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Determination of *Escherichia coli* Phylogenetic Group Isolated from Women with Vaginitis in Hilla City, Iraq.

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ABSTRACT

Escherichia coli is one of the common organisms in the microbial flora of pregnant as well as non-pregnant women. Vaginal colonization with *E. coli* is associated with various genitourinary, obstetric and neonatal complications, such as the severe form of pelvic inflammatory disease, urinary tract infections, very-low-birth-weight, Infants, and early-onset neonatal septicemia and meningitis. A total of 32 *E. coli* isolates recovered from vaginal swab of women with vaginitis. DNA Extraction of *E. coli* isolates were performed using Colum kit. Agarose gel electrophoresis performed to check the DNA ready for PCR. Three primer sets were used for Phylogeny: *chuA*, *yjaA* and *TspE4C2*. The results showed that all isolates where have all genes and belong to extra intestinal group B2 and subgroupB23**.** No one of the isolated *E.coli* belong to intestinal group and this reveal infection with highly virulent *E.coli* not with normal flora that may come from intestine.

Keywords: Vaginitis, *E. coli*, Phylogeny.

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INTRODUCTION

Vaginitis is an inflammation of the vaginal lining with or without an associated discharge. It can be treated if the source of irritation is diagnosed. Infection vaginitis is common disorder in women particularly important in pregnant women which include the three vaginal infection trichomoniasis, bacterial vaginosis, and candidiasis [1].

Escherichia coli is one of the common organisms in the microbial flora of pregnant as well as non pregnant women. *E. coli* have been reportedly identified in 9±28% of non-pregnant women. Vaginal colonization with E. coli is associated with various genitourinary, obstetric and neonatal complications, such as the severe form of pelvic inflammatory disease, urinary tract infections, very-low-birth-weight, Infants, and early-onset neonatal septicemia and meningitis. Vaginal E. coli has also been reported to be sexually transmissible to a male partner [2].

Escherichia coli is a commensal organism of humans and other warm-blooded animals. It can also cause various diseases, both intestinal and extra intestinal, in these hosts. Variations in genetic backgrounds and the presence or absence of specialized virulence factors in the bacteria may contribute, in part, to the commensalism-versus-virulence duality of *E. coli*. *E. coli* populations have a clonal structure [3], and indeed, various intestinal or extra intestinal *E. coli* infections have been linked to specific clones or groups of related clones [4]. Phylogenetic studies have shown that *E. coli* can be divided into four main phylogenetic groups, designated A, B1, B2, and D [5]. Most *E. coli* strains responsible for urinary tract infections (UTI) and other extra intestinal infections belong to group B2 or, to a lesser extent, to group D [6]. In addition, pathogenic E. coli strains are often marked by the presence of special virulence determinants. For example, 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 [7]. Overall, strains of phylogenetic group B2 and D often carry virulence determinants that are lacking in group A and B1 strains [8].

MATERIALS AND METHODS

Isolates

A total of 32 *E. coli* isolates recovered from vaginal swab of women with vaginitis. Identification firstly done by cultivation on MaCconky agar and Eosin Methylene Blue (EMB) agar. The pink colony and green metallic shin colonies then cultured on Luria Bertani (LB) broth overnight at 37°C for DNA extraction.

DNA Extraction and PCR

Table 1: Primer sets sequence and amplicon size

Gene	Primer sequence (5'-3')	Amplicon size(bp)
chuA	F:GACGAACCAACGGTCAGGAT	279
chuA	R:TGCCGCCAGTACCAAAGACA	
yjaA	F:TGAAGTGTCAGGAGACGCTG	211
yjaA	R:ATGGAGAATGCGTTCCTCAAC	
TspE4C2	F:GAGTAATGTCGGGGCATTCA	152
TspE4C2	R:CGCGCCAACAAAGTATTACG	

Table 2: PCR conditions

Steps	Temperature	Time	No. of cycles
Initial denaturation	95 C°	4 min	1
Denaturation	94 C°	30 sec	30
Annealing	59 C°	30 sec	
Extension	72 C°	30 sec	
Final extension	72 C°	5 min	1

DNA Extraction of *E. coli* isolates were performed using Colum kit (Favorgen/Taiwan). Agarose gel electrophoresis performed to check the DNA ready for PCR. Three primer sets were used for Phylogeny: *chuA,*

yjaA and *TspE4C2* [9]. According to the instruction provided by primer manufacturer (Bioneer / Korea) the TE buffer were added to get 100 picomole/microliter concentration of primer stock solution. The working solution prepared from stock by dilution with TE buffer to get 10 picomole/microliter. The primer sequence and conditions were shown in tables (1) and (2) respectively.

The results were interpreted according to Abdul-Razzaq *et. al.* 2013 [10] as mentioned in the table (3) below.

Table 3: Phylogeny interpretation table

Phylogenic groups	Phylogenic subgroup	ChuA	yjaA	TspE4cC2
Group A	Subgroup A_0	-	-	-
	Subgroup A_1	-	+	-
Group B1	None	-	-	+
Group B2	Subgroup $B2_2$	+	+	-
	Subgroup $B2_3$	+	+	+
Group D	Subgroup D1	+	-	-
	Subgroup D2	+	-	+

RESULTS

The results showed that all isolates where have all genes and belong to extra intestinal group B2 and subgroup$B2_3$ as showed in figure (1, 2, 3).

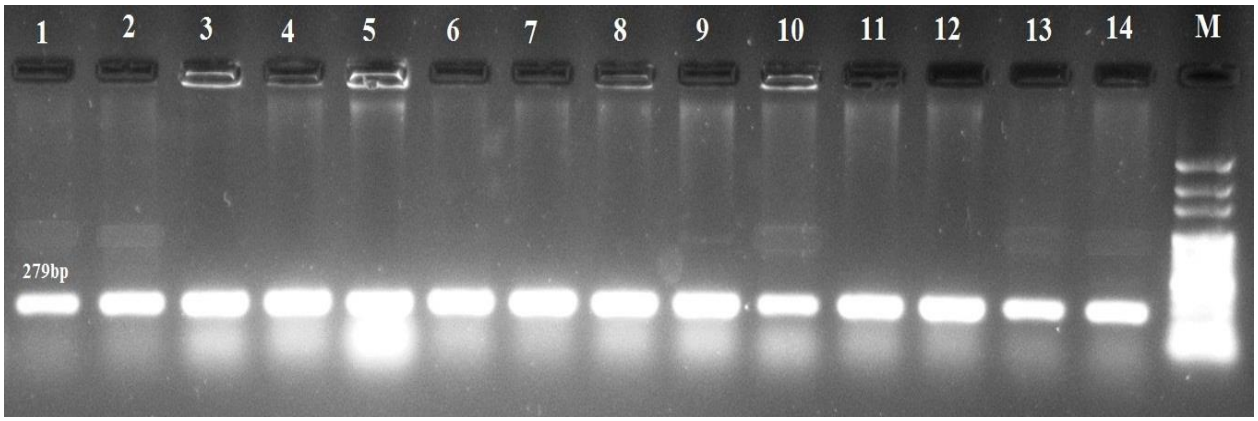

Figure 1: 2% Agarose gel electrophoresis. *chuA* amplicon size 279bp, lane 1-14 represent the samples and lane M represent Ladder.

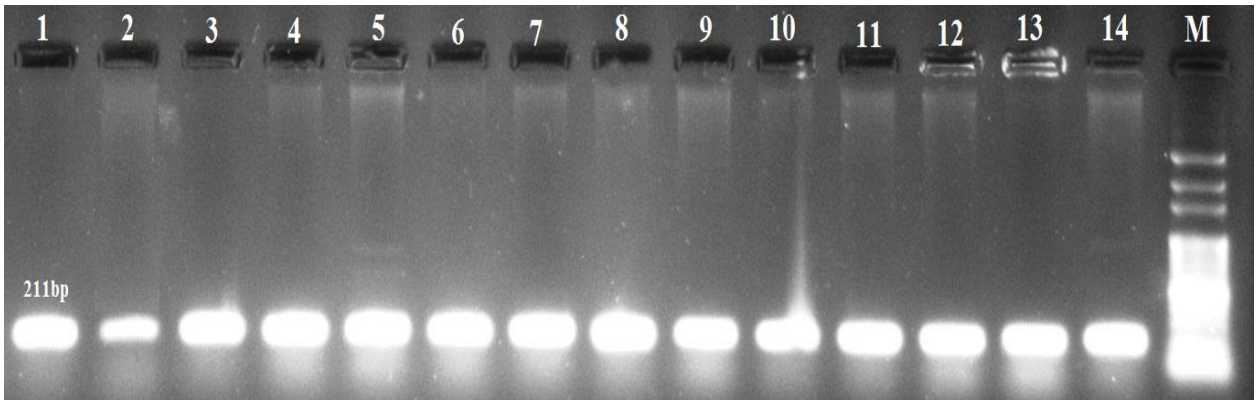

Figure 2: 2% Agarose gel electrophoresis. *yjaA* amplicon size 211bp, lane 1-14 represent the samples and lane M represent Ladder.

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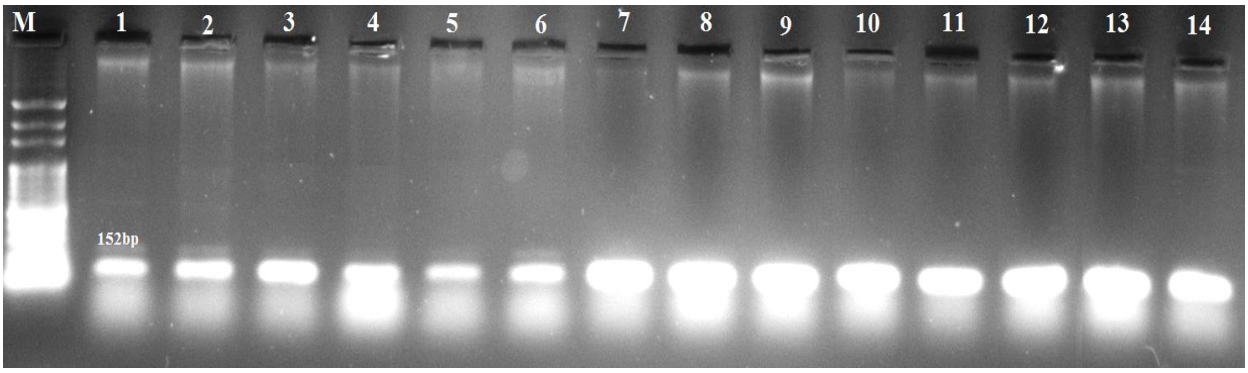

Figure 3: 2% Agarose gel electrophoresis. *TspE4C2* amplicon size 152bp, lane 1-14 represent the samples and lane M represent Ladder.

DISCUSSION

Bacterial vaginosis (BV) is a common reproductive tract infection amongst women of reproductive age and has been implicated as a risk factor for adverse pregnancy outcomes such as preterm birth, recurrent abortions, post-abortal sepsis, early miscarriages and still births [11,12] .Infections leading to preterm birth and other complications of pregnancy may extend beyond delivery and create serious and sometimes life-threatening consequences for the neonate. However, many infections are subclinical and so our knowledge of the role played by BV in PTB remains limited and inconclusive [13].

E. coli is important causative agent of symptomatic and asymptomatic BV The highly virulent E. coli mostly belong to the extraintestinal group B2 or D. Among extraintestinal phylogenetic groups , group B2 is dominant and many of E. coli isolates that cause neonatal meningitis and septicemia belong to this group [14]).

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