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ORIGINAL ARTICLE

Study of Virulence and Antibiotic Resistance of the Most Common Bacterial Species Causing Aerobic Vaginitis

[1]Rasha G. Mostafa, [2]Reem M. Elkholy, [3]Amany T. Elfakhrany, [4]Safa H. Elkhalsh, [1]Amira H. Elkhyat

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University, Egypt
[2]Department of Clinical Pathology, Faculty of Medicine, Menoufia University, Egypt
[3]Department of Clinical Pharmacology, Faculty of Medicine, Menoufia University, Egypt
[4]Department of Family Medicine, Faculty of Medicine, Menoufia University, Egypt

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Corresponding Author:
Amira Hamed Abou El-Soud ELkhyat
MD of Medical Microbiology and Immunology, Faculty of Medicine, Menoufia University
Address: Tala City, Menoufia Governorate, Egypt
Tel.: 01111156488
amirahamed61@yahoo.com

ABSTRACT

Background: *Vaginitis is one of the most common causes of women's visits to a family physician and gynecologist. If untreated it may lead to serious complications.* ***Objectives:*** *To detect the prevalence of aerobic vaginitis among women of reproductive age attending at Family Medicine and Gynecology Clinics in Menoufia University Hospital, Egypt and determine the most common bacterial isolate and its virulence profile in both pregnant and non- pregnant women.* ***Methodology:*** *High vaginal swabs were obtained from 350 women (200 pregnant and 150 non pregnant) who visited Family Medicine and Gynecology Clinics in Menoufia University Hospital during the study period and suspected of having vaginitis. Identification of isolated micro-organisms was done by standard microbiological methods. Identification and antimicrobial susceptibility testing of the most common aerobic bacterial species isolated from vaginal samples were performed. Also, 15 fecal E. coli strains were isolated from healthy women. The prevalence of Virulence genes fim H, iucC, hly F, papC, afa, ibe A and cnf among Escherichia coli (E. coli) isolates was examined by multiplex PCR.* ***Results****: The prevalence of aerobic vaginitis was 43% in pregnant women and 26% in non-pregnant women. E. coli was the most common isolated aerobic bacterial spp. Antibiotic resistance of E. coli isolated from non-pregnant women was higher than those isolated from pregnant women with a highly statistically significant difference. Higher rate of virulence genes was detected among E.coli isolated from pregnant women when compared with those isolated from non-pregnant women with a highly statistically significant difference (P<0.001). Comparing virulence factors of total vaginal E. coli isolates (50) with fecal E. coli isolates (15), the vaginal E. coli strains harbored higher percentage of virulence genes than did fecal E. coli strains with a highly statistically significant difference (P<0.001).* ***Conclusions:*** *Escherichia coli from pregnant women with aerobic vaginitis is more virulent than those from non-pregnant women, thereby increasing possible maternal and neonatal complications.*

INTRODUCTION

Vaginitis is the most prevalent genital infection recognized among women in the primary health care sector and in gynecology clinics. The classical etiological agents of vaginitis are *Candida albicans*, *Trichomonas vaginalis* and pathogens causing bacterial vaginosis (BV) [1].

Aerobic vaginitis (AV) is a clinical entity was first defined in 2002 as the isolation of aerobic bacteria from women with symptoms of vaginitis. It is diagnosed when vaginal smear is deficient in lactobacilli, presence of cocci or coarse bacilli, positive for parabasal epitheliocytes and/or positive for vaginal leucocytes (with their granular aspect). The identification of the causative agents of AV and their antimicrobial sensitivity pattern is very important for proper management of this infection[2]. The predominant pathogens causing AV are *E. coli*, group B Streptococci, *S. aureus*, and Enterococci. These aerobes increased by 3 to 5 folds and were associated with inflamed vaginal mucosa when compared with the normal vaginal flora. Untreated AV are commonly associated with a significant risk of morbidity in women in the form of pelvic inflammatory disease (PID), which can cause tubal infertility, ectopic pregnancy, reproductive dysfunction and adverse pregnancy outcomes (e.g., preterm labor and low birth weight) [3].

E.coli are enteric Gram-negative bacilli accounting for 80 to 90% of AV in pregnant women and frequently isolated as the sole organism. *E. coli* had also been more

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commonly reported in mothers who delivered preterm than those delivering full term. *E.coli* had also been accused in various infections in pregnant women, as intra-amniotic, puerperal and even neonatal sepsis both early and late neonatal sepsis. The mother having AV with *E.coli*, may transmit it to her newborn after colonization or infection of amniotic fluid, after membrane rupture or on passage of the neonate through the vaginal canal during labor leading to neonatal sepsis[4].

The ability of some *E. coli* strains to adapt to the vaginal conditions and the amniotic fluid and to invade the blood or the CSF of neonates correlates with an enrichment of virulence genes. However, data on the virulence factors of *E. coli* strains having the ability to cause infection of genital tracts of women and then affect their babies are scarce. Virulence genes include type 1 Fimbriae (fimH), haemolysin (hly), cytotoxic necrotizing factor type 1 (cnf1), P-fimbriae (pap), afimbrial adhesin (afa), invasin (ibeA), aerobactin (iuc). These genes are acquired by horizontal gene transfer. They are present on plasmids and/or in pathogenicity islands (PAIs) on the chromosome, and account for the pathogenicity of *E. coli* [5].

For better understanding of the biological role of *E.coli* in vaginitis, it is very important to determine virulence factors. Furthermore, to initiate proper treatment, it is important to determine its antimicrobial susceptibility pattern. So, this study aimed to determine the prevalence of aerobic vaginitis and the most frequent bacterial species causing AV in women of reproductive age (pregnant and non- pregnant) attending at family medicine and gynecology clinics of Menoufia University Hospital, Egypt and to compare among *E. coli* isolates from vaginal samples of pregnant and non-pregnant women and fecal *E. coli* isolated from healthy women in terms of the prevalence of virulence genes pap C, hly F, iucC, afa, fimH, ibeA and cnf genes to determine their virulence potential.

METHODOLOGY

Collection of samples and identification of aerobic microorganisms:

This study is a cross sectional study conducted at Microbiology and Immunology Department and Clinical Pathology Department, Faculty of Medicine, Menoufia University over a twelve months period (from February 2018 – February 2019) after obtaining written consents from all participants. The study was approved by the Local Ethics Committee, Faculty of Medicine, Menoufia University.

The study included 350 women (200 pregnant and 150 non-pregnant women) attended family medicine and gynecology clinics during period of the study complaining of vaginitis symptoms (discharge or itching) after exclusion of those who were already on antibiotics or unmarried, all participants were subjected to full history taking (medical, menstrual, obstetric, contraceptive and social history) and thorough clinical examination. Vaginal examination was performed. Then two high vaginal swabs were obtained from the posterior fornix of each woman. The swab stick was immediately replaced in its casing and labeled appropriately. One sample was used for microscopic diagnosis of AV as shown in Table 1[4]. The other swab was cultured on different media (Oxoid, UK); blood agar, chocolate and MacConkey's agar plates and incubated at 37°C for 24-48 hours at Microbiology laboratory. The aerobically incubated organisms were identified by standard microbiological methods. Also, 15 fecal *E. coli* strains were isolated from healthy women. The growing *E.coli* isolates were identified by culture characters, biochemical reactions and VITEK 2 System. Confirmed *E.coli* isolates were suspended in nutrient broth supplemented with 16% glycerol and stored frozen at -80°C[6].

Table 1: Criteria for the microscopic diagnosis of aerobic vaginitis (by Donders' score) [4]

AV score	Lactobacillary grades (LBG)	Number of leukocytes	toxic leukocytes	Background flora	parabasal epitheliocytes (PBC)
0	I and IIa	≤10/hpf	None or sporadic	Unremarkable or cytolysis	None or <1%
1	IIb	>10/hpf and; ≤10/epithelial cell	≤ 50% of leukocytes	Small coliform bacilli	≤ 10%
2	III	>10/epithelial cell	>50% of leukocytes	Cocci or chains	>10%

Hpf: high power field (400× magnification).

LBG: lacto bacillary grades. I: predominant lactobacilli ''healthy micro flora''. IIa: mixed flora, but lactobacilli predominate. IIb: a mixed flora, but the proportion of lactobacilli is severely depressed. III: severely decreased or absent lactobacilli with overgrowth of other bacteria. A total score of 1–2 means normality. A score of 3–4 corresponds to slight aerobic vaginitis, a score of 5–6, moderate vaginitis. Score C7 means 'severe AV'. In practice, a score of 8–10 corresponds to so-called 'desquamative inflammatory vaginitis' the most extreme form of AV.

Antibiotic susceptibility test of *E. coli* vaginal isolates

Antimicrobial susceptibility testing for *E. coli* isolates was performed using Kirby-Bauer disk diffusion method against different antimicrobial agents (Oxoid) as recommended by CLSI, 2018. tested antimicrobials included amoxicillin/clavulanate (AMC, 20µ/10µg), cefoxitin (FOX,30µg), cefepime (FEP, 30µg), cefotaxime (CTX, 30µg), ceftriaxone (CRO, 30µg), ceftazidime (CAZ, 30µg), imipenem (IPM, 10µg), meropenem (MEM, 10µg), ertapenem (ETP, 10µg), aztreonam (ATM, 30µg), gentamicin (CN, l0µg), amikacin (AK, 30µg), ciprofloxacin (CIP, 5µg), Levofloxacin (LEV, 5 µg)[7] .

Detection of *E. coli* virulence genes

***DNA extraction*:** Bacterial DNA was extracted and purified using the gene JET™ genomic DNA purification kit (Therm Fisher Scientific, UK). Primers were shipped and received in a lyophilized state (Invitrogen, Thermo Fisher, UK). The volume of nuclease-free H2O added to the lyophilized primer was determined by reading the number of nmol of primers in the tube and multiplied by 10 to make a 100 µmol/L primer stock [8]

Determination of virulence genes Each gene was amplified with the primers described in table 2 in a total volume of 25ul containing 1x PCR buffer, 0.1 mM (each) deoxyribonucleoside triphosphate, 0.5uM (each) primer, 0.5 U of Taq polymerase, and 25 ng of DNA. The reaction conditions were as follows: initial denaturation at 94°C for 3 min followed by 25 cycles of denaturation at 94°C for 1 min, annealing at the melting temperature of each primer for 1 min, and extension at 72°C for 1 min, followed by a final 10-min extension period at 72°C. The amplification products were separated by electrophoresis in a 1% agarose gel and visualized after ethidium bromide staining. A 100-bp DNA ladder (Life Technologies) was used in each gel as a molecular size marker. The results were considered to be positive if the amplification product was of the expected molecular size, negative controls were used and the assays were performed twice.[8]

Table 2: Primers for detection of virulence genes:

Genes	Sequences of primers (5´ to 3´)	Reference	Size of product (bp)
hly F	F′ GGCCACAGTCGTTTAGGGTGCTTACC R′ GGCGGTTTAGGCATTCCGATACTCAG	8	449
fim H	F′ TCGAGAACGGATAAGCCGTGG R′ GCAGTCACCTGCCCTCCGGTA	9	506
papC	F′ ATATCCTTTCTGCAGGGATGCAATA R′ CTGTAATTACGGAAGTGATTTCTG	9	328
iuc C	F′ AAACCTGGCTTACGCAATTGT R′ ACCCGTCTGCAAATCATGGAT	9	269
cnf	F′ TTTCCTTTTTATATCTC R′ ACTGCTGGGTATATCAA	10	585
afa	F′ GGC AGA GGG CCG GCA ACA GGC R′ CCC GTA ACG CGC CAG CAT CTC	10	559
ibe A	F′ TTACCGCCGTTGATGTTATCA R′ CATTAGCTCTCGGTTCACGCT	10	171
aer	F′ TACCGGATTGTCATATGCAGACCGT R′ AATATCTTCCTCCAGTCCGGAGA AG	8	602

Statistical analysis

Computer SPSS program version 17 was used. The results were expressed as ranges and mean± SD. Chisquare test was done and p value <0.05 was considered as significant

RESULTS

Of the 200 pregnant women, 86 (43%) had aerobic vaginitis. However, only 39 (26%) of non-pregnant women had AV. Of the 86 pregnant women with AV, 79 had a single growth, and 7 had mixed growth. Of the 39 non- pregnant women with AV, 23 had a single growth, and 16 had mixed growth.

The prevalence of AV among pregnant women was higher than that in non-pregnant women with a highly statistically significant difference ($P < 0.001$) as shown in table 3.

Table 3: Results of aerobic cultures of vaginal swabs obtained from pregnant and non- pregnant women

	Positive culture		Negative culture		X^2	P value
	No	%	No	%		
Pregnant (N=200)	86	43	114	57	10.78	0.001 HS
Non pregnant (N=150)	39	26	111	74		
Total (N= 350)	125	35.7	225	64.3		

During this study, 148 aerobic bacteria were isolated (93 from pregnant women and 55 from non-pregnant women. The most frequent isolates in pregnant and non- pregnant women were *E. coli* (33.3%, 34.5%) followed by *Klebsiella* spp. (17.2%, 16.4%) and *Staph aureus* (16.1%, 14.5 %) respectively with no statistical significant difference between the two groups regarding isolated bacterial spp. as shown in table 4

Table 4: Number and percentage of isolated aerobic bacteria from positive cultures:

			Pregnant		Non pregnant			
Aerobic bacterial growth		**Single growth**	**79**		**23**			
		mixed growth	**7**		**16**			
		Growth	**86**		**39**			
			Bacterial isolates from pregnant		**Bacterial isolates from non- pregnant**		X^2	**P value**
			No	**%**	**No**	**%**		
Isolates	**Gram-positive**	***Staph aureus***	15	16.1	8	14.5	2.18	00.94 NS
		Group B streptococci	10	10.8	5	9.2		
		Enterococci	7	7.5	3	5.5		
	Gram-negative	***E coli***	31	33.3	19	34.5		
		Klebsiella spp.	16	17.2	9	16.4		
		Enterobacter spp.	6	6.5	7	12.7		
		Proteus spp.	5	5.4	2	3.6		
		Pseudomonas spp.	3	3.2	2	3.6		

Isolated *E. coli* from pregnant women were highly resistant to amoxicillin/clavulanic acid (64.5%) followed by Cefotaxime (48.4%), cefoxitin (41.9%) and Ceftriaxone (38.7%). On the other hand, a high sensitivity to carbapenems (100%) followed by fluoroquinolones (Levofloxacin 93.5% and Ciprofloxacin 87.1%) was observed. Regarding *E. coli* isolated from non-pregnant women , antibiotic resistance was higher than those isolated from pregnant women with a highly statistically significant difference ($P < 0.001$) as shown in table 5

Table 5: Antimicrobial susceptibility of vaginal *E. coli* isolates by disc diffusion method:

Resistance to antimicrobial agents by disk diffusion method	***E. Coli* isolates**								**Z test**	**P value**
	Pregnant (N=31)				**Non- pregnant (No. =19)**					
	S		**R**		**S**		**R**			
	No	**%**	**No**	**%**	**No**	**%**	**No**	**%**		
Amoxicillin/clavulanic acid	11	35.5	20	64.5	0	0	19	100	6.70	<0.001 HS
Cefoxitin	18	58.1	13	41.9	8	42.1	11	57.9		
Ceftazidime	20	64.5	11	35.5	6	31.6	13	68.4		
Cefotaxime	16	51.6	15	48.4	6	31.6	13	68.4		
Ceftriaxone	19	61.3	12	38.7	6	31.6	13	68.4		
Cefepime	20	64.5	11	35.5	8	42.1	11	57.9		
Aztronam	21	67.7	10	32.3	6	31.6	13	68.4		
Ertapenam	31	100	0	0	19	100	0	0		
Imipenam	31	100	0	0	19	100	0	0		
Meropenam	31	100	0	0	19	100	0	0		
Amikacin	23	74.2	8	25.8	7	36.8	12	63.2		
Gentamycin	25	80.6	6	19.4	7	36.8	12	63.2		
Ciprofloxacin	27	87.1	4	12.9	10	52.6	9	47.4		
Levofloxacin	29	93.5	2	6.5	10	52.6	9	47.4		

The frequency of virulence genes (hly F, fim H, iuc C, cnf, pap, afa and ibeA) in *E.coli* isolated from pregnant and non- pregnant women, were (77.4%, 36.8%) (32.3%, 31.6%) (51.6%, 21.1%) (58.1%, 26.3%) (45.2%, 15.8%) (12.9%, 10.5%) and (6.5%5.3%) respectively. Higher rate of virulence genes was detected among *E.coli* isolated from pregnant women when compared with those isolated from non-pregnant women with a highly statistically significant difference (P<0.001). Comparing virulence factors of total vaginal isolates (50) with fecal isolates (15), the vaginal *E. coli* strains harbored higher percentage of virulence factors than did fecal *E. coli* strains with a highly statistically significant difference (P < 0.001) as shown in table 6 & Fig 1.

Table 6: Prevalence of hly F, fim H, iuc C, cnf, pap, afa and ibeA virulence genes among *E. coli* strains isolated from vaginal samples of pregnant and non- pregnant women and fecal *E.coli* isolated from healthy women:

Virulence genes	Pregnant women *E.coli* isolates (31)		Non pregnant women *E.coli* isolates (19)		Total *E.coli* Vaginitis isolates (50)		Control isolates(15)		*Z test*	*P value*
	No	%	No	%	No	%	No	%		
hly F	24	77.4	7	36.8	31	62	3	20		
fim H	10	32.3	6	31.6	16	32	0	0		
iuc C	16	51.6	4	21.1	20	40	0	0	3.85*	<0.001*
cnf	18	58.1	5	26.3	23	46	1	6.7	4.83**	<0.001**
pap	14	45.2	3	15.8	17	34	3	20		
afa	4	12.9	2	10.5	6	12	1	6.7		**HS**
ibe A	2	6.5	1	5.3	3	6	1	6.7		

* Between vaginal *E.coli* (pregnant and non- pregnant women)
**between vaginal *E.coli* and fecal *E.coli*

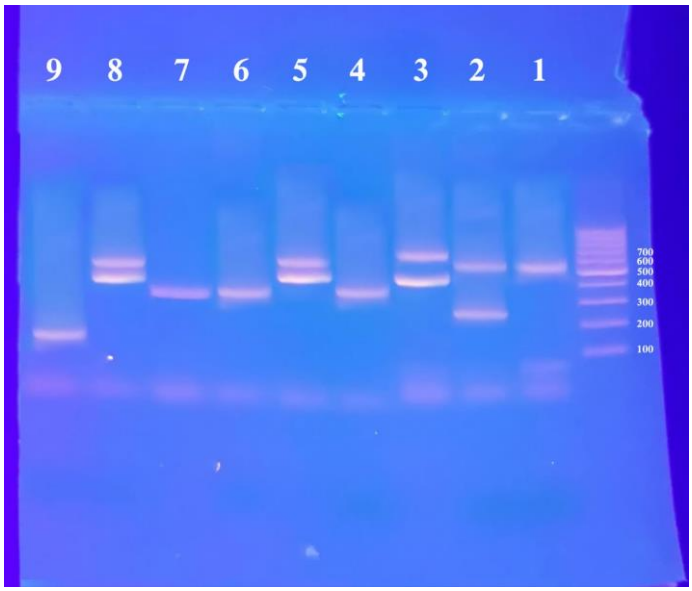

Fig 1: Agarose gel electrophoresis for the PCR amplified products of *E. coli* virulence genes hly F, fim H, iuc C, cnf, pap, afa and ibeA
Lane (M) 100 bp DNA ladder. Lanes 1&2 showed fim H (at 506pb), also lane 2 showed iuc C (at 269pb).
Lane 3 showed two genes; hly F (449pb)& aer (602pb)
Lanes 4, 6&7 showed pap C gene (328)
Lane 5 and 8 showed two genes ; fim H (449pb) & cnf (585pb)
Lane 9 showed ibe A (171pb)

DISCUSSION

Vaginal infections are usually caused by alteration of normal vaginal flora, where lactobacilli are displaced by other proliferating bacteria, including potentially pathogenic. So, diagnosis of these infections is mandatory to provide treatment according to the etiological agent detected by the microbiologist and not only to identify the classical etiological agents (*Trichomonas vaginalis*, *Candida albicans*, and bacterial vaginosis pathogens) [1]. Aerobic vaginitis (AV) has been described recently and characterized by abnormal vaginal flora with proliferation of aerobic enteric bacteria, vaginal inflammation and deficient epithelial maturation. Misdiagnosis of AV leads to treatment failure and serious complications including spontaneous abortions, preterm labor, premature rupture of membranes, chorioamnionitis, pelvic inflammatory disease, and post-operative infections after gynecological surgery and easier acquisition of sexually transmitted diseases[4].

In this study, the observed prevalence of aerobic vaginitis in pregnant women (43%) was higher than that in non-pregnant (26%) with a highly statistically significant difference (P<0.001). This result was in agreement with the data published by Anderson et al. [11], Dermendjiev et al. [12] and Han et al[13]. This result may be explained as mucosal immunity of the genital tract is compromised during pregnancy which may be due to immunologic alterations or hormonal changes leading to higher rate of AV in pregnant women. Another explanation is that hemorrhoids frequently occur in pregnancy. So, the bacteria colonizing the intestine may spread locally and colonize the vagina as the rectal mucosa is exposed to the perineum in women having external hemorrhoids. Also, Personal hygiene habit may play a serious role in the pathogenesis of AV. On the other hand, Mulu et al. [14] found that the prevalence of AV was significantly higher in non-pregnant compared to pregnant women (p = 0.03) and explained this result by lowering of immunity in non-pregnant women included in their study due to use of steroid drugs as contraceptive.

In our study, *E.coli* was the most common isolated aerobic bacteria from both pregnant (33.3 %) and non-pregnant women (35.5 %) followed by *Klebseilla* spp. and *Staph aureus*. The same result was obtained by Mulu et al[14]. Also, Kaambo et al[3]. mentioned that *E.coli* accounts for 80 to 90% of AV in pregnant women and this might be attributed to the fact that *E.coli* is a main member of the normal fecal flora and various virulence factors contributing to its pathogenicity. However, Mumtaz et al[15]. found that *Staph aureus* was the most prevalent vaginal pathogen and Han et al. [13] reported that the most common isolate of AV was *Enterococcus faecalis* (40%). Since *E. coli* was the most isolated bacterial pathogen, this work focused on *E.coli.*

Antibiotic resistance in *E. coli*, the main etiological agent of aerobic vaginitis, is a serious problem that leads to ineffective treatment and persistent infection. So, antimicrobial susceptibility test should be performed for proper treatment[16]. The isolated *E.coli* strains from pregnant women in the current study showed a high resistance to amoxicillin/clavulanic acid (64.5%) followed by Cefotaxime (48.4%), cefoxitin (41.9%) and Ceftriaxone (38.7%). On the other hand, a high sensitivity to carbapenems (100%) followed by florquinolones (Levofloxacin 93.5% and Ciprofloxacin 87.1%) was observed. Similar results were obtained by Al-Mayahie[17] and Mulu et al.[14] Regarding *E. coli* isolated from non-pregnant women, antibiotic resistance was higher than those isolated from pregnant women with a highly statistically significant difference (P < 0.001). The same observation was get by Al-Mayahie [17] who suggested that genital tract of pregnant and non-pregnant women represent different environments for *E. coli* in terms of antibiotics that can be used for treatment of each. Limited types of antibiotics can be used during pregnancy in order not to affect the fetus. So, there is difference in selective pressure that selects for emergence of resistance[17].

The ability of *E. coli* strains to cause a disease is influenced by the carriage of virulence factors. Little is known about virulence properties of *E. coli* that may promote bacterial colonization and cause aerobic vaginitis[8]. In this study, we examined *E. coli* strains isolated as the causative agent of aerobic vaginitis from pregnant and non-pregnant women and fecal *E. coli* strains isolated from normal healthy women.

Comparing virulence factors of 50 vaginal isolates with 15 fecal isolates, the vaginal *E. coli* strains harbored higher percentage of virulence factors than did fecal *E. coli* strains with a highly statistically significant difference (P < 0.001). The same result was obtained by Guiral et al[18]. In contrast to our result, Hilbert et al. [19] did not find any significant differences regarding prevalence of these virulence factors between rectal and vaginal *E. coli* isolate. This can be explained as the rectal isolates they examined are not representative of fecal *E. coli* and they are enriched in virulence factors[19].

The frequency of virulence genes (hly F, fim H, iuc C, cnf, pap, afa and ibeA) in *E.coli* isolated from pregnant and non-pregnant women, were (77.4%, 36.8%) (32.3%, 31.6%) (51.6%, 21.1%) (58.1%, 26.3%) (45.2%, 15.8%) (12.9%, 10.5%) and (6.5%5.3%) respectively. Higher rate of virulence genes was detected among *E.coli* isolated from pregnant women when compared with those isolated from non-pregnant women with a highly statistically significant difference (P<0.001).The same result were reported by Padilla et al. [5] who found that the most common genes detected in

both pregnant and non-pregnant women were fimH, hly, and cnf1 and Guiral et al. [18] who reported that hly, cnf, pap and iroN genes were the most common detected in both pregnant and non-pregnant women. Whatever the type of most common virulence genes detected in different studies, all authors stated that *E. coli* strains isolated from vaginal and/or endocervical samples of pregnant women are more virulent than those from non-pregnant women.

Our previous results regarding the comparison between three groups of *E.coli* isolates can be explained by the general thought that *E. coli* strains incriminated in neonatal infections originate from the vagina, which was colonized from a rectal source. Our data indicate that the physiological conditions of different ecosystems crossed by *E. coli,* make a strong selection on *E. coli* and this pressure leads to progressive changes in *E. coli* subpopulation. This population change was first imposed by the vaginal ecosystem. The vaginal *E. coli* strains harbored a lot more virulence factors than did the fecal *E. coli* strains isolated from healthy women. Thus, vagina acts as a partial filter that favors colonization and infection by *E. coli* strains that present a high risk for neonatal infections[9].

Genes, iucC and ibeA, are associated with CSF strains and may play a major role in the pathogenesis of neonatal meningitis**.** Also, *E.coli* strains having genes hly F and fimH, afa and pap C gave hemolytic and adhesion properties and higher percentage of genes associated with pathogenicity islands (PAIs), such as the hly, cnf1 and pap genes were observed among isolates causing neonatal infections[20]. In this study, higher rate of these virulence genes was detected among *E.coli* isolated from vaginal samples. This finding may be significant for three reasons. First, persistence of these *E. coli* strains in the vagina of pregnant women can expose neonates to a higher risk of infection. Second, *E. coli* is the most frequent pathogen isolated from bacterial prostatitis patients. So, vaginal carriage of these virulent strains could expose sexual partner to a risk of prostatic infection. Third, women themselves are more prone to recurrent urinary and genital tract infection in association with these virulent strains. [20] So, it would be interesting in future studies to follow up of pregnancy outcomes of untreated patients with aerobic vaginitis

CONCLUSIONS

- *Escherichia coli* strains isolated from pregnant women are more virulent than those from non-pregnant women developing severe infections, thereby increasing possible neonatal sepsis. Further studies are needed to determine the role of each virulence factor in the transmission of *E. coli* from mother to baby and subsequent complications
- A better understanding of the virulence and antibacterial sensitivity of *E. coli* strains isolated from vaginitis patients can lead to improved diagnosis and treatment of disease.

Conflicts of interest:

The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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