Phylogentic Analysis of E. coli Bacteria Isolated from Iraqi UTI Patients

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Abstract
A total of 200 clinical specimens of midstream urine were collected from Iraqi patients suffering from UTIs from Al-Salam teaching hospital. An extra only 10 of mid-stream urine specimens were collected from apparently healthy Iraqi individuals; hence considered as the control group. All specimens were collected from November 2021 to February 2022. All mid-stream urine specimens for patients and control groups were cultured on blood agar and MacConkey agar. All control specimens yield no growth, on the contrary, all patient specimens were culture positive. According to the cultural, morphological, biochemical characteristics, and VITEK, the results showed 86.5% of UTI patients have bacterial growth and 13.5% have not bacterial infection, the isolated bacteria was distributed as 57 (28.5%), 46 (23%), 25 (12.5%), 19 (9.5%), 11 (5.5%), and 15 (7.5%) were identified as Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia., Enterococcus farcalis, Proteus mirabilis., and other types (low number of bacteria lower than103), respectively. Also, the results noted the most UTI patients were female, while in control group were male.
Keywords: E. coli, UTI, ChuA, yjaA, TSPE4.C2.

1. Introduction

The most frequent illnesses seen in clinical practice around the world are urinary tract infections (UTIs). Despite several efforts, over 150 million people globally experience UTIs each year, with significant morbidity and huge medical expenses in the United States, UTIs are responsible for over 10 million office visits, over 2 million visits to emergency rooms, and 100,000 admissions to hospitals each year (Flores-Mireles et al., 2015). Acute, chronic, and recurrent infections, as well as asymptomatic and symptomatic bacteriuria are all disorders that can be brought on by UTIs (Klein et al., 2020). The most prevalent etiological agent, uropathogenic E. coli (UPECs), is responsible for more than 75% of UTI infections (Flores-Mireles et al., 2015). Prior to colonizing the urinary tract, UPECs keeps urea, unstable pH levels, urine flux (which is challenging due to adhesiveness and persistence), and low levels of oxygen availability in the environment (Neugent et al., 2020). E. coli consist of various groups as described below.
Enteripathogenic E. coli (EPEC) is one of the diarrheagenic E. coli pathotypes, is one of the most serious infections affecting children globally (Abba et al., 2009). Shiga-toxin producing E. coli (STEC), also known as verocytoxin-producing Escherichia coli (VTEC), is a food-borne zoonotic agent linked to outbreaks all over the world and a severe threat to the public's health. Despite the fact that fewer than 380 distinct VTEC serotypes have been isolated from both humans and animals, most of them are not connected to human disease (Karmali et al., 2010). Enteroinvasive Escherichia coli (EIEC), a subgroup of intestinal pathogenic Escherichia coli, are intracellular pathogens that can penetrate colon epithelial cells, multiply there, and migrate between neighboring cells via a similar method to Shigella, the bacillary dysentery etiological agent (Pasqua et al., 2017). A pathogenic variant of E. coli known as enterotoxigenic E. coli (ETEC) is characterized by the development of heat-stable (ST) enterotoxins and diarrheagenic heat-labile (LT). Nearly 50 years ago, these bacteria were first linked to cholera-like watery diarrhea. Children's diarrhea and traveler's diarrhea continue to be mostly caused by enterotoxigenic Escherichia coli (ETEC) strains (Hosangadi et al., 2019). ETEC bacteria can colonize small intestines by attaching to host cell receptors with the help of colonization factor antigen (CFA) and coli surface antigen (CS) adhesins (Nataro et al., 1998). And finally the uropathogenic e.coli (UPEC) which our study is based on.
Escherichia coli is classified phylogeneticaly into intestinal and extraintestinal, the intestinal E. coli normally live in the intestine of people and animals, most of it are harmless and actually are an important part of a healthy human intestinal tract. however, some of them are pathogeneic they can cause illness either dirrhea or illness outside the intestinal tract, In human, the main prevalent pathogenic of Gram-negative bacteria is Escherichia coli extraintestinal pathogenicity (ExPEC). The majority of urinary tract infections, the second most common cause of neonatal meningitis, and the primary cause of adult bacteremia are all caused by ExPEC (UTIs). ExPEC infections are linked to an increasing number of hospitalizations, fatalities, and rising healthcare

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expenses. One important barrier to treatment is the growing antibiotic resistance among ExPEC strains in humans (Poolman et al., 2016).

2. Material and Method

Ethical statement

All of the people participating agreed to give the researcher urine samples. In accordance with the Helsinki Declaration, all participants gave their informed consent.

Patient group

Between November 2021 to February 2022, a total of 200 clinical samples were collected from patients suffer from UTI who were attended to Al-Salam Teaching Hospital in the city of Mosul. The group of patients showed signs of painful and frequent urination or blood in urine along with abdominal pain. The age of the patients under study ranged between 15-55 years, including 86 males and 114 females.

Specimen collection

The mid-stream urine samples of patients with UTIs were collected. By telling patients to clean their genitalia first before collecting urine and to throw away the first and last samples they collected, mid-stream urine samples might be obtained. As a result, midstream urine was isolated in the top of a sterile container and collected.

Identification of E. coli Bacteria

Unless otherwise stated, morphological traits on culture medium, microscopic examination, Vitec 2 were used to identify isolates, according Wanger *et al.* (2017).

Molecular Identification

Primers

The primers (Bioner/Korea) used in the current study's conventional polymerase chain reaction (PCR) are listed in Table 2-1.

Table 2-1: The primers and their sequences used in the conventional PCR

Target gene		Sequences of primers (5´-3´)	Product Size	Reference
ChuA	F	5´-GACGAACCAACGGTCAGGAT-3´	279 bp	Clermont et al., 2000
	R	5´-TGCCGCCAGTACCAAAGACA-3´		
yjaA	F	5´-TGAAGTGTCAGGAGACGCTG-3´	211 bp	
	R	5´-ATGGAGAATGCGTTCCTCAAC-3´		
TSPE4.C2.	F	5´-GAGTAATGTCGGGGCATTCA-3´	152 bp	
	R	5´-CGCGCCAACAAAGTATTACG-3´		

PCR amplification and detection of bacterial genes

Tables 3-5 show the primer sequences for the ChuA and YjaA genes, as well as the TSPE4.C2 DNA fragment. The primers were lyophilized and dissolved in sterile nuclease-free water to a final concentration of 100M before being stored at -20 °C until use. The PCR mixture was prepared of 10 µl of PCR Master Mix (2X), a template of DNA (5µl), sense (0.5 µl) and antisense (0.5 µl) primers, and nuclease-free water (4µl) to make a final volume of 20µl.

The vortex rapidly mixed the Eppendorf PCR tubes before inserting them into the PCR system. For 5 min, the reaction mixture was subjected to one round of initial denaturation at 95 °C. The cycling conditions programs for bacterial genes were listed in Table 2-2.

Table 2-2: PCR conditions for bacterial genes Chua, Yjaa and TSPE4.C2 in the current study.

Time	Temperature	Cycle	PCR Steps
5 min	95	1	Pre-Denaturation
30 sec	95	30	Denaturation
30 sec	59		Annealing
30 sec	72		Extension
5 min	72	1	Final extension
∞	4	-	Hold

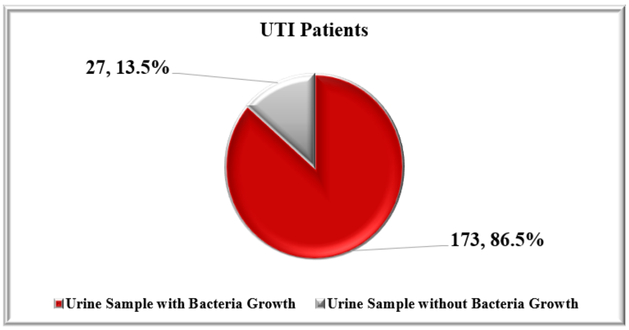
3. Statistical Analysis

The data of the current study were statistically analysis by SPSS version 26, based in using non-parametric and descriptive cross table Chi-Square and Odds Ratio at p. value < 0.05.

4. Results and Discussion

Prevalence of Bacterial Growth in UTI Patients

The current results investigated among 200 UTI patients 86.5% of which have bacteria growth, while 13.5% of which have not bacterial infection. The results also noted a significant difference according to bacterial growth in UTI patients at p. value < 0.05 as in figure 3-1.

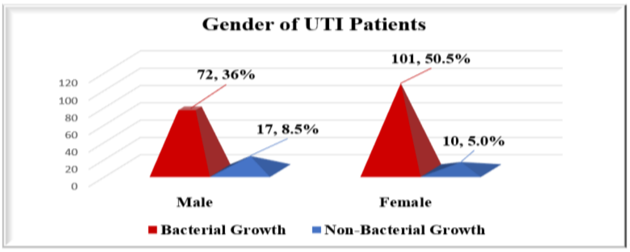


CalX2= 106. 58 TabX2= 3.84 DF= 1 p. value < 0.0001

Figure 3-1: Prevalence of growth bacteria in UTI patients

Prevalence of Bacteria Growth in UTI Patients According to Gender

The current results illustrated the most UTI patients have bacterial infection was female 50.5%, followed by male 36%. While the most UTI patients have not bacteria growth was male 8.5%, and the lowest was in female 5.0%. the results also noted a significant difference between growth and non-bacteria growth according to gender, the odds ratio was increased in female than male at p. value < 0.05 as showed in figure



CalX2= 4.308 TabX2= 3.84 DF= 1 p. value 0.038, Odds Ratio = 2.385 (1032-5.510)

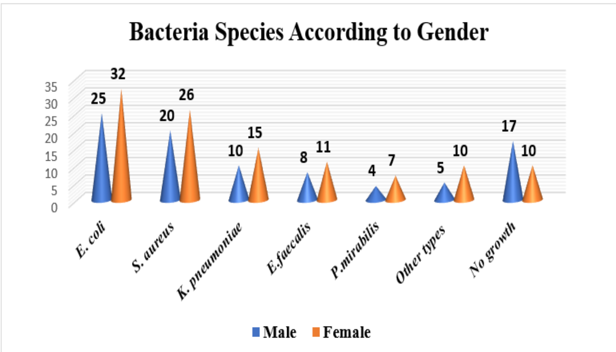
Figure 3-2: prevalence of culture of urine according to gender.

Prevalence of the bacteria genus isolated from urine samples

The bacterial species that were isolated from UTI patients of the present study were shown at table (3-1), fifty-seven (28.5%) of the isolates were found to be E. coli, 46(23%) were found to be Staphylococcus aureus, and there were 25(12.5%) Klebsiella pneumoniae isolates. In addition, 19(9.5%) of the isolates belonged to the genus Enterococcus faecalis, while 11(5.5%) of them belonged to the genus Proteus mirabilis. In addition, 15(7.5%) of the culture results showed a variety of bacteria, while twenty-seven (13.5%) of the culture results showed no sign of growth.

Table 3-1: the total number of bacterial isolates that were isolated and diagnosed (no. = 173 isolates)

Isolates	No	Percentage %
E. coli	57	28.5%
Staphylococcus aureus	46	23%
Klebsiella pneumoniae	25	12.5%
Enterococcus faecalis	19	9.5%
Proteus mirabilis	11	5.5%
Other types (not verify the infection due to low number of bacteria lower than 103)	15	7.5%
No growth	27	13.5%
Total	200	100%
CalX2= 59.91 TabX2= 12.59 DF= 6 p. value < 0.0001		



CalX2= 5.057 TabX2= 12.59 DF= 6 p. value 0.537

Figure 3-3: prevalence of bacteria species according to gender

Prevalence of Bacteria Species According to Gender

The current study showed the most isolated bacteria was E. coli, followed by S. aureus bacteria, the results also noted all UTI female patients have bacterial infection more than UTI male patients. While UTI patients have not bacterial infection in male than female. The results also noted a non-significant difference according to gender at p. value < 0.05 as in figure 3-3.

In Iraq, the current results are closely related to those of Alsamarai et al. (2016) who reported that E. coli caused 46.42% of UTI cases, followed by Coagulase-negative staphylococci (33.9 %), K. pneumoniae (8.03%), and other bacterial isolates for the remaining percentage of cases. Martin et al. (2019) findings, which showed that S. aureus and E. coli were the two most common bacterial uropathogens. On the other hand, the current findings were in contrast to those of Fadhel et al. (2013), who showed that Staphylococcus spp had a greater prevalence rate in UTI cases. In some regional countries, according to Safar et al. (2009) in Iran, staphylococci accounted for roughly 8% of UTI cases. Similar to this, Alshabi et al. (2019) found that 10.52% of pregnant women had UTIs caused by S. aureus in Al-sudia Arabia.

The results of the current study are in agreement with those of Tamadonfar et al. (2019), who revealed that E. coli frequently causes recurrent infections of UTIs. The current study's findings also agree with those of Kodner et al. (2010), Ana et al. (2015), Hof (2017), and Riedel et al. (2019), who have determined that E. coli is the most frequent species responsible for UTIs, followed by other species. In other words, patients are more likely to get severe urinary tract infections if they have certain risk factors. In addition, The University of Sarajevo evaluated the prevalence of bacterial isolates that cause urinary tract infections in the same way as prior retrospective research was conducted at the Faculty of Medicine, and the most common isolates were identified in 405 initial samples taken from UTIs outpatient clinics. According to the findings of this research, E. coli was determined to be the predominant kind of bacteria discovered (67.21%), followed by Proteus spp. (9.83%), Enterococcus (7.37%), then Enterobacter (5.73%). The number of Klebsiella spp., Streptococcus spp., as well as Acinetobacter spp (1.63%) was less than the occurrence of Pseudomonas spp., which were discovered in (2.45%) of the samples (Vranic et al., 2017).

Positive urine culture results led to the isolation of ten different species of bacteria. E. coli made up 55.38% of all of the isolated bacteria, which was followed by various species of Enterobacter (29.61%), Pseudomonas (4.9%), and S. aureus (3.21%). E. coli was found to be the most often isolated uropathogen among Gram-negative bacilli (55.3%), whereas S. aureus was found to be the most frequently isolated uropathogen among Gram-positive cocci (3.21 %) (Angoti et al., 2016). This dominance is brought on by a variety of E. coli virulence factors, including endotoxins in all strains, adhesins (pili) and capsules present in some strains associated with UTIs, and colonization factors (Terlizzi et al., 2017). Exotoxins, invasins, and siderophores are virulence-related characteristics of E. coli that aid bacteria in attaching to and invading host tissue (Johnson et al., 2005). Previous research has connected the rise in Staphylococcal UTIs to a rise in the usage of instruments such bladder catheters (Iregbu et al., 2013).

The abuse of antibiotics, which increases the virulence and invasiveness of bacterial isolates, and cultural differences in awareness may be to blame for this discrepancy.

Concerning the prevalence isolates, the recent results agree with those of Hadi et al. (2014), who found that in the Basra governorate, Gram-positive bacteria account for just 18.7% of UTI cases whereas Gram-negative bacteria account for 81.3% of UTI cases. Gram-negative and Gram-positive bacteria were present in UTI patients in Duhok governorate at rates of 52.48% and 47.5%, respectively, according to Mahde et al. (2015). Moreover, the present findings were in line with those made by Alshabi et al. (2019), who discovered that E. coli, the most prevalent bacterium, occurs more frequently in Gram-negative isolates than in Gram-positive isolates.

E. coli is shown to be the prevalent microbe in the majority of investigations carried out in various parts of the world, while the pattern may vary from place to place and from time to time. Additionally, the most common uropathogen that causes UTIs in pregnant women or in clinical settings is E. coli.

One of the most frequent reasons for people in the community to seek medical assistance is because they have a bacterial infection of the urinary system. The successful treatment of people who have bacterial UTIs often depends on determining the kind of organisms that caused the condition and selecting an antibiotic drug that is effective against the organism in question. This is known as the "identification and selection" approach to treatment (Kebira et al., 2009).

This variation can be attributed to geographic variation and antibiotic abuse, which results in multidrug-resistant bacteria that contribute to the spread of bacteria.

E. coli phylogenetic distribution

In this study, the *chuA* and *yjaA* genes as well as a DNA fragment known as TSPE4 were detected using the PCR method to perform phylogenetic grouping of the *E. coli* isolates. *E. coli* can be categorized into four groups based on the presence or lack of one or more of these genes, with further subgroups within each of those four groupings.

Table 3-2: Percentage of *E. coli* isolates phylogenetic subgroups

Phylogenetic groups No. (%)		Phylogenetic subgroups	No. (%)
Intestinal Groups	Group A 5 (8.92)	Subgroup A0 chuA - / yjaA - / TspE4.C2 –	2(3.57%)
		Subgroup A1 chuA - / yjaA + / TspE4.C2 –	3(5.35%)
	Group B1	chuA - / yjaA - / TspE4.C2 +	2(3.57%)
Extra-intestinal Groups	Group B2 40(71.42)	Subgroup B21 chuA + / yjaA + / TspE4.C2 –	7(12.5%)
		Subgroup B2 chuA +, - / yjaA + / TspE4.C2 +	33(58.92%)
	Group D 9(16.07)	Subgroup D1 chuA + / yjaA - / TspE4.C2 –	1(1.78%)
		Subgroup D2 chuA + / yjaA - / TspE4.C2 +	8(14.28%)
Total			56 (100%)

The A group included 5 of the isolates, accounting for 8.9%, of which 2 (3.57%) belonged to subgroup A1 and 3 (5.35%) belonged to subgroup A2. Group B1 contained 2 (3.57%) of the isolates, accounting for 8.92%. In contrast, group B2 consists of 40 (71.42%) isolates, of which 7 (12.5%), 33 (58.92%) belong to Subgroup B21, and 33 (58.92%) belong to Subgroup B22 respectively. In addition, 9 (16.07%) isolates belong to group D, 1 (1.78%) belong to subgroup D1, and 8 (14.28%) belong to subgroup D2. The information presented in table no (4.2).

The results of a prior research that used the PCR technique to categorize the isolates concur with our findings, which revealed that group B2 (129 isolates; 36.7%) and group D (110 isolates, 31.3%) were the two phylogroups with the highest frequency (Hashemizadeh et al., 2017).

Additional research included the isolation of 160 E. coli strains from human patients suffering from UTI. Of these, 100 came from females and 60 came from male patients. The phylogenetic groupings were distributed throughout the females in the population. 46% of the isolates were members of group B2, 33% of the isolates were members of group D, 13% of the isolates were members of group A, while 8% were members of group B1. Among males, phylogroup B2 was the most common (48.3%), followed by phylogroup D (25%), group A (20 %), as well as phylogroup B1 (4.8 %) (6.7 %) (Staji et al., 2019).

The agarose gel electrophoresis for chuA, yjaA and fragment TspE4.C2 are illustrating in the Figures (4.4, 4.5 and 4.6), respectively.

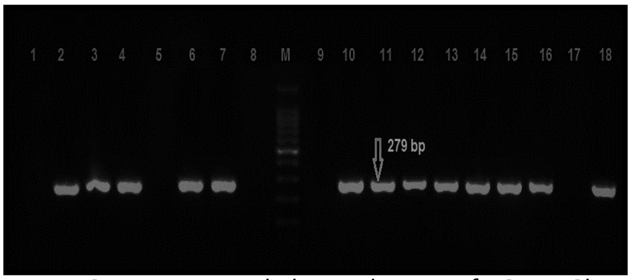


Figure 3-4: Agarose gel electrophoresis of PCR to ChuA amplicon (279bp).

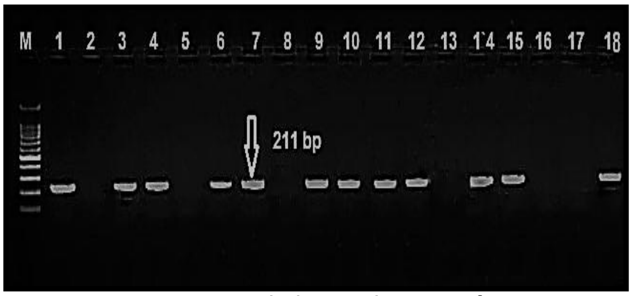


Figure 3-5: Agarose gel electrophoresis of PCR to yja A amplicon (211bp).

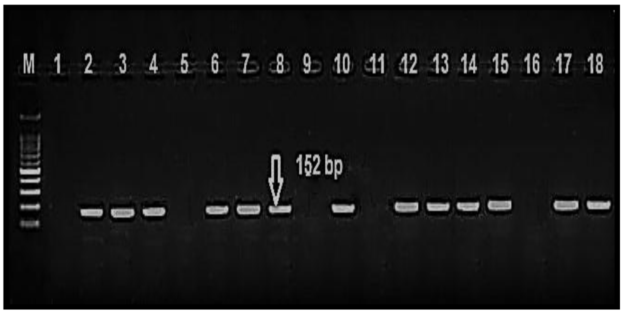


Figure 3-6: Agarose gel electrophoresis of PCR to TspE4.C2 amplicon (152bp).

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