Molecular detection of *Escherichia coli* Cause Urinary Tract Infections among Pregnant Women at Thi-Qar province, Iraq

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Abstract

The prevalence of antibiotic resistance in Enterobacteriaceae has increased sharply in recent years. Extended-spectrum ß-lactamase-producing Enterobacteriaceae include *Escherichia coli* has become especially common. Although traditionally linked to risk factors such as prior hospitalization and antibiotic use, these bacteria have become increasingly recognized in the community, especially as pathogens in urinary tract infections (UTIs). In this study urine samples from 150pregnant women clinically diagnosed with UTIs were used for Gram staining, culture, API 20 E and singleplex PCR. Singleplex PCR was performed with primers targeted to chuA and yjaA genes and anonymous DNA fragment TspE4C2 of *E. coli*. The positive singleplex PCR products were identified by presence of 279 bp, 211 bp and 152 bp amplicons of chuA and yjaA genes and anonymous DNA fragment TspE4C2 for of *E.coli*. Conventional methods of Gram staining, culture and API 20E test showed positive result for *E. coli* in 35 (40%) out of 150 pregnant clinically diagnosed with urinary tract infection. PCR detected 24 (27.5%) out of the 35 (40%) samples that were positive for *E. coli*. The majority of UTIs caused by spectrum ß-lactamase-producing Enterobacteriaceae include *E. coli* was acquired in the community, so rapid, specific and sensitive molecular are urgently needed to better prevalence, prevent and treat these infections in Iraq.

Keywords: *UTIs, E. coli, pregnant women, ChuA gene, yjaA gene, TspE4C2 fragment*

Introduction

Urinary tract infections are either acquired from the acquired infections community, or Nosocomial Infections which is the second largest cause of Bacteraemia in hospitalized patients(1). Urinary tract infection (UTIs) is one of the most common diseases caused by different types of pathogens. Women are often more likely to be infected than men for physiological and anatomical reasons of the urinary system(2). Urinary Tract Infections (UTIs) are important and common health problems in developing and developed countries and the major proportion of them are bacterial pathogens. UTIs are an infection caused by the presence and growth of microorganisms anyplace in the urinary tract. It is usually causes by bacteria from the digestive tract, which the reach the opening of the urethra and begin replication to cause infection(3) Pregnancy is one of the factors that increase the risk of urinary tract infection, which is one of the most common complications that women suffer during pregnancy, especially when the injury develops to kidney infections, which leads to complications and risks to the mother and fetus, especially when not treated(4). Urinary tract infection occurs in all ages in pregnant women and in various stages, and the third stage of pregnancy is the most common stage of pregnancy with urinary tract infections(5). Bacteria are the most frequent cause of UTIs and aerobic Gram negative bacilli predominant(6) Urinary tract infections occur when bacteria invade the tissues of the urinary system, which extends from the opening of the urethra to the renal cortex, and that clinical symptoms, diagnosis, treatment, complications and their long-term significance vary depending on the site of the injury and the presence or absence of birth defects in the urinary system(7).

Identification of bacterial isolates is an essential task of clinical microbiology laboratories, and its`

relies on phenotypic tests. Traditional phenotypic identification is difficult and time consuming, while genotypic identification is emerging as an alternative or complement to established phenotypic methods. Typically, genotypic identification of bacteria involves the use of conserved sequences within phyelogenetically informative targets[8]. Phylogeny is the study of the evolutionary history and relationships among individuals or groups of organisms. Molecular phylogeny has also revealed that horizontal transfer plays an important and unexpected role in evolution[9]. The aim of present study is to review the impact of *E. coli* in causes of urinary tract infections in pregnant women, and to study the role of chuA and yjaA genes and anonymous DNA fragment TspE4C2 in the molecular detection of *E. coli* diagnosed with urinary tract infections.

Materials and Method

Mid-stream urine samples were obtained from pregnant women clinically diagnosed with urinary tract infection in Al-Hussain Teaching Hospital, Thi-Qar, south of Iraq for a period from March to July 2020. The samples (150) of urinary tract infections were investigated. Patients' ages ranged from 18 to 42 years.

Identification of the bacteria

Urine samples were obtained from150 pregnant women clinically diagnosed with urinary tract infection and directly processed for bacterial isolation and identification using standard methods according to[10]. The urine samples were cultured on blood and MacConkey agars as an enrichment and deferential media for *E. coli* from other Enterobacteriaceae members and incubated aerobically at 37°C for 24 hrs. After that the bacterial isolates from positive samples were activated using brain heart infusion broth and incubated at 37°C for 18 hrs. Gram stains were performed. API 20 E was used as a further conventional diagnosis for the *E. coli* isolates from other Enterobacteriaceae isolates.

DNA extraction.

DNA was extracted from the isolates using Quantiphore® Bacterial DNA Extraction spin kit according to manufacturer's instructions (Anatollia, Turky)

The primers for ChuA and YJa genes and anonymous DNA fragment TspE4C2 of E. coli as the target genes for this study were selected according to [11].

Table 1: Primer name, sequence and expected product size of ChuA and YJa genes and anonymous DNA fragment TspE4C2 of *E. coli*

Primer name	sequence (5'→3')	Size product
chuAF	GACGAACCAACGGTCAGGAT	279bp
chuA R	TGCCGCCAGTACCAAAGACA	
yjaA F	TGAAGTGTCAGGAGACGCTG	211bp
yjaA R	ATGGAGAATGCGTTCCTCAA	
TspE4C2 F	GAGTAATGTCGGGGCATTCA	152bp
TspE4C2 R	CGCGCCAACAAAGTATTACG	

Table (2):- PCR conditions of chuA, yjaA and TspE4C2.

PCR step	Temp. °C	Time	Repeat
Initial Denaturation	94	3mim	1
Denaturation	95	30sec	
Annealing	4	30sec	35cycle
Extension	72	5min	
Final extension	72	5min	1

Results

The result of conventional methods showed positive result for *E. coli* in 35 (40%) out of 150 pregnant women clinically diagnosed with urinary tract infection. PCR detected 24 (27.2%) out of the 35 (40%) samples that were positive for *E. coli* samples that were positive using Gram staining, culturing on MacConky and blood agar, The result of ChuA and YJa genes and anonymous DNA fragment TspE4C2 amplification by singleplex PCR using ChuAF, ChuAR, YJaF, YJaR, TspE4C2F and TspE4C2R primers, respectively. Pregnant women (150) clinically diagnosed with urinary tract infection, a positive result for E. coli was detected in 24 (27.5%) out of the 35 (40%) samples who were gave positive result by the conventional methods. The singleplex PCR products and 50-1500 bp DNA ladder were resolved by electrophoresis, signleplex PCR product were loaded on 1.5% agarose gel and run at 70 volt/ cm for one hour. The gel was stained with ethidium bromide solution (0.5 µg/ ml) for 15-30 minutes.

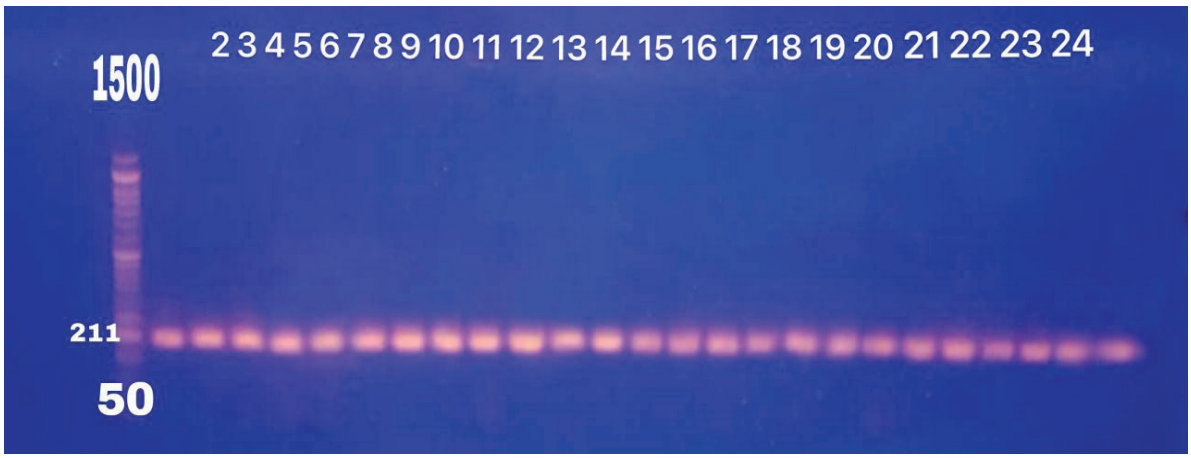

Figure 1: Gel electrophoresis of singleplex PCR products 211 bp of YJa gene for *E. coli* isolates using 1.5% agarose gel at 7 volt/ cm for 1hour. Lane 1: 50-1500bp DNA ladder, lane 2-24: PCR products of YJa gene.

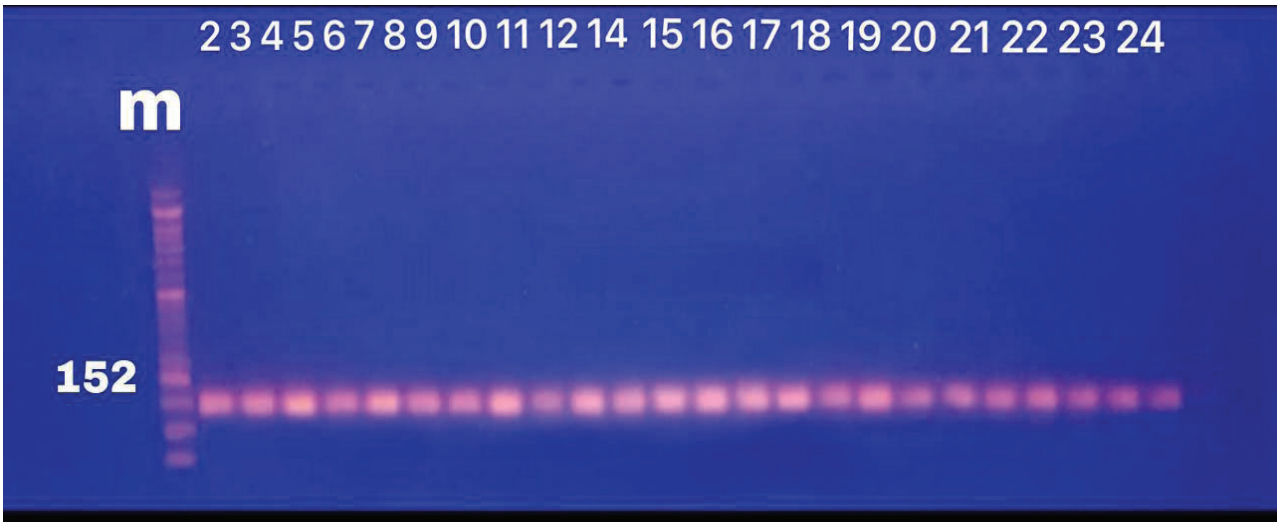

Figure 2: Gel electrophoresis of singleplex PCR products (152 bp) of TspE4C2F DNA fragment for E. coli isolates using 1.5% agarose gel at 7 volt/ cm for 1 hour. Lane 1: 50-1500 bp DNA ladder, lane 2-24: PCR products of TspE4C2F DNA fragment

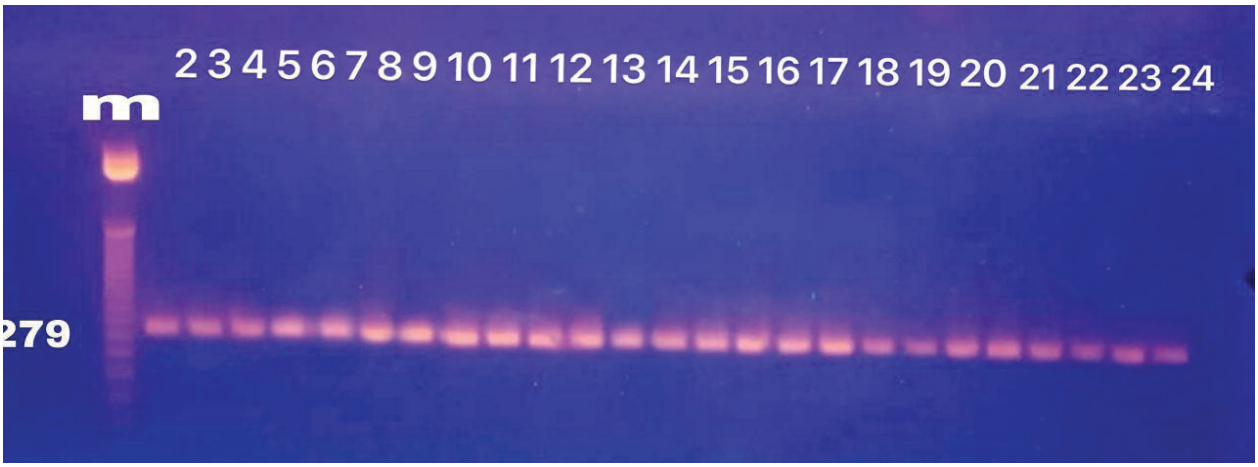

Figure 3: Gel electrophoresis of singleplex PCR products of ChuA gene (279bp) for *E.coli* isolates using 1.5% agarose gel at 7 volt/ cm for 1 hour. Lane 1: 50-1500 bp DNA ladder, lane 2-24: PCR products of ChuA gene.

Discussion

The results showed that *E. coli* was the most isolated bacteria that causes UTIs in pregnant women. these results came close to the results obtained by the researcher[11] showed that E. coli was the most common causative agent as a cause of UTI in pregnancy (46%), also came close to the result recorded by[11] study on adult patients, the largest isolation rate of E. coli was 31.5%. and in the study of the researcher [12] that have conducted on pregnant women with diabetes, the incidence of E. coli was(37.7%.). Also these results agree with data from various medical records showed that the epidemiology of the pathogens of UTI is represented by E. coli the most common causative agent which was 51.70%, In Iraq[13], have found that the most prevalent bacteria in the urinary tract infections were E. coli. Another study by[14] on UTIs, revealed that the E. coli was (52.2%) which represent the most common bacterial isolates .The benefits of molecular methods are more sensitive, more qualitative for results, materials available, but the drawback of molecular methods is costly. These explanations made molecular methods relatively more accurate than conventional methods [15]. The data of present study agrees with the study conducted by [16] that confirms the efficacy of the PCR

assay compared to conventional methods of diagnosis in the clinical setting [17].

In conclusion, this study has demonstrated the efficacy of the ChuA and YJaF genes and TspE4C2F DNA fragment as molecular markers to detect of the *E. coli* in pregnant women with urinary tract infection offers the possibility of reducing the risk to the mother and fetus, also the present study referred to possibility of using the singleplex PCR-based test as a rapid, specific and sensitive method to prevalence the E. coli

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: None

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References

1- Okonko IO, Ijandipe LA, Ilusanya AO, Donbraye-Emmanuel OB, Ejembi J, Udeze AO, Nkang AO. Detection of urinary tract infection (UTI) among pregnant women in Oluyoro Catholic Hospital, Ibadan, South- Western Nigeria. *Malaysian journal of Microbiology . 2010;6*(1):16-24

2- Tan CW, Chlebicki M P. Urinary tract infections in adults. *Singapore medical journal . 2016*;*57*(9):485

3- Mike LA, Smith SN, Sumner CA, Eaton KA, Mobley HL. Siderophore vaccine conjugates protect against uropathogenic Escherichia coli urinary tract infection. *Proceedings of the National Academy of Sciences. 2016*;*113*(47): 13468-13473

4- Andersson SH, Rymer J, Joyce DW, Momoh C, Gayle CM. Sexual quality of life in women who have undergone female genital mutilation: a case–control study. *BJOG: An International Journal of Obstetrics & Gynaecology . 2012*;*119*(13):1606-1611

5- Fareid MA. Frequency and susceptibility profile of bacteria causing urinary tract infections among women. *New York Science Journal. 2012*;*5*(2):72.

6- El-Naggar W, Hassan R, Barwa R, Shokralla S, Elgamal A. Molecular diagnosis of Gram negative bacteria in urinary tract infections. Egyption J. of Med. Microbiology.2010; 19(1): 59-66

7- Singh RS, Bhari R, Kaur HP. Characteristics of yeast lectins and their role in cell–cell interactions. *Biotechnology advances. 2011*;*29*(6):726-731

8- Noor AF, Shams F, Munshi SK, Hassan M, Noor R . Prevalence and antibiogram profile of uropathogens isolated from hospital and community patients with urinary tract infections in Dhaka city. Journal of Bangladesh academy of sciences . 2013;37(1): 57-63

9- Wertz JE, Goldstone C, Gordon DM , Riley MA . Molecular phylogeny of enteric bacteria and implications for a bacterial species concept. Journal of evolutionary biology . 2003;16(6):1236-1248

10- Forbes BA, Daniel FS, Alice SW. *Bailey and Scott s Diagnostic* Microbiology.2007; 12th ed., Mosby Elsevier company. USA

11 Clermont O, Bonacorsi S, Bingen E . Rapid and simple determination of The Escherichia coli phylogenetic group. Applied and environmental microbiology . 2000;66(10):4555-4558

12- Abeer H , Adeeba Y. Bacteriological Study On The causes of Urinary Tract Infection In Pregnant Women In Mosul And Study Of Some Immunological Variables. 2013;5(2):72

13- Subramanian M, Singh M. Antimicrobial susceptibility pattern of urinary tract infection causing human pathogenic bacteria. *Asian Journal of Medical Sciences .2011*;*3*(2):56-60.

14- Tajbakhsh E, Tajbakhsh S, Khamesipour F. Isolation and molecular detection of Gram negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. *Iranian Red Crescent Medical Journal*. 2015;*17*(5):24779

15- Al- Dahmoshi HOM. Genotypic analysis of ESBLs among extraintestinal isolates of enterobacter cloacae recovered from patients with CA-UTI, Hilla-Iraq. *Adv. Life Sci. Technolgy . 2015*;*28*:34-43

16- Basima Q , Sanaa J , Seham H . Isolation of uropathogens from pediatric associated UTI, with special focus on the detection of Proteus vulgaris. Iraqi J. of Biotech. 2015;14(1): 77-84

17- Kim JY, Hahn HJ, Choe YB, Lee YW, Ahn K J , Moon KC. Molecular biological identification of Malassezia yeasts using pyrosequencing. *Annals of dermatology . 2013*; *25*(1):73-79