Original paper

Virulence factors genes and phylogenic groups of *Escherichia coli* isolated from High Vagina and Endo-cervix of Women from Kerbala

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Abstract

Background: *Eschrechia coli* (*E.* coli) is the most common colonizing bacteria of the genital tract however, its status as a cause of genital tract infections is questionable.
Aims of the study: To study the virulence and phylogenic groups of *E coli* strains isolated from high vagina and endo-cervix.

Materials and Methods: A total of 100 female patients were enrolled in this study. All patients were attendants of the Gynecology and Obstetrics Teaching Hospital in Kerbala , holy kerbala. Iraq in the period from December 2013 to January 2015. *E. coli* isolates were identified by standard methods. Molecular diagnosis and characterization for virulence and phylogenic genes were detected by Multiplex PCR.

Results and Discussion: Among 65 isolates recovered in this study, the most frequent phylogenic group of *E. coli* was "B2" which comprised 61.5% (no= 40) followed by "Phylogenic group A" that comprised 33.8% (no= 22). Previous studies have shown that phylogenetic groups B2 and D are virulent because these groups are associated with the presence of several virulence factors. Interestingly, 9 out of 10 isolates from women who their husband using condoms were shown to be of B2 phylogenic group. Frequencies of virulence genes of adhesion *Pap*, *Sfa* and *Afa*, among *E.coli* isolates were 14, 42 and 5, respectively. In addition, *Pap* C and *Sfa* were found in higher rates in phylogenetic group B2 isolates, and this gives more support to the hypothesis that B2 is the pathogenic group.

Conclusions: Most of the *E. coli* isolates were belong to the virulent phylogenic group "B2" and that those isolates harbor several virulence factors. Accordingly, *E coli* may be considered as a true genital tract pathogen and its colonization entails great risk for vertical transmission to the fetus and fetal membrane..

Key Words: Virulence factor genes, Phylogenic groups.

Introduction

The genital tract infections are among the most frequent disorders for which female patients seek care of gynaecologists. These infections may be transmitted to neonates before or during delivery. The cervico-vaginal epithelium is extensively colonized by microorganisms; however, the upper genital tracts are usually considered to be sterilized. Presence of microorganisms in these places is linked with development of several diseases (endometritis or pelvic inflammatory disease) [(1)]. During pregnancy, the developing fetus is protected from invading pathogens by the placenta, fetal membranes, and cervical mucus. Introduction of microorganisms into the intrauterine cavity results in several obstetric conditions such as miscarriage [(2)], chorioamnionitis [(3)], premature rupture of membranes (PROM) [(4)] and preterm birth [(5)]. These conditions are hypothesized to results from the maternal and, occasionally, fetal inflammatory response to bacterial pathogens. In addition, colonization of the birth canal may lead to neonatal sepsis. Early onset neonatal

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sepsis is linked to gaining of microorganisms from the mother, through transplacental route, or a climbing infection from the cervix, or may be caused by organisms that colonize the genito urinary tract of the mother [6].

E. coli is a common colonizing bacteria of the genital tract [7, 8] , however, its status as a cause of genital tract infections is questionable. It is reported that aginal colonization by *E coli* as a risk factor for very low birth weight delivery and other perinatal complications [9]. Vertical transmission of vaginal infections during pregnancy may entail high risk on the fetus as well as the pregnant female. Furthermore, neonatal infections acquired during delivery through passage in the birth canal are of high clinical importance. Neonatal infections usually result from infection or colonization of the birth canal with potential pathogens. The cervico-vaginal epithelium is close to the fetus and, therefore, cervico-vaginal epithelium comprises the most important site from which microorganism can be transmitted to the fetus or newborn).

The aim of this study was to explore the virulence and phylogenic groups of *E coli* strains isolated from high vagina and endo-cervix. .

Materials And Methods

Study patients: A total of 100 females patients complaining of high vaginal discharges were enrolled in this study. The study conducted in the period from December 2013 to January 2015. All patients were attendants of the Gynecology and Obstetrics Teaching Hospital in Kerbala , holy kerbala. Iraq.

Specimen collection: Patients with abnormal vaginal discharges and showing signs of cervicitis were identified by consultant Gynecologists and referred to High Vaginal and endo-cervical swabs. The swabs were taken by specialist nurses after obtaining verbal consent from each participant. The swabs were transferred to the laboratory in the same day and cultured according to standard microbiological protocols for aerobic bacteria. In addition, a detailed questionnaire was filled for each participant at the time of taking the swabs.

Identification of *E.coli* isolates: *E. coli* isolates were identified to species level based on morphological properties, cultural characterization and standard biochemical tests. The identification was confirmed using API 20E strips (Biomereux, USA).

E. coli were isolated from 60 patients. In addition, 5 patients were found to harbor 2 different type (strains) of *E. coli* (that are different on API20E system and phylegenic group by PCR; see later). Therefore, the total *E. coli* isolates in this study was 65 isolated from 60 patients.

DNA extraction of *E.coli* isolates: Bacterial DNA was prepared by boiling methods as previously described [11] with few modifications. Bacteria were harvested from 1 ml of an overnight broth culture, suspended in 200 µl of sterile water, and incubated at 100°C for 10 min to release DNA from whole organisms. DNA extracts were resuspended in Tris-EDTA (10 mM Tris-HCl, 0.10 mM EDTA [pH 8.0]) buffer and stored at -20 ^{o}C until used.

Primers sequences for PCR: Two sets of primers were used in this study (listed in table 1). First set "set A" contains primers used to amplify 3 sequences specific to adhesins-encoding operons *pap, afa*, and *sfa*. Those primers were adapted from study of Soto SM, et al. (2008) [12]. Second set "set B" was used to determine the phylogenetic grouping of the *E.coli* isolates by targeting two genes, *Chu*A, *Yja*A and anonymous DNA fragment *TspE4.C2* as previously described [13]. Each set was used in multiplex PCR (M-PCR) in separate tube. The PCR running conditions and detection was conducted as previously described [12, 13].

Table1. Primers sequences of Virulence genes and Phylogenetic groups.

	traget		Primer sequences(5́-3́)	Product size (bp)	References
Set A	*PapC*	F	GACGGCTGTACTGCAGGGTGTGGCG	328	Soto *et al.*,2011 (12)
		R	ATATCCTTTCTGCAGGGATGCAATA		
	Sfa	F	CTCCGGAGAACTGGGTGCATCTTAC	410	
		R	CGGAGGAGTAATTACAAACCTGGCA		
	Afa	F	CGGCTTTTCTGCTGAACTGGCAGGC	672	
		R	CCGTCAGCCCCCACGGCAGACC		
Set B	*ChuA*	F	GACGAACCAACGGTCAGGAT	279	Clermont *et al.*, 2000 (13)
		R	TGCCGCCAGTACCAAAGACA		
	Yja	F	TGAAGTGTCAGGAGACGCTG	211	
		R	ATGGAGAATGCGTTCCTCAAC		
	TspE4.C2	F	GAGTAATGTCGGGGCATTCA	152	Bonacorsi *et al.*, 2000 (14) [15]
		R	CGCGCCAACAAAGTATTACG		

The phylogenetic grouping of *E.coli* isolates was made on the basis of the presence of specific PCR-amplified fragments as previously described (13) and as follows:

- (*chu* A - , *yja* A +/-, *TspE4C2* -) → group A
- (*chu* A -, *yja* A+/-, *TspE4C2* +) → group B1
- (*chu* A+, *yja* A +, *TspE4C2* +/–) → group B2
- (*chu* A+, *yja* A -, *TspE4C2* -/+) → group D

Statistical analysis: Data were entered in SPSS (Statistical Package) version-18. A chi-square test was used to identify the correlation between the variables of this study.

Ethical approval and consent: The necessary ethical approval from Karbala hospital that include in present study was obtained. Moreover, all subjects involved in this work were informed and the agreement was obtained from each one before the collection of samples.

Results

Identification of *E. coli* isolates: Out of the 100 female patients included in this study, *E. coli* was isolated from 60 patients. In addition, 5 patients were found to harbor 2 different type (strains) of *E. coli* (that are different on API20E system and genotyping by PCR). Therefore, the total *E. coli* isolates in this study was 65 isolated from 60 patients.

Molecular Detection of phylogenic groups of *E.coli*: The phylogenic group genes of *E.coli* isolates such as *chuA*, *YjaA* and the DNA fragement *TspE4.C2* were detected by Agarose-electrophoreses technique.

Among of 65 *E .coli* isolates in this study, the frequencies of the phylogenic groups A, B1, B2 and D were 22 (33.84%), 2 (3.07%), 40 (61.53%) and 1 (1.53%) respectively (Table 2).

Molecular detection of *E.coli* virulence genes: The results of this study showed that the frequencies of *Pap*, *Sfa* and *Afa*, among *E.coli* isolates were 14, 42 and 5, respectively. A total of 48 isolates had at least one virulence gene and 17 isolates had none of the studied genes. Furthermore, 34 isolates carrying just one virulence factor, and 14 isolates carrying 2 genes, however, none of the isolate out has found to carry all the three genes.

Relationship between *E.coli* phylogenetic and virulence factors: When the relationship of phylogenicity and virulence was studied, the prevalence of virulence genes was showed in Table(3). *Pap*C was found higher in phylogenetic group B2 isolates. *Sfa* gene was prevalent in phylogenetic group B2. *Afa* gene was absent in groups B2 isolates.

Discussion

A phylogenetic analysis using PCR was performed to determine which phylogenic group is most prevalent among *E. coli* isolates and to find if there is a specific phylogenic group is associated with any of the studied parameters. The most frequent phylogenic group of *E. coli* was "Phylogenic group B2"

which comprised 61.5% followed by "Phylogenic group A" that comprised 33.8%. Thus, this study shows that most of the *E. coli* isolates from the cervico-vaginal epithelium belong to the virulent group "B2".

Table 2. Phylogenetic distribution of *E. coli* isolates

Phylogenetic Groups	*Chu*A gene	*Yja*A gene	TspE4.C2 Fragment	Number of profiles within Phylogenetic group	No. of *E.coli* isolate N=65
Group A	-	+	-	9	22(33.84%)
	-	-	-	13	
Group B1	-	-	+	0	2(3.07%)
	-	+	+	2	
Group B2	+	+	+	38	40(61.53%)
	+	+	-	2	
Group D	+	-	-	1	1 (1.53%)
	+	-	+	0	

Table 3. Prevalence of virulence related genes in various phylogenetic groups of *E. coli* isolates.

Phylogenic groups	**Distribution of virulence genes**		
	PapC	***Sfa***	***Afa***
Group A	**1**	**8**	**2**
Group B2	**12**	**30**	**3**
Chi-square **Fisher s Exact Test**	**0.035** **0.032**	**0.016** **0.018**	**Nc***

***Not calculated**

Previous studies have shown that phylogenetic groups B2 and D are virulent because these groups are associated with the presence of several virulence factors [12]. Regarding this study, the majority of patients were infected by *E. coli* isolates belong to B2 group. These results may highlight the importance of *E. coli* as a pathogen in the cervico-epithelium 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.

Data on the features and virulence factors of *E. coli* colonizing the cervico-vaginal epithelium are very limited. The study of the virulence factors of *E.coli* strains could be necessary to understand the potential risk for vertical transmission to neonates during pregnancy and to design interventions to address such risks adequately. In this study, adhesins genes of *E coli* such as *papC*, *afa*, and *sfa* were analyzed by multiplex PCR method. The results showed that most of the isolates belong to the virulent group B2 harbor several virulence factors. In addition, *papC* and *sfa* adhesion genes were prevalent among the *E coli* strains.

Previous study has shown that neonatal sepsis caused by *E. coli* is related to a limited number of phylogenic groups B2 and D, both considered virulent, and the pathogenicity of these groups is associated with the presence of several virulence factors [12]. Furthermore, epidemiological studies have clearly reported a good correlation between the occurrence of certain human diseases and the presence of specific virulence factors in *E. coli* [15].

In conclusion, this study shows that most of *E. coli* strains colonizing the cervico-vaginal epithelium belong to the virulent group "B2" and that those isolates harbor several virulence factors. Accordingly, *E coli* may be considered as a true genital tract pathogen and its colonization entails great risk for vertical transmission to the fetus and fetal membrane. According to this, it could be recommended to investigate the actual causes for the high prevalence of *E.coli* and to document their sources, whether it is external or endogenous, in addition to highlighting the actual role of urinary system in *E.coli* transmission the cervico-vaginal epithelium.

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