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Identification of *Escherichia coli* by analysis of *16S rRNA* and study some Virulence genes isolated from female genital tract Infections

(1) Enas Razaq ALwaeli, (2) Rahman Laibi Chelab, (3) Enaas Saleh Jawad

(1) Department of Anesthesia Techniques, College of Medical and Health Technology, AL-Ayen University.
(2) Department of Biology, College of Education for Pure Sciences, Thi-Qar University.
(3) Department of Gynecology and obstetrics. Medical college. Thi-Qar University

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Abstract:

Vaginal diseases such as trichomoniasis, vulvovaginal candidiasis (VVC) and bacterial vaginosis (BV) are widespread worldwide. Accurate detection and administration of effective medications are essential, since such infections are related to negative effects for women during pregnancy and for newborns However, the presence of *Escherichia coli* in such infections is controversial, and hence it is important to identify these genetic and typical characteristics of *E. coli* strains isolated from genital infection, this review will focus on the diagnosis of *E. coli* isolates by 16SrRNA, discovering the effectiveness of some antimicrobials and studying some genes of virulence factors that affect the female reproductive system, In addition to its effect on fetuses in pregnant women. include with its impression share (*fimH),* type 1 fimbriae (%76.36), (*cnf1),* cytotoxic necrotizing factor (36.36%), (*usp*) uropathogen specific protein (58.18%) and (*ibeA*), invation brain endothelium (32.72%), from (300) female genital tract secretions specimens isolate ,The number of bacterial isolates was 165 (55%) and the number of yeast isolates was 110 (36,6%) no growth 25(8,33%) and number of *E.coil* isolates(55) ,The results also showed that the resistance and sensitivity of the different types of antimicrobial such as ,amoxicillin-clavulanic acid(AMC) (54.54%) doxycycilin (DO) (32.72%) ,cefixime (CFM)(54.54%) ceftriaxone(CRO)(50.90%), cephalothin (KF) (14.54%) ,Cefotaxime (CTX) (54.54%) ,Nitrofurantoin (NI) (9.09%) ,ciprofloxacin (CIP) (78.18%) , gentamicin (CN) (41.81%) trimethoprim /sulfamethoxazole (TS) (27.27%),imipenem(IPM) (89.09%) meropenem,(MEM) (90.90%), Amikacin (AK) (58.18%) ,Netilmicin (NET) (32.72%) ,lincomycin (L) ,(14.54%) Tobramycin (TN) (20%).

Key words: vaginitis, *Escherichia coli*, antimicrobial susceptibility, virulence genes

Introduction:

Vaginitis is one of the most important reasons why women, are looking for a gynecologist. [1] The effects of vaginitis are complex and include psychiatric disorder caused by spontaneous abortion, premature membrane rupture, premature birth, chorionic and amniotic inflammation, pelvic inflammatory disorder, endometriosis after childbirth, and ease of HIV infection. [2,3] The strength of *E. coli* strains that because

disease is affected by the distribution of virulence factors that give rise to the disease [4] for example, UTI in non-immunocompromised adult women is often correlated with *E. coli* strains that contain several virulence factors. [5].

Many of the virulence factors that caused the development of colonization of the vagina or cervix, which included many specific infections in pregnant women, as well as in puerperal necrosis that causes neonatal infection in both early and late sepsis, urinary tract infections or meningitis transmission *E. coli* From mother to infant after the invasion or contamination of the amniotic fluid, separation of the membrane or during the passage of the newborn through the vaginal canal at birth, the formation of bacteria in the vagina is complicated and depends on changes in multiple events in a woman's life, and any change in this balance contributes to inflammation. [1]

E. coli isolated from pathogens have specific properties that allow them to colonize and stay on infection sites. These characteristics are specific to many pathogens of *E. coli*, in any case of the site of infection. For example, type 1 fimbriae (FimH) make easy adhesion to the walls of the bladder and leads to cystitis. [5] and (ibeA) that gene related with the brain epithelium invasion ,(CNF1) cytotoxic necrotizing factor 1 causes tissue damage that facilitates bacterial diffusion, releases host nutrients, and may also modulate host signaling pathways that affect many of the processes correlated with inflammatory reactions, host cell survival, and fundamentals cytoskeletal dynamics, both genes, ibe, cnf, raise the likelihood that their newborns may develop neurological disorders or meningitis [6,7], and uropathogenic specific protein gene, (usp), related to prostatitis, pyelonephritis, and urinary tract bacteremia [8]

The equilibrium between *Escherichia coli* and the immune. system of its hosts is responsible for colonizing the intestine without seriously damaging it. However, *Escherichia coli* are microorganisms with a high capacity to adapt to new environments through their high genetic resiliency, which leads to the formation of new patterns, such as diarrheagenic and uropathogenic [9,10] . *E. coli* strains that cause extraintestinal infections have a virulent reservoir that helps them to cause infection [11,12] little renowned a both The virulence factors properties of *E. coli* in vagina which can facilitate bacterial invasion and it causes of GTIs. The following study, isolated *E. coli* strains were investigated as a causative agent (GTIs) by identifying virulence genes and antimicrobial sensitivity.

Materials and methods:

Samples collection and identification of bacteria and yeasts from vaginal swabs:

swabs of Vaginal were collected from 300 female patients who come to the consulting clinic at the Maternity and Children Hospital in Nasiriyah, Thi-Qar for the period 30/12/2018 to 30/9/2019, The aged of patients ranged between 17-50 years. Form each patient two vaginal swabs were taken and transported to the laboratory by inoculating the swab into a sterile tube containing transport medium ,one of the swabs was directly examinated by Gram stained and wet mounted film, the other swab was used for inoculation onto the SDA medium ,CHROM agar *Candida* ,CHROM agar orientation CHROM agar Staph , Eosin methylene blue, MacConkey's agar, for microbiological investigation and Phenotypic diagnosis .Finally, the biochemical diagnosis was used Vitek 2 system

Antimicrobial susceptibility test:

The susceptibility test was carried out using the disk diffusion method according to [13] The following antimicrobials were tested: amoxicillin-clavulanic acid(AMC,30mg), doxycycilin (DO,10mg), cefixime(CFM,5mg), ceftriaxone (CRO,30mg), cephalothin (KF,30mg) ,Cefotaxime (CTX,30mg) ,Nitrofurantoin (NI,300mg) ciprofloxacin (CIP,10mg), gentamicin (CN,10mg), Amikacin (AK,10mg),

trimethoprim/ sulfamethoxazole (TS,1.25,23.75 mg), imipenem (IPM,10mg), meropenem (MEM,10mg Netilmicin (NET,30mg) ,lincomycin (L,10mg) ,),Tobramycin(TN,10mg) (Fitted by USA company ,Bioanalyse)

Determination of virulence genes:

All of E. *coli* isolates were cultured at (37 ° C) in BH broth (10 mL), centrifuged (8000 gm) and the sediments obtained were used to extract the genomic DNA, (which was created by the Geneaid company), following the manufacturer's directions. The DNA was processed at (4 ° C) before usage ,in this research study *Escherichia coli* genes are often associated with vaginitis : type 1 fimbriae (FimH) ,brain epithelium invasion (ibeA), (CNF1) cytotoxic necrotizing factor .All virgin genes were studied in (55) strains of *Escherichia coli* and all PCR primer nucleotide were designed for this study ((**16SrRNA** gene, **F**, TGGTAGTCCACGCCGTAAAC, **R**, AGTTGCAGACTCCAATCCGG (524bp), (**usp) gene**, **F**,TACCTGGACGCCGGAAAATC,**R**,CCTCAGGGACATAGGGGGAA(500bp),(**ibeA)gene**,**F**,GCGG ATGCAATGCTTGAACA,**R**,TTGTTCCCACCGTCATACCG(655bp),(**fimH)gene**,**F**,CTGGTCGGTAA ATGCCTGGT,**R**,GCACTGAACCAGGGTAGTCC(560bp),(**cnf1)gene**,**F**,GGTGGTGGTCTAAAAGG GGG,**R**,ACCTAAGCTTGACTGTGGGC(351bp)) Products amplified by electrophoresis and detected in an agarose gel (1.5%), Then visualized with (0.25 µg / ml) bromide ethidium DNA ladder (100 bp) (Bioneer) was used as a molecular scale marker for each gel, the gel was analyzed and photographed on a UV trans illuminator[14].

DNA sequencer method:

DNA sequencing system was used for confirmatory genetic identification and study of phylogenetic trees analysis as well as deposited in National Center for Biotechnology Information (NCBI) Genbank data base of 16S ribosomal RNA gene *E. coli* isolates. The DNA sequencies of the target genes were detected by PCR technique and the PCR product Submit to Macrogen company (Korea) for implement the DNA sequencing by Applied Biosystems (AB) DNA sequencing system , the NCBI-Blast Alignment Identification used to analyzed and processed the acquired nucleotide sequencing gene by (http:/www.ncbi.nlm.gov/BLAST/Blast.cgi) and phylogenetic tree analysis based on the Maximum Likelihood Process and Molecular Evolutionary Genetics Analysis by use (Mega v. 6).

Statistical analysis:

The data obtained through the surveys was processed using the SPSS were analyzed by Chi-squared

Result and Discussion:

After conducting biochemical tests for isolates . The results of this study showed that , number bacterial isolates 165 (55%) and the number of yeast isolates was 110 (36,6%) and no growth 25(8,33%) and only 55(33.33%) isolate *E .coli* from 165 specimens were positive culture for bacteria, this results depends on the phenotypic diagnosis They appear metallic green on (EMB), on CHROM agar orientation appear violet (which is a differential medium between bacteria types according to the enzymatic color reactions and finally diagnosis by used Vitek 2 system .

The vagina is a complex and multiple habitat of microorganisms, and vaginal infections are the leading cause of diseases worldwide from microbial flora. Vaginal studies reveal that the microorganisms usually found in this habitat play a key role in preventing colonization of exogenous bacterial species. [15] normally

high number of *Lactobacillus* species are great importance in maintaining a vaginal pH at about 4.7 (or less), which prevents the growth of other microorganisms [16]. Bacterial vaginosis is the most common cause of vaginal discharge and is described as a bacterial disorder of the vaginal micro flora where opportunistic species multiply [17] *Escherichia coli* multiply in healthy intestinal canal of, humans and different animals. It is accepted that the pathogenic *E. coli* strains are derived from dual strains by obtaining chromosomes or virulence factors outside of the chromosomes, despite the pathogenic *E coli* strains are mostly opportunism, it may cause a number of serious illnesses. [18] and the presence of *E. coli* in the vagina is controversial in terms of his potential, pathogen role. Some studies have indicated their presence as an opportunistic, microorganism [19] and another studies have concluded that this type has not been find out in a healthy vaginal epithelium [20].

susceptibility of Antimicrobial:

Prevalence of resistance is associated with hereditary mobile elements, such as plasmids, that may also load virulence determinants [21] .When bacteria often resist standard treatments, they lead to long-term illness and greater risk of death, and increase the chances of spreading resistant microorganisms by transmitting resistance to other strains of the same or other types. Moreover, the costs associated with staying in the hospital for long periods and used the last generation of antibiotics increases dramatically, in addition to the side effects associated with the use of these antibiotics [22] A greater number of sensitive strains were observed for meropenem, imipenem, ciprofloxacin, cefotaxime, cefixime, Additionally, a large number of antibiotic resistant strains were found in (Table 1) Most of the *E. coli* strains were sensitive to fluoroquinolones, carbapenems, quinolines, These findings are similar to those published in the previous study [23,24] but vary from other *E. coli* Isolated strains from non-vaginal environments [25,26] We believe that the cause of high antibiotic resistance is random and excessive use that leads to creating resistant strains, these results are very important when the doctor begins prescribing the correct antibiotic to treatment.

Table (1). Antimicrobial resistance to *E. coli* strains isolated from vaginitis

Antimicrobial		S(N/%)	R(N/%)	X2	P
1	AMC	30(54.54)	25(45.45)	0.455	0.500
2	DO	18(32.72)	37(67.27)	6.564	0.010
3	CFM	30(54.54)	25(45.45)	9.618	0.002
4	CRO	28(50.90)	27(49.09)	0.018	0.893
5	KF	8(14.54)	47(85.45)	27.655	0.000
6	CTX	30(54.54)	2545.45)	0.455	0.500
7	NI	5(9.09)	50(90.90)	36.818	0.000
8	CIP	43(78.18)	12(21.81)	17.473	0.000
9	CN	23(41.81)	32(58.18)	1.473	0.225
10	AK	32(58.18)	23(41.81)	1.473	0.225
11	TS	15(27.27)	40(72.72)	11.364	0.001
12	IPM	49(89.09)	6(10.90)	33.618	0.000
13	MEM	50(90.90)	5(9.09)	36.818	0.000
14	NET	18(32.72)	37(67.27)	6.564	0.010
15	L	8(14.54)	47(85.45)	27.655	0.000
16	TN	11(20)	44(80)	19.800	0.000

Amoxicillin, clavulanicacid (AMC) (30mg),
doxycycilin (DO) (10mg),
cefixime (CFM) (5mg),
ceftriaxone (CRO) (30mg),
cephalothin (KF) (30mg)
Cefotaxime (CTX) (30mg),
Nitrofurantoin (NI) (300mg)
ciprofloxacin (CIP) (10mg),
gentamicin (CN) (10mg),
Amikacin (AK) (10mg),
trimethoprim /sulfamethoxazole (TS) (1.25,23.75 mg),
imipenem (IPM) (10mg),
meropenem (MEM) (10mg)
Netilmicin (NET) (30mg)
, lincomycin (L) (10mg),
Tobramycin (TN) (10mg).

Detecting of gene 16S rRNA:

The function of the 16S rRNA gene has not changed over time, indicating that changes in the random sequence are a more accurate measure of time (development) and gene measurement (1500 bp) which is large enough to store information and useful for bacterial classification [27]. Performed DNA multiplication test for the 16SrRNA by using the initiator at 524bp, this gene confirms the diagnosis of this bacterial, after PCR reaction the product carried over to agaroes gel at 1.5% concentration and UV examination Purple showed molecular size packages of 524 base pairs of all Bacterial isolates (55) This means that the target gene for these isolates is present (Figure 1) through the results, this gene is found in all *E. coli* strains, and it carries a special genetic code to diagnose these bacteria and the results are in accordance with the routine results therefore diagnostic tests are dispensed using this gene to save time and effort also, the molecular diagnosis of bacterial isolates is important and gives accurate and sensitive results [21].

It exists Three Ribosomal genes 16S rRNA, 23S rRNA ,5S rRNA genes, historically ,16S rRNA gene is generality the most widely used in to the purpose of diagnosis, due to its high ability to own a number of copies moderate genetic that belongs to the bacterial that it owns As this gene is found in all types of bacteria even mutated ones because the 16S rRNA gene contains Unique proteins that give information or instructions for any bacteria that contain it, which ultimately facilitates diagnosis [27]

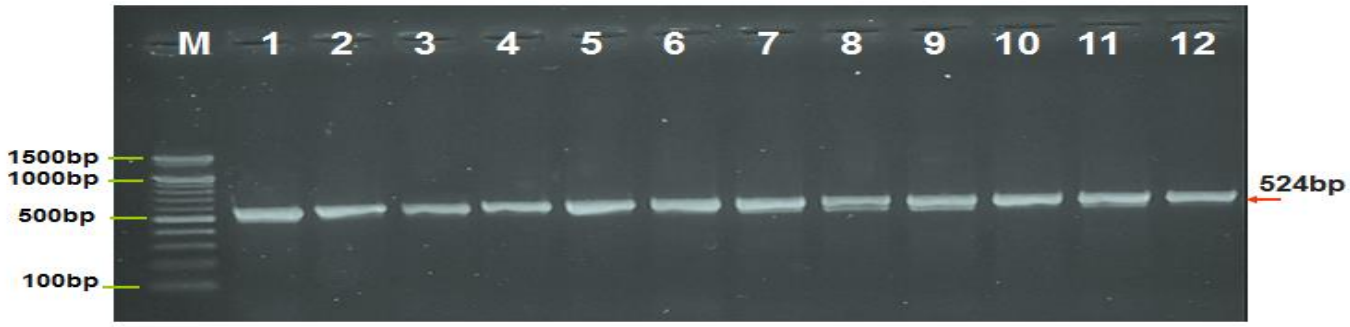

Figure (1): Agarose gel electrophoresis image that show the PCR product analysis of 16SrRNA gene in Escherichia coli isolates. Where Marker ladder (1500-100bp), lane (1-12) positive Escherichia coli isolate 16SrRNA gene at 524bp

Detection of virulance genes by PCR:

These genes encode the virulence associated factors of *E. coli, such* as *fimH* gene (560 pb), *cnf1*gene (350pb) usp (500pb) and ibeA(655pb). Each gene is appearing a single band in the identical DNA ladder region. All *E. coli* isolates were undergoing to standard PCR to detect these virulence-related genes. Not all isolates were positive., were as (*fimH)* (%76.36), (*cnf1)* (36.36%), (*usp*) (58.18%), (*ibeA*) (32.72%) .as in figure (2,3,4,5). All of them showed significant differences (p, 0.000, 0.224, 0.043, 0.010) Respectively. *Fim H* , include the mannose-specific adhesin, which plays a role in assisting pathogenesis through specific interactions with α-D-mannosylated proteins current on the cell surfaces of eukaryotic and decay by free mannose, *FimH* bind with its receptors mediates adhesion and ability to interactions between *E.coli* and epithelial cells is a multifactorial and complicated phenomenon which include several adhesins produced according to the phase of infection [28,29] presence of these genes was high and this is consistent with a study [6] The *ibeA* gene, correlating with invasion of the epithelium, is found a limited number of *E. coli* strains in this study that carry this gene and is especially important for pregnant women with *E. coli*. where

genes, *ibeA* and cnf, raised the possibility of neonatal meningitis or other neurological disorders. [30] The percentage of occurrence that comes close to studying [31] *CNF1*, Cytotoxic necrotizing factor 1, a single-chain toxin molecule, N-terminal region of the *CNF1* contains the cell-binding domain of the toxin and the C-terminal region is responsible for its catalytic activity, a chromosomally encoded is a recently described type III toxin belonging to the "dermonecrotizing" toxins group, *CNF1* stimulates the reorganization of F-actin structures through the p21 ras-like GTPase Rho pathway and binds to its cell surface receptor with high affinity, and requires an acidic compartment to translocate its enzymatic domain into the cytosol and leads to the disruption of human brain microvascular endothelial cells (HBMEC) and penetration of the central nervous system (CNS) by contact with its receptor [32,33] Usp,uropathogenic specific protein, a urovirulence factor that was more considerably found strains of UPEC associated with different symptoms in humans, which includes a C-terminal that shows similarities to DNase-like colicins (released into the environment to reduce competition from other bacterial strains) and pyocins (ability to oxidise and reduce other molecules), In its role and working mechanisms, DNAdamage caused by genetic toxins activate the response mechanism to DNA damage and interrupt the subsequent cell cycle, resulting in irreversible DNA damage Programmed cell death or aging. exposure of the DNA to the factors that because damage may lead to genomic sensitivity and instability, which increases the risk to development of tumor, and enhances the incidence of cancer [34,35].

In fact, it is conceivable that the strains *E. coli* derived of vaginitis may gradually transform into an unprecedented pathogen during the process of co-evolution of humans. However, it may also be likely that the tested genes are not usable under various physico-chemical conditions in the vaginal environment. It can also be remembered that the bacteria analyzed isolated from women with vaginitis, indicating that some these genes could be vigorous.

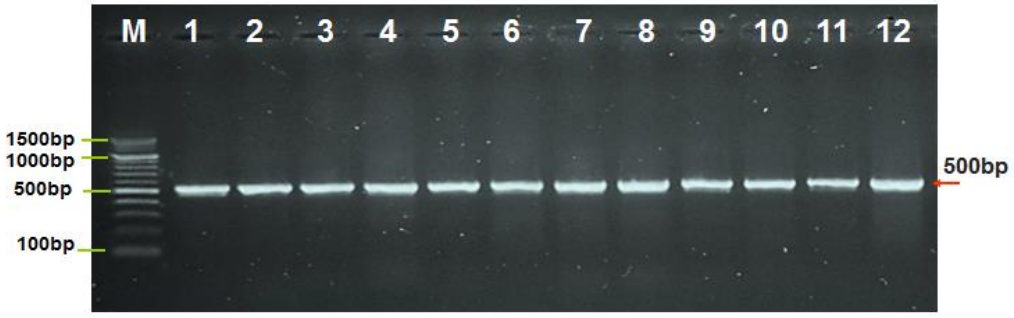

Figure (2): Agarose gel electrophoresis image that show the PCR product analysis of usp gene in Escherichia coli isolates. Where Marker ladder (1500-100bp), lane (1-12) positive Escherichia coli isolate usp gene at 500bp.

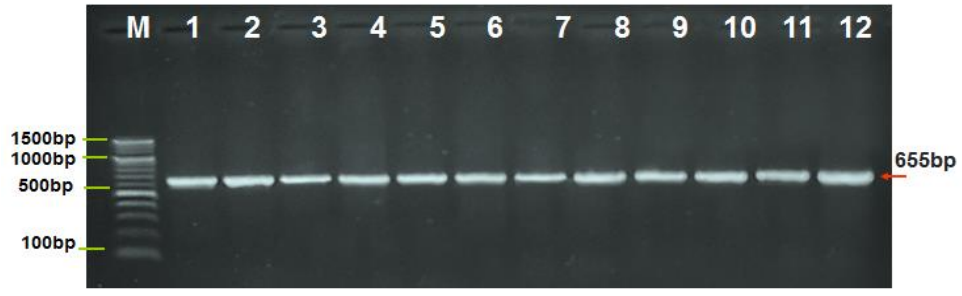

Figure (3): Agarose gel electrophoresis image in *Escherichia coli* isolates that shows the PCR product analysis of the ibeA gene. Where Marker ladder (1500-100bp), lane (1-12) isolate ibeA gene at 655bp.

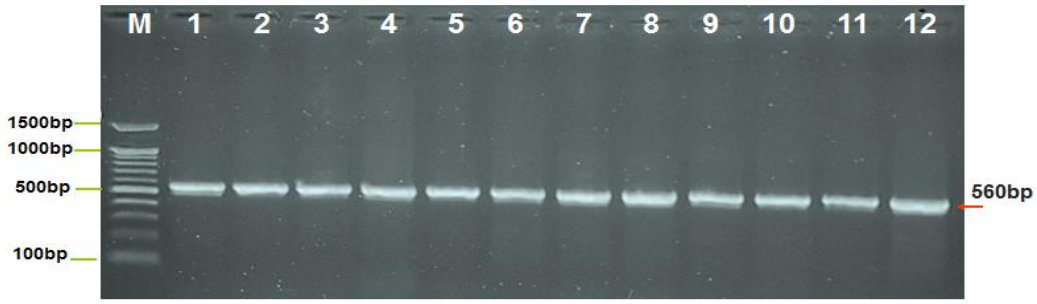

Figure (4): Agarose gel electrophoresis image that show the PCR product analysis of fimH gene in Escherichia coli isolates. Where Marker ladder (1500-100bp), lane (1-12) positive Escherichia coli isolate fimH gene at 560bp.

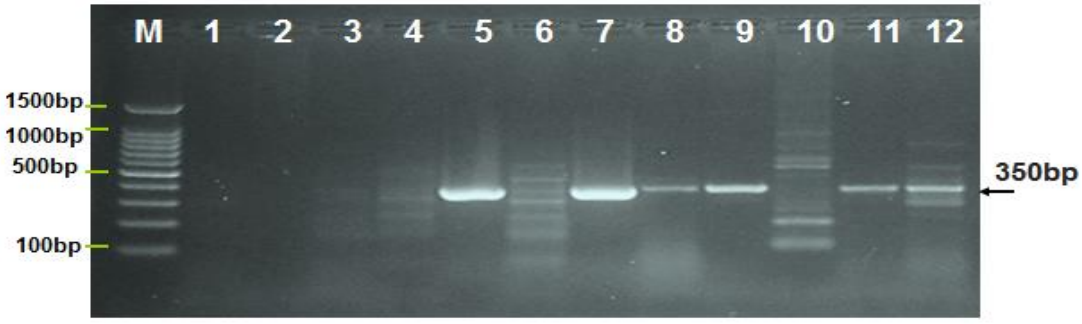

Figure (5): Agarose gel electrophoresis image that show the PCR product analysis of cnf1 gene in Escherichia coli isolates. Where Marker ladder (1500-100bp), lane (1-12) some positive Escherichia coli isolate cnf1 gene at 350bp

Sequencing analysis of *16Sr RNA gene* to *E. coli:*

According to the results the 16S rRNA gene of two isolate from vaginitis (MN741162), (MN741163) Figure (6,7), table(2) showed light heterogeneity , Phylogenetic tree showed a genetic affinity of all local *E.coli* isolates where relatively more similar sequences and genetically closed related to 10 isolates as in figure (8) especially (AF527827)Where Isolation source was bovine, in studying on the capacity of the strains *E. coli* to transmit resistance to rifampicin between the farm cattle in Canada [36] , we noticed matching was 99.59% for isolation NO.1 and 99.79% for isolates NO.2 and the total genetic change was (0.002-0.0005%). In addition to the strain (LC487866) Isolation source India from contaminated soil [37], and (MK880639) Isolation source India from drinking water that study the ability of the *Escherichia coli* strain to cause Diarrhea and Dysentery.[38]

These results indicate that the most common cause of vaginitis infection is the use of contaminated water for personal hygiene through direct or indirect contact with water with infected animals, or mixing wastewater with clean water and it is known that most of the non-harmful *Escherichia coli* strains live in the intestine of healthy humans , And some strains produce strong virulence factors that help them cause severe diseases, and with limited urethra in women[5], which helps bacteria reach to the vagina and cause infection

Table (2) the NCBI-BLAST Homology Sequence identity (%) between local *Escherichia coli* IQN isolates and NCBI-BLAST isolates

Local isolate No.1	Genbank Accession number	NCBI-BLAST Homology Sequence identity (%)		
		Identical NCBI BLAST gene related isolate	Genbank Accession number	Identity (%)
Escherichia coli IQN. No.1	MN741162	*Escherichia coli* strain RREC I	AF527827.1	99.59%
Escherichia coli IQN No.2	MN741163	*Escherichia coli* strain RREC I	AF527827.1	99.79%

Conclusions:

Studying *E. coli* strains need to understand the potential risk factors for the vertical transmission of pregnant women's neonatal infection and the cause of pregnancy complications, In addition the diseases that affect the nervous system of the fetus ,To design interventions that adequately address such risk factors, And also its effect on the occurrence of infection in non-pregnant women ,and know the rapid development of antibiotic resistance due to bad and random use without knowing the danger

Score	Expect	Identities	Gaps	Strand
885 bits(479)	0.0	483/485(99%)	0/485(0%)	Plus/Plus

```
Query  1    ACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGT  60
Sbjct  1    ............................................................  60
Query  61   CGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAAATTGACGGGGGCCCGC  120
Sbjct  61   ............................................................  120
Query  121  ACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGA  180
Sbjct  121  ............................................................  180
Query  181  CATCCACAGAACTTTCCAGAGATGGATTGGTGCTTCGGGAACTGTGAGACAGGTGCTGCA  240
Sbjct  181  ...................................................A........  240
Query  241  TGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCT  300
Sbjct  241  ............................................................  300
Query  301  TATCCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGG  360
Sbjct  301  ............................................................  360
Query  361  AGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCT  420
Sbjct  361  ............................................................  420
Query  421  ACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGT  480
Sbjct  421  .................................T..........................  480
Query  481  CGTAG  485
Sbjct  481  .....  485
```

Figure (6): Multiple sequence alignment analysis of the 16S rRNA gene in local *Escherichia coli* IQN_No.1 isolate and NCBI-Genbank isolate. The multiple alignment analysis was constructed using (NCBI BLAST Database). That display the nucleotide alignment likeness as (*) with substitution mutations in 16S rRNA

Score	Expect	Identities	Gaps	Strand
885 bits(479)	0.0	481/482(99%)	0/482(0%)	Plus/Plus

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Query  4    ATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACGCGTTAAGTCGA  63
Sbjct  1    ............................................................  60
Query  64   CCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAAATTGACGGGGGCCCGCACA  123
Sbjct  61   ............................................................  120
Query  124  AGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGTCTTGACAT  183
Sbjct  121  ............................................................  180
Query  184  CCACAGAACTTTCCAGAGATGGATTGGTGCTTCGGGAACTGTGAGACAGGTGCTGCATGG  243
Sbjct  181  ............................................................  240
Query  244  CTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTAT  303
Sbjct  241  .....................................A......................  300
Query  304  CCTTTGTTGCCAGCGGTCCGGCCGGGAACTCAAAGGAGACTGCCAGTGATAAACTGGAGG  363
Sbjct  301  ............................................................  360
Query  364  AAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGACCAGGGCTACACACGTGCTACA  423
Sbjct  361  ............................................................  420
Query  424  ATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGTGCGTCGT  483
Sbjct  421  ............................................................  480
Query  484  AG  485
Sbjct  481  ..  482
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Figure (7): Multiple sequence alignment analysis of the 16S rRNA gene in local *Escherichia coli* IQN_No.2 isolate and NCBI- Genbank isolate. The multiple alignment analysis was constructed using (NCBI BLAST Database). That display the nucleotide alignment likeness as (*) with substitution mutations in 16S rRNA.

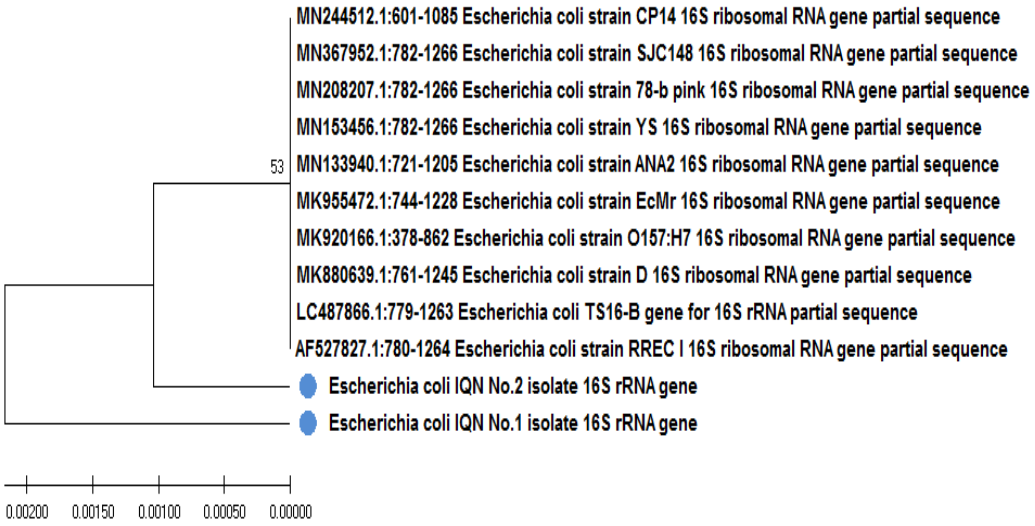

Figure (8): Phylogenetic tree analysis based on 16SrRNA gene partial sequence in local *Escherichia coli* IQN_No.1-2 isolates that used for genetic relationship analysis. The phylogenetic tree was constructed using Maximum Likelihood method (MEGA 6.0 version). The local *Escherichia coli* IQN No.1-No.2 isolates were clear genetic different related to the NCBI-BLAST Escherichia *coli* at total genetic changes (0.002-0.0005%).

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