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Determination of Phylogenetic Groups and Antimicrobial Susce-Ptibility Patterns for *Escherichia coli* Isolated From Patients With Urinary Tract Infection

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Abstract

Escherichia coli (E. coli) are one of the most widespread microbes in the world, capable to cause intestinal or extraintestinal disease and lead to a wide range of diseases. This study was conducted to isolate *E. coli* from urine of patients with urinary tract infections at Al-HusseinTeaching Hospital, during the period from November 2017 to March 2018, to detect Phylogenetic groups for isolates and susceptibility to antibiotic. A total of 152 samples were collected from urine gave 46 (30%) *E. coli.* Phylogenetic analysis based on the presence or absence of *chuA, yjaA* and *TspE4.C2* marker genes, was carried out to 46 isolates of UPEC, showed that Phylogenetic group B2 was most predominant, which included 34(74%) isolates, group A 6(13%) isolates and group D 6(13%) isolates. Antibiotic resistance exhibited group B2 was resistance 100% to amoxicillin/clavulanic acidacid, 94% trimethoprim, 82% tetracycline and ceftriaxon 76%. Group A and D 100% resistance for each of the amoxicillin/clavulanic acid, trimethoprim and tetracycline. Group A resistance 33% to ceftriaxone and group D was 100%. In This study the phylogentic group B2 the majority predominated in UPEC and more resistance to antimicrobial.

Key words: Uropathogenic *E. coli* , Phylogenetic typing groups, Antibiotic resistance

Introduction

Escherichia coli (*E. coli*) is one of the most widespread microbes in the world, a group of genetically heterogeneous bacteria, usually colonizes the digestive system of human infants through a few hours after birth and remain mutual benefit for decades, *E. coli* wherever found it indicates a fecal contamination of water or food (Brooks *et al.*, 2013) and can be divided into pathogenic and non pathogenic.

Non pathogenic strain also called Commensal *E. coli* are benign because they live together with the human host with mutual benefits ; do not cause disease but it may cause disease if the host is compromised immunologically or if the gastrointestinal barriers are damaging it (Russo and Johnson, 2003). Commensal *E. coli* strains efficiently exchange genetic material with pathogenic *E. coli* strain or other pathogens such as *Salmonella*, *Shigella*, *Yersinia* and *Vibrio*(Betteridge *et al.*, 2011; Schroedern *et al.*, 2017) and have the ability to transfr, resistance genes between different *E. coli* strain and other species of bacteria within the gastrointestinal tract (Bartoloni *et al.*, 2006; Vittecoq *et al.*, 2017)**.** The selective pressures in the habitant of commensal strains may be enhanced by coincidence the emergence of virulence factors and antibiotic resistance, rendering commensal *E. coli* strains reservoirs of virulent and resistant strains (Tenaillon *et al.*, 2010).

Pathogenic strains divided according to the site of intestinal infection into: intestinal pathogenic *E. coli* which, causes diarrhea that divided in six strain based on type of diarrhea and virulence factor; and extraintestinal pathogenic *E. coli* which causes several types infections; named based on site of infection such as: uropathogen *E. coli*(UPEC) that causes urinary tract infection Kwak *et al.* (2016). The primary, route of UTI is ascending way that results from contamination, by fecal that contain commensal, and pathogenic bacteria (Giray *et al.*, 2012; Jafri *et al.*, 2014).

(Clermont *et al.*, 2000) have divided *E. coli* strain to the four separated phylogenetic groups according to their differences in genetic composition. It was reported that the group(B2 and D) *E. coli* have (100% positive) *chuA* gene, a gene required for heme transmit in O157:H7 *E. coli* and was absent from group A and B1 strains (King-sun, 2007). In addition to the *yjaA*, a gene at first identified in the recent complete genome sequence of *E. coli* K-12, the gene was present in group B2 (100% positive) but not present in group D isolates. An unspecified DNA fragment, *TspE4.C2*, was also examined to be existe in group B1 but not existe in group A. ExPEC belongs at most to group B2 and to a lower extent to group D while intestinal commensal strain tend to belong to groups A and B1 (Clermont *et al.*, 2013). UPEC most common to cause infection to human, in the present study isolation of UPEC to detect hylogenetic groups for isolates and susceptibility to antibiotic.

Material and Methods

Sample collection a total of 152 samples were collected from urine of patients with UTI in clean cup, of both sexes (female, male). Samples were collected during the period from November 2017 to March 2018 in Al-husain Teaching Hospital, Nassiriyah city, thi-Qar province, south of Iraq.

Isolation of bacteria the samples were derived from fresh midstream urine, cultured on MacConkey agar as well as blood agar and incubated at 37 °C for 24 hr. The *E. coli* colonies appear smooth colonies with distinct edges and pink colonies on the MacConkey agar (lactose ferment) and on the blood agar Milky to white color with or without zone of hemolytic, or most UPEC appearance β-hemolytic (Brooks *et al.*, 2013). The microscopic examination has been showed that all *E. coli* isolates

were by stain gram stain short gram negative rode, non-spore forming after 24 hours post incubation at 37ºC and vary in length.

Biochemical test all the used, was performed as(Mac Faddin, 2000), which included Indol test, methyl red test, Voges-Proskauer test negative, citrate utilization test, Kligler iron test, catalase and oxidase test. *E. coli* isolated was confirmation by using Api-20E system (Analytical profile index for Enterobacteriaceae test (BioMerieux /France), it was done according to (Leboffe and Pierce, 2005).
Preservation *E. coli* isolates in this study, which type of preservation, it was long or short term or both. *E. coli* isolates positive cultures were stored on brain heart agar slant at 4˚C for short term storage and were frozen in brain heart infusion broth with 15% glycerol, stored at -20˚C, for long term storage as described by (Thomas, 2008). From frozen stock cultures stored at -20°C make subcultured on brain heart infusion agar plates, then incubated in aerobic condition at 37˚C overnight for molecular examination.

Antimicrobial susceptibility test

Susceptibility to nitrofurantoin (NIT) 30 mg, ciprofloxacin (CIP) 5 mg, amoxicillin/clavulinic acid (AMC)30 mg, gentamicin (GEN) 10 mg, amikacin (AK) 30 mg, ceftriaxone (CRO)30 mg, Tetracycline (T) 30 mg, nitilmicin (NET) 30 mg and Trimethoprim (TMP)30 mg, were determined by disc diffusion, method as described by (Bauer *et al.*, (1966). The selection of antibiotic disc was a done according to the guidelines recommended by the Clinical and Laboratory Standard (CLSI, 2016).

DNA extraction DNA

Cultures grown for 18 hours in 10 ml brain heart infusion were utilized for DNA extraction by using company DNA extraction (Geneaid / USA). After DNA extraction was finished, DNA was examined by using Nanodrop (THERMO. USA) that measurement DNA concentration (ng/µL) and examined the DNA purity was detected by reading the absorbance at (260 /280 nm).

Detection of phylogeny groups by PCR amplification

Multiplex-PCR was conducted to determine the phylogenetic grouping of the isolates by targeting three genes marker: *chuA*, *yjaA* and anonymous DNA fragment *TspE4.C2* (Baponi *et al*,. 2016). PCR master mix was prepared by using (MaximeTM PCR PreMix Kit (i-Taq)) and this master mix done according to company instructions for each gene, each 20 µl of PCR reaction mixture for PCR contained 1µl of forward primer, 1 µl of reveres primer, 13µL of free nuclease water and 5 µl of DNA extraction. Thermal cycler (Biorad, Germany) conditions were as follows: 95°C for 5 min Initial Denaturation, 35 cycles of denaturation at 94ºC for 30sec., annealing at 59 ºC for 10sec., extension at 72ºC for 30sec. and final extension at 72ºC for 7 min.
The primers used for PCR amplification as shown in table (1) were *chuA* (279bp), *yjaA*(211bp) and *TspE4.C2*(152 bp\). The PCR product was visualized by electrophoresis on 1.8% agarose gels containing 10 mg/mL ethidium bromide at 80 V for1 hour.
The data of the three amplification results to the determinant pylogenetic group in isolated *E. coli* of the isolates as follows: *chuA*+, *yjaA*+, *TspE4.C2*+/- group B2; *chuA*+, *yjaA*-, *TspE4.C2*+/- group D; *chuA*-, *yjaA*+/-, *TspE4.C2*- group A and group B1 *chuA*-, *yjaA*±, TspEC2+.

Table 1. Primers sequences used for genes amplification.

Primer Name	Primer Sequences (5'-3')		Product size bp	Ref
ChuA	F	GACGAACCAACGGTCAGGAT	279	Clermont et al., 2000
	R	TGCCGCCAGTACCAAAGACA		
YjaA	F	TGAAGTGTCAGGAGACGCTG	211	Clermont et al., 2000
	R	ATGGAGAATGCGTTCCTCAAC		
TspE4C2	F	GAGTAATGTCGGGGCATTCA	152	Clermont et al., 2000
	R	CGCGCCAACAAAGTATTACG		

Result

Isolation of *Escherichia coli*

The One hundred and fifty-two samples of urine, Only 46 (30%) sample gave growth of *E. coli*. The phylogenetic analysis of UPEC a total of 46 *E. coli* strains isolated from patients with UTI were assigned to three phylogenetic groups (A, B2 and D) and No strains were found belong to group B1, as shown in table (2). According to triplex PCR-based phylotyping, group B2 contained the majority of the collected isolates, which included in UPEC group B2 have 34(74%) isolates, group A 6(13%) isolates and group D 6(13%) isolates, as show some isolated UPEC in figure (1).

Table 2: Phylogenetic Analysis to 46 isolates of UPEC.

Phylogenetic groups	*ChuA*	*YjaA*	*TspE4.C2*	UPEC(%)
A	-	+	-	6 (13)
	-	-	-	0
B2	+	+	+	34 (74)
	+	+	-	0
D	+	-	-	6 (13)
	+	-	+	0
Total (%)	40 (87)	40 (87)	34 (74)	46 (100)

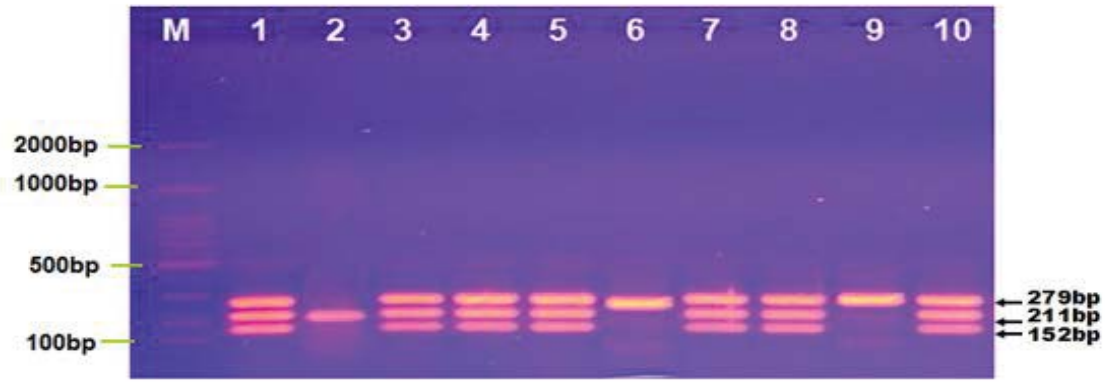

Figure 1. Agarose gel electrophoresis image that shown the PCR product of genes marker.

Where M: Marker (2000-100bp), lane (1-10) some *Escherichia coli* UPEC isolates that show *chuA* gene at 279bp, YjaA gene at 211bp, and *TspE4.C2* gene at 152bp, PCR product.

Association between phylogeny and antibiotic susceptibility the results demonstrate phylogenetic group varied in their resistance and sensitivity to the antibiotics, as the explain in the table (3). All phylogenetic group do not produce any resistance against amikacin .Group B2 resistance 94% to amoxicillin/clavulanic acidacid, 94% trimethoprim 82% tetracycline and ceftriaxon 76%. Group B2 less resistance to ciprofloxacin 35%, gentamicin 29%,nitrofuranution12% and netilmicin 6%. Group A resistance 100% to amoxicillin/clavulanic acidacid, trimethoprim and tetracycline. Group A less resistance to ceftriaxon and ciprofloxacin, was 33%. Group D resistance 100% to amoxicillin/clavulanic acid, trimethoprim, tetracycline and ceftriaxone respective.

Table 3: Antibiotic resistance pattern among phylogenetic groups in Uropathogenic *E. coli*.

	B2 No = 34 %			A No = 6 %			D No = 6 %		
	R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
AK	**0** 0	**2** 6	**32** 94	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0	**6** 100
CRO	**26** 76	**2** 6	**6** 18	**2** 33	**0** 0	**4** 67	**6** 100	**0** 0	**0** 0
CIP	**12** 35	**0** 0	**22** 65	**2** 33	**0** 0	**4** 67	**0** 0	**0** 0	**6** 100
GEN	**10** 29	**2** 6	**22** 65	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0	**6** 100
NIT	**4** 12	**6** 18	**24** 71	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0	**6** 100

NET	**2** 6	**0** 0	**32** 94	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0	**6** 100
T	**28** 82	**2** 6	**4** 12	**6** 100	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0
AMC	**32** 94	**0** 0	**2** 6	**6** 100	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0
TMP	**32** 94	**0** 0	**2** 6	**6** 100	**0** 0	**0** 0	**6** 100	**0** 0	**0** 0

R: resistance; I : intermediate; S: sensitive

Discussion

E. coli are genetically heterogeneous group of bacteria, some subgroups of these bacterial species have acquired genes that enable them to cause intestinal or extraintestinal disease (Bacon *et al.*, 2000; Abdalla *et al.*, 2009).

In present study number of the UPEC isolates was 46 (30%) from 152 patients in Nassiriyah city. This result is in line with many studies in a local study by Hussein (2017) *E. coli* isolated 27.2% from 104 patients in Al-Nasiriyah city and With the study by Al-hamadani (2010) where found *E. coli* was (35.9%) in Diwaniyah and also consistent, with the study Bayati (2009) in Baghdad, the percentage of *E. coli* (33.3%). In the United Arab, the result of present study agree with study by Najwa Al Mously *et al.* (2016) was *E. coli* isolated 27.4% in Saudi Arabia and different from the study in the Emirates by Al-Zarouni *et al.* (2008), where 63.8% were isolated of UPEC from 130 sample urine.

Several studies have revealed that isolates responsible for extraintestinal diseases belong chiefly to the group B2 and to a lesser extent, to the group D. In both groups have a higher prevalence of virulence factor determinants, than the strains in the groups A and B1(Picard *et al.*, 1999; Ejrnaes *et al.*, 2005), in contrast, previous studies of the phylogenetic groups A and B1 *E. coli* strains can also cause disease of extra-intestinal sites (Escobar-Páramo *et al.*, 2004; Rijavec *et al.*, 2008; Regua-Mangia *et al.*, 2010).

In present study when revealed phylogenetic group in UPEC, we found group B2 have 34(74%) isolates, group A 6(13%) isolates and group D 6(13%) isolates. This result is in line with previous study indicated that the dominance of group B2 phylogenetic type among *E. coli* isolates by Abood (2015) which showed, among 65 *E. coli* isolated, phylogenetic group B2 represented 61.5% of the isolates, followed by phylogenetic group A 33.8% , group D 1.5% and group B1 3.0% in Kerbala. The result approaching with study in Iran by Ghauor and Salehzadeh (2017) distribution of phylogenetic groups (B2, D, A and B1)in the isolates were (64%, 24%, 12%, and 0%), respectively and with study by Usein *et al.*,(2011) in India phylogenetic groups in *E. coli* isolates (A, B1, B2, and D) accounted for (20%, 2%, 65%, and 13%) respectively.

The result of present study, different than study by Abdulridha and Al-khafaji (2015) in Karbala was phylogenetic groups (A, B1, B2, and D) in UPEC (16 %, 10.7%, 39.2% ,33.9%) respectively and also, with study by Nielsen *et al.* (2014) phylogenetic groups (A 6%, B1 4%, B2 60%, and D 27%) in UPEC. The absence of the appearance of phylogenetic group B1 in this study, concurrence with study by ALameer et al. (2015) in AL-Nasiriyiah city where was from total 90 *E. coli* isolation the group A &D (40%)for each, group B2 (20%) and no isolated belong to group B1. May be indicates that *E. coli* is dependent on the geographical region and population distribution, as stated by the study Naji (2017).

In current study demonstrated high resistance of phylogenetic groups *E. coli* to antimicrobials that commonly used as amoxicillin/clavulanic acid, Ceftriaxone, Tetracycline and Trimethoprem. That high resistance may be due to extensive and long term use antibiotics, this opinion like to what mention in the study of (Bajaj *et al.,* 2016).

The three phylogenetic group of *E. coli* in this study highly sensitive to amikacin, netilmicin and nitrofurantion agree with study Naji (2017) and study Karami *et al*. (2017), amikacin and netilmicin antimicrobial activity against a wide spectrum of different microorganisms, including Gram-positive or Gram-negative bacteria. The lack of resistance may be related to nitrofurantoin has multiple mechanisms of action, requiring of organisms to develop more than a single mutation in order to develop resistance (Al-jelehawy, 2014).

The phylogenetic group of *E. coli* isolated B2, A and D(respectively) of *E. coli* high resistance to amoxicillin/clavulanic acid, Trimethoprim, Tetracycline and Ceftriaxone agree with study by Almohana (2010) in Najef and with study by Navidinia *et al.* (2014) through search phylogenetic grouping of urine and fecal *Escherichia coli* in Iran. Lee *et al.* (2015) explain through study phylogenetic group distributions and antimicrobial resistance properties of *E. coli* isolates in South Korea, was high resistance to this antibiotic.

The phylogenetic group B2 resistance to gentamicin was 29%, but all isolates in group A and D was sensitive, this result agree with study Abdulrasheed, (2018) and different from study by Kazemnia *et al.* (2014) which high resistance to gentamicin in three phylogenitic group.

The phylogenetic group B2, A and D resistance to ciprofloxacin was 35%, 33% respectively and all isolates in group D was sensitive ,this result different from study by Moreno *et al.* (2006) in Spain was 11% ,56% and 17% respectively.

Ejrnaes in (2011) which explain phylogenetic group A was associated with resistance to many of the tested antimicrobials, whereas group B2 was associated with susceptibility to many of these antimicrobials. These findings are consistent with previous studies showing resistance to antimicrobials to be associated with phylogenetic group A in UPEC (Johnson *et al.*, 2005; Houdouin *et al.*, 2006; Bukh *et al.*, 2009).In addition study by Grude *et al.* (2007) demonstrated the phylogenetic group A predominance among UPEC in Russia.

Through the current study we can conclude the phylogentic group B2 was the majority predominated in UPEC and more resistance to antimicrobial agent.

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