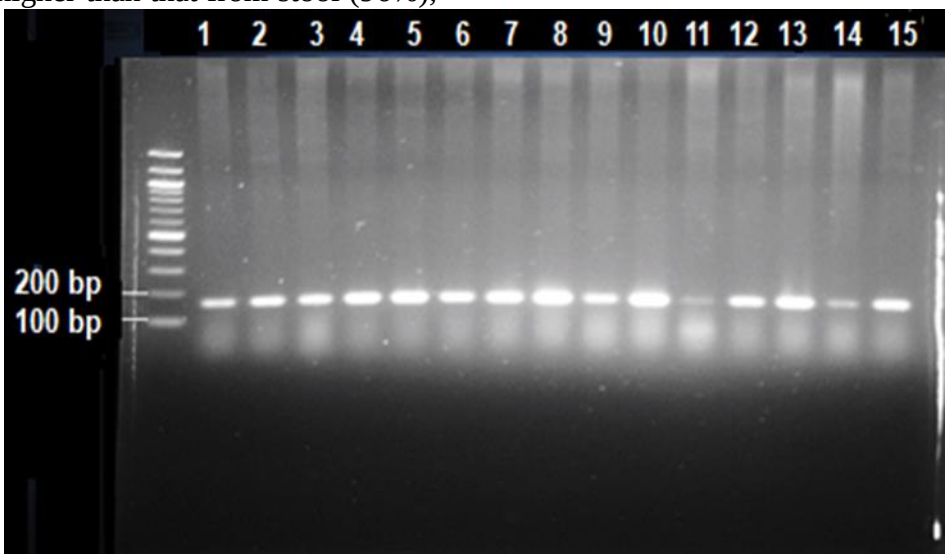

Figure 5. Gel electrophoreses of the *FimH* gene on 2% agarose gel for 70 min at 80 volts with a DNA ladder of 1000 bp

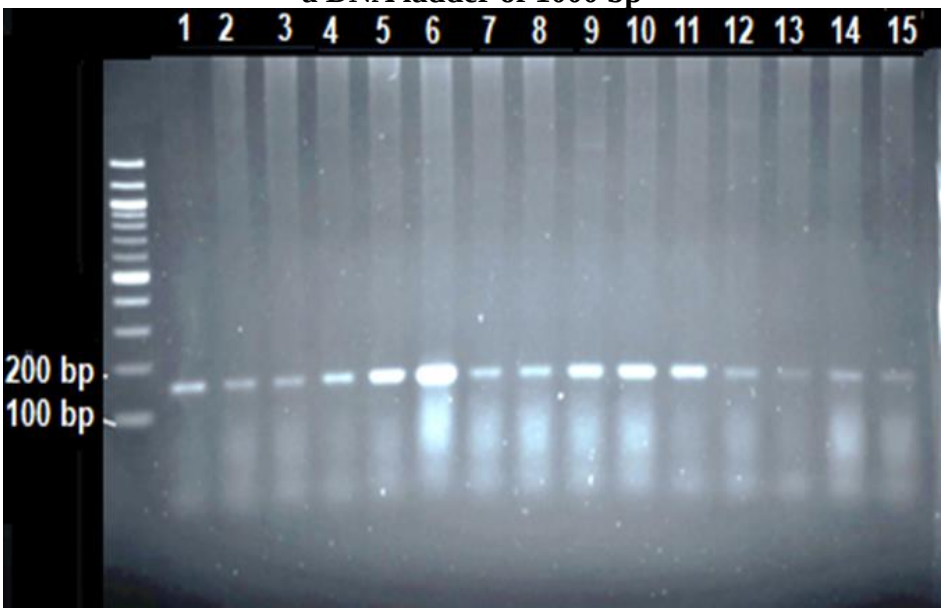

Figure 6. Gel electrophoreses of the *IutA* gene on 2% agarose gel for 70 min at 80 volts with a DNA ladder of 1000 bp

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