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STUDY OF PHYLOGENY GROUPS OF *ESCHERICHIA COLI* BACTERIA ISOLATED FROM WOMEN VAGINA

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Abstract

Detection of phylogeny group of *Escherichia coli* isolated from vagina of women suffered from vaginitis infection. In this study, collection about 130 vaginal swabs were obtained from women suffering from vaginitis infection with age (18-40 years) .Samples swab were cultured on culture media such as MacConkey and EMB agar for diagnosis, also used molecular method for identification phylogeny groups. Molecular identification based on PCR products were detected by electrophoresis by using 1% agarose for 45mints with 70 volt and by using 100bp marker ladder for determine size of amplification PCR products. In this study from 130 swabs isolated only 20(14.8%) isolates diagnosis *E.coli* bacteria after culture on diagnosis culture media while by depending on molecular detection appear 14 (70%) isolates belonging to groups B_2 *Escherichia coli*, 5(25%) isolates belong to group A and 1(5%) belong to group B_1 bacterial type while no appear any isolates belong to group D bacterial type.

Key words : Vaginosis *E. coli* infection, phylogeny, women vagina.

Introduction

Escherichia coli bacteria present in several sites of the body such as vagina and colon and other, *E. coli* that present in vagina has been poorly characterized when compartment with other sites colonization it. These bacteria have ability to causes several infections either symptomatic or asymptomatic, also studies appear present relationships between *Escherichia coli* normal flora in genital tract and those causing intra-amniotic infection (Sáez-López *et al*., 2016).

There are four phylogeny *E. coli* groups (A, B_1, B_2, D) (Carlos *et al*., 2010; Clermont *et al*., 2013). The *chuA* is gene responsible for iron transport in enterohemorrhagic O157 : H7 while *yjaA* gene play essential role in cellular response to hydrogen peroxide and acid stress and the function of *TspE4.C2* is not yet understand (Lee, 2011).

However, *Escherichia coli* strains categorized in four phylogeny groups, the strain that causes extra-intestinal infection are acute strain that belong to group B_2 and D, while symbiotic group are belong to A_1 and B_1 (Clermont and Bonacorsi, 2011; Baponi *et al*., 2016).

Nagarjunal *et al*. (2015) show the commensal *E. coli* strains belong to groups A and B_1, whereas the strain causes extra-intestinal infection belong to group B_2 and D. The intestinal *Escherichia coli* are mixture of all phylogenic groups and may consider as a reservoir for the pathogenic isolates.

Materials and Methods

Patients

130 vaginal swabs collected from women suffering from vaginal and urinary tract infections with age (18-40 years) were submitted to hospital of maternity and children in Hilla city through period from (October 2016 to April 2017).

Laboratory diagnosis and identification of bacterial isolate

Vaginal swabs collection immediately inoculation into transport media tube for preserved from dryness until transport to laboratory, then the swabs were culture on growth media such as MacConkey and EMB agar and incubation at 37^0C for 24 hours. By depended on (MacFaddin, 2000) diagnosis procedure recommended take single colony from each positive culture and noting the morphology properties (color production, colony shape texture and edge). Also using Gram stain procedure for

detection bacterial belong to Gram positive or Gram negative (Winn *et al*., 2006).

Molecular identification

DNA was extracted by using gene aid kit specific for DNA extraction from Gram negative bacteria as *E. coli* belong to gram negative bacterial type and accordance with gene aid protocol.

Detection of phylogeny groups by PCR

The major phylogenic groups of *E. coli* (A, B_1, B_2 and D) were identification by depended on specific genes *chuA*, *yjaA* and DNA fragment *TspE4.C2* (Clermount *et al*., 2000).

The primer used were *chuA*, *TspE4.C2* and *yjaA* which generated 279, 152 and 211 bp fragment, respectively. The information of the three PCR amplification result in assignment of the isolates to phylogenetic groups as follows : $chuA^+$, $yjaA^+$, group B_2; $chuA^+$, *yhaA*-group D; $chuA^-$, $TspE4.C2^+$, group B_1 and $chuA^-$, $yjaA^+$, $TspE4.C2^-$, group A. The polymerase chain reaction products size were determined by using molecular marker 100pb.

Results

Twenty (14.8%) vaginal *E. coli* (VEC) isolates were recovered from 135 female suffering from vaginitis and urinary tract infection. All vaginal *E. coli* isolates were subjected to phylogrouping by PCR.

In this study, it was noticed that *E. coli* isolated from women with vaginitis belongs mainly to the phylogeny group B_2 (14/20) followed by the phylogeny group A(5/20) and only one isolate (1/20) for group B_1.

Detection of *ChuA*

All *E. coli* 20 (14.8%) isolates are subjected for detechion of gene*chuA* by using specific primer at 279 bp. It was found that 14 isolates gave postive for this marker as shown in fig. 1.

Detection of *yjaA*

All *E. coli* isolates are subjected for detection of *yjaA* gene by using specific primer at 211 bp. It was found that 17 isolates gave postive for this Marker as shown in fig. 2.

Detection of *TspE4.C2*

All *E. coli* isolates are subjected for detechion of *TspE4.C2* gene by using specific primer at 152 bp. It was found that 14 isolates gave postive for this marker as shown in fig. 3.

Concisely, the results in this table showed that most *E.coli* isolate, belong to group B_2 14 isolate (70%) followed by group A (5 isolates) (25%) and only one for B_1 only one isolate (5%).

Table 1 : Contents of the reaction mixture.

No.	Contents of reaction mixture	Volume
1)	green master mix	12.5 µl
2)	forward primer	2.5 µl
3)	reverse primer	2.5 µl
4)	DNA template	5 µl
5)	Nuclease free water	2.5 µl
	Total volume	25 µl

Table 2 : Distribution of *E. coli* isolated according to phylogeny groups.

Phylogeny groups	*TspE4.C2*	*yjaA*	*chuA*	No. of isolates
A	-	-	-	1
B_2	+	+	+	2
B_2	+	+	+	3
B_2	+	+	+	4
B_2	+	+	+	5
B_2	+	+	+	6
B_1	+	+	-	7
B_2	+	+	+	8
B_2	+	+	+	9
B_2	+	+	+	10
A	-	-	-	11
B_2	+	+	+	12
B_2	+	+	+	13
B_2	+	+	+	14
B_2	-	+	+	15
A	-	+	-	16
A	-	-	-	17
B_2	+	+	+	18
A	-	+	-	19
B_2	+	+	+	20

Discussion

The presence of *E. coli* in healthy vagina and also in patients with vaginitis may have many explanation where the source of its existence in vagina may be as a contaminant from faces or it may be true pathogen of this disease, as a result of opportunistic capability of this bacteria to do various type of infections in women such as UTI and vaginitis (Ahmed, 2015).

The phylogentic groups of *E. coli* isolated from vagina was detected by identifying the presence of specific PCR amplified fragment (*chuA, yjaA* and *TspE4.C2*).

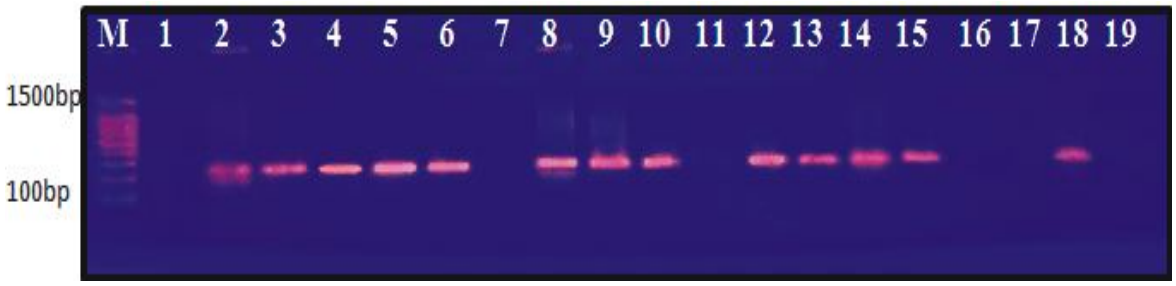

Fig. 1 : Gel electrophoresis of PCR product of *chuA* gene (1-19) : No. of isolates obtained from vaginal samples. M = Molecular marker (100bp).

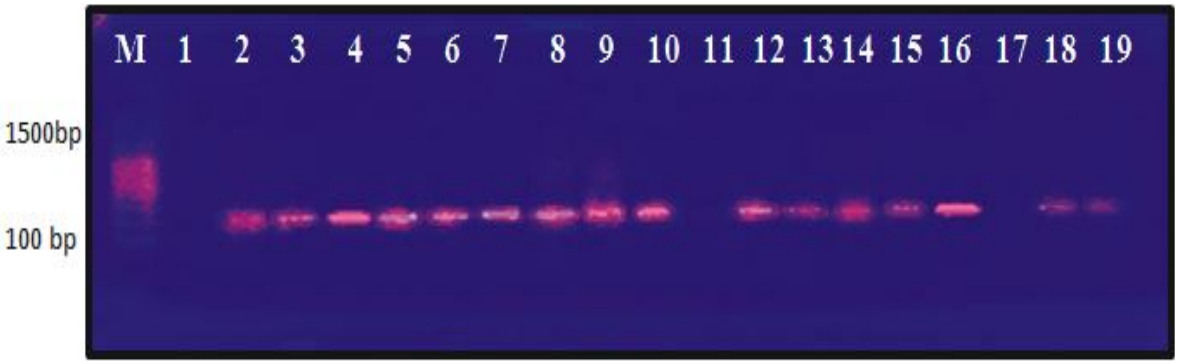

Fig. 2 : Gel electrophoresis of PCR product of *yjaA* gene(1-19) : no. of isolates obtained from vaginal samples . M= Molecular marker (100bp).

Fig. 3 : Gel electrophoresis of PCR product of *TspE4.C2* gene(1-19) : no. of isolates obtained from vaginal samples. M= Molecular marker (100bp).

All the fourteen isolates belonging to group B_2 are considered as extra-intestinal and pathogenic to man, and this will confirm their role in vaginitis. Whereas the isolates belong to group A and B_1 are considered as commensal, not true pathogen that they may be isolated as a contaminants from anal area. However, no isolates was classified within the group D.

The study carried out by Abdul-Lateef (2011), who found that most *E. coli* isolates belong to the group B_2 (30%), A (30%) & D (30%). At the same rate this is not identical to the results of this study which no isolates was found to be related to group D. According to the data obtained. *E. coli* isolates were distributed in four phylogeny groups depending on the results of the presence and absence of phylogeny genes (*chuA, yjaA, TspE4.C2*).

Group D is not found in this study, although many studies confirmed that most *E. coli* strains that are isolated from extraintestinal regions are enither belong to phylogeny group B_2 or D (Da Silva and Mendonca, 2012; Rasmussen *et al*., 2012; Johnson *et al*., 2012). But this study showed that most isolates of vaginal *E. coli* belong to the group B_2. However, Al Safar (2016) have found all vaginal *E. coli* belong to the group B_2 not for A, B_1 or D. However, other studies (Al-Khaqani *et al*., 2017; Guiral *et al*., 2011; Usein *et al*., 2011) have found that most vaginal *E. coli* belong mainly to the group B_2 that may indicate that source of these isolates is not from the faeces.

These results may highlight the importance of *E. coli* as a pathogen in the Vaginitis with high risks of transmission to the upper parts of the genital tract and, if the women are pregnant, it may cause serious infections to the fetus, or the newborn (Al-Khalide *et al*., 2015).

The results of this study appeared that the *chuA* gene was present in all isolates belonging to groups B_2 and D and was absent from all isolates belonging to groups A and B_1. The *yjaA* gene allowed perfect distinguish between group B_2 and group D and it was present in all isolates belonging to group A. Also, the *TspE4.C2* is present in group B_1 strains and absent from all group A isolates (Abdul-Razzaq and Abdul-Lateef, 2011).

Phylogeny groups was detect the source of infection where the groups A& B_1 are commensal, so the source of *E. coli* in cases of vaginitis may be the intestinal because *E. coli* is commensal mostly in human intestinal (Cribby *et al*., 2008).

Conclusion

Most bacterial isolates belong to phylogeny group B_2 and then to group A.

Recommendation : Study the phylogeny groups and its relation to gene conversion in bacteria.

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