



Isolation and identification of *E. coli* from urinary tract infection in women in waist province

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ABSTRACT

One hundred and seventy-six specimens (urine) from women anage range of 17–40 years were collected from October 2021 to April 2022 in sterilized containers from AL-KUT hospital. All isolates were identified according to their morphological characteristics Biochemical tests and Vitek 2 System were used to confirm the final diagnosing. Results were show 70 (77.77) *E. coli* isolates were obtained from 176 urine samples from women suffering from UTI symptoms, 45 (64%) being of Symptomatic Samples and 25 (36%) of Asymptomatic Samples women, and 50 (71.42) Pregnant and 20 (28.57) non-pregnant, diabetes 27 (3.85) and not present 43 (61.5), were also detected from the obtained isolates. Susceptibility test was determined for all pathogenic *E. coli* isolates to 16 different antibiotics (Ampicillin, Piperacillin, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Tigecycline, Nitrofurantoin, and Trimethoprim). The result showed that all pathogenic *E. coli* isolates had high resist (100%) to Ampicillin, Cefazolin, Ceftriaxone, Cefepime, Trimethoprim and Ceftazidime. 40% of isolates were resist to Cefoxitin, 15% of isolates were resist to Gentamicin, 66% of isolates were resist to Nitrofurantoin, while 100% of isolates were sensitive to Piperacillin, Ertapenem, Imipenem, Amikacin, Ciprofloxacin, Levofloxacin and Tigecycline.

Keywords:

Escherichia coli, urinary tract women

Introduction

Urinary tract infections (UTIs) are common bacterial infections in women, with half of all women experiencing at least one in their lifetime [1]. Of the women affected, 25-30% develop recurrent infections unrelated to any functional or anatomical abnormality of the urinary tract, Most UTIs in women are episodes of acute uncomplicated cystitis which occur in women of childbearing age [2].

Although acute uncomplicated cystitis may not be thought of as a serious condition, it affects the patient's quality of life by causing an estimated six days of discomfort [3]. Acute cystitis refers to symptomatic infection of the bladder in the lower urinary tract, It can occur alone or in conjunction with pyelonephritis, i.e. infection of the kidney in the upper urinary tract. Most

episodes of cystitis and pyelonephritis are considered to be uncomplicated infections when occurring in otherwise healthy non-pregnant women. A complicated UTI can occur in either the upper or lower urinary tract, but is accompanied by an underlying condition which increases the risk of therapy failing, such as obstruction, an anatomical abnormality, urological dysfunction, pregnancy or resistant pathogen [3].

Escherichia coli and *Staphylococcus saprophyticus* account for about 80% of community-acquired uncomplicated urinary infections (UTI), particularly in women under 50 years of age [4]. *E. coli* is a Gram-negative commensal of the distal colon which also harbors other anaerobic bacteria, including *Bacteroides* and *Bifidobacteria* [5]

.Uropathogenic *E. coli* differs from intestinal pathogenic *E. coli* with regard to the presence of specific virulence factors. Among the various serotypes of *E. coli*, 01, 02, 04, 06, 07, 08, 016, 018, 025, and 075 are commonly recovered from patients with UTI. About 80% of uropathogenic *E. coli* express P fimbriae which anchor to the glycolipid of the outer membranes of urothelial cells localized in the kidney[3].

Material and Method

176 of urine samples were collected from women suffering from symptoms (These include increased urine frequency along with pain and the presence of cloudiness in the urine, Other symptoms comprise dysuria, cramps in the lower abdominal region, back pain, chills, fever, and general weakness accompanied by nausea and vomiting. Generally, urine contains not only bacteria, but also salts, wastes, and fluids. It has been seen that pathogenic bacteria entering and multiplying in the bladder or kidneys eventually become a cause of UTIs. Asymptomatic bacteriuria, if left untreated, might cause acute cystitis and also pyelonephritis, which could eventually lead to serious consequences such as prematurity, low birth weight, and increased foetal mortality rates. from different hospitals around wasit province (Al -kut Hospital for Gynecology and obstetrics and pediatrics) from 15 October for 15 desumber . The women were chosen based on their general urine examination results. Samples were placed in sterile urine collection tubes then cultured on MacConkey agar using the streaking method, then they were incubated at 37°C for 24 hours. The identification of the isolate included morphological characteristics and biochemical tests which carried out depending on Bergy's Manual of Systematic Bacteriology, 2nd edition [7] Epi20E, Vitek 2 system.

Identification of the isolates by API20E system:

It was carried out according to the manufacturer's (BioMerieux) instructions. The bacterial inoculum was prepared by suspending 3-5 colonies on EMB agar in 5 ml of saline to obtain a turbidity equivalent to 0.5 Macferland turbidity standard (1.5×10^8 CFU/ml), then the cupules were inoculated with

sterile Pasteur pipettes, and the strips were incubated at 37°C for 24 h.

Vitek2 System:

Vitek 2 System was used to confirm *E. coli* from a diagnostic group specific to the system, and this requires a diagnostic card specific to Gram-negative bacteria containing 64 slots and in each slot, a dried color-indicator. These indicators react to the sample given, and the System records the changes that were happening due to bacterial growth on the slots. According to the given changes in color, the System identifies the bacterial sample according to the guidance given by bio Merieux [8].

Results and Discussions:

Culture results:

Urine samples (176) were collected from symptomatic (101) and asymptomatic (75) women with urinary tract infections (UTI) from women who attended to Al-Kut Hospital for Gynecology and obstetrics and pediatrics during the period from 15 October, 2021 to 15 April, 2022. The women had an age range between 17-40 years. The positive growth from all samples was 121 (69.0%) samples: 70 (57.8%) were *E. Coli* isolates followed by other gram negative bacteria 11 (9.0%) *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were 9 (7.4%). While gram positive bacteria were *staphylococcus aureus* and *streptococcus pneumonia* 18 (14.8%), 7 (5.7%) respectively, as well as *Candida albicans* was 6 (4.9%) as shown

Figure (1-3) . - Growth percentage from all samples collected

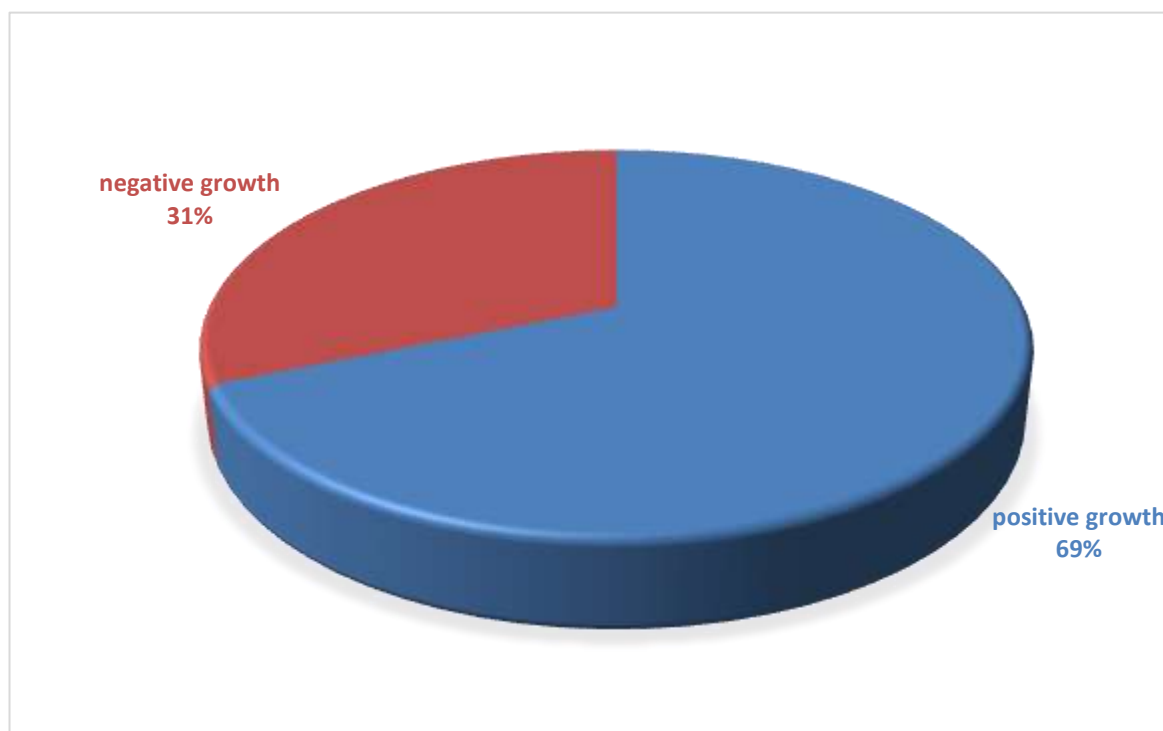


Table: Distribution positive culture isolates according to their group and genus.

Isolates	No. and %	Type of bacteria	No. and %	X ²	P value
Gram negative bacteria	90	<i>E. coli</i>	70(77.77)	119.4	0*
		<i>Klebsiella pneumonia</i>	11(12.22)		
		<i>Pseudomonas aeruginosa</i>	9(10)		
Gram positive bacteria	25	<i>staphylococcus aureus,</i>	18(72)	9.68	0.002*
		<i>streptococcus pneumonia</i>	7(28)		
Yeast	6	<i>Candida albicans</i>	6(100)		
Total No.	121				
X ²	144.3				
P value	0*				

* Significantly difference at P<0.05

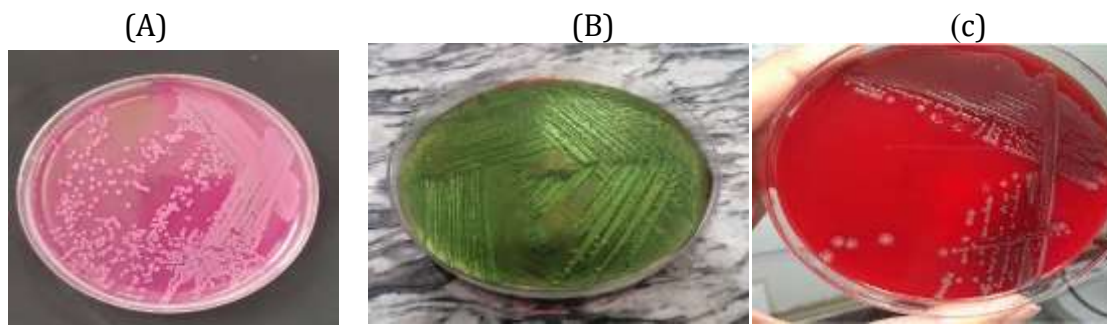
3.2. Isolation and identification of *E. coli* cultural characteristics:

E. coli isolates were identified depending on their physical characteristics. The isolate appeared as bright pink colonies when cultured on MacConkey agar and the colonies appeared green metallic sheen on EMB media. This results

due to MacConkey agar containing crystal violet and bile salts which allows Gram-negative bacteria to grow while inhibiting Gram-positive bacteria growth. It was found that the bacteria were sugar fermenters as shown in Figure3.2A, on Eosin Methylene Blue (EMB), which it was differential medium for *E. coli* used to

differentiate it from other Enterobacteriaceae members. Sheen green metallic colonies were observed, which means that the colonies produced organic acids due to glucose and lactose fermentation which in the presence of eosin and methylene gives a sheen green

metallic color as shown in Figure 3.2B (Singha and Prakash, 2008). The isolates were also cultured on sheep blood agar to test their ability to lyse red blood cells, and as shown in Figure 3.2C, the isolates did lyse red blood cells and cause hemolysis



Figure(3.2):- Bacterial growth on 3 different mediums. A: Bacterial colonies on MacConkey agar, notice the bright pink colonies. B: Sheen green metallic colonies on EMB media. C: Hemolysis on blood agar plates, circular, convex and smooth colonies.

3.2.1 Microscopic Examination:

A smear was made from a colony grown on MacConkey agar and it was stained with Gram’s stain. The bacteria appeared short rods, negative to Gram’s stain, and not spore forming and this is what was mentioned in(Levinson *et al.* 2018)

,appendices 1-A. the results showed that all E. coli isolates were had given positive test for catalase, indole, Methyl Red, but negative for oxidase, Voges-Proskauer, Simmon citrate ,Urea positive and TSI test showed A/A with gas , without H2S . Further confirmation done using API 20E system depending on 20 biochemical tests related with E. coli metabolism activities show in(appendices1-B).

3.2.2 Biochemical Tests :

The E. coli isolates also confirmed by biochemical test as shown in table(3-1)

Table:- Identification of E. coli by biochemical tests.

Id	Biochemicaltest	Result
1	Gramstain	-
2	Citratedutilization	-
3	Catalaseproduction	+
4	Indoleproduction	+
5	Voges-Proskauertest	-
6	Methylred	+
7	Motility	+
8	Oxidaseproduction	-

9	KliglerIronAgar(KIA)	A/A,withgas ,No H ₂ S
10	Ureaseproduction	-
11	Hemolysis	α - β -hemolys is

Vitek 2 System:

To confirm our identification of the bacterial isolates, Vitek 2 System was utilized (shown in appendice2). A GNID card was used for the Gram-negative bacteria. The System provides 64 tests during a 5 – 8 hours period which provides a practical time period to identify the isolates without any mutations happening and with high accuracy (about 99%) and very low chance of any error (Pincus, 2011).

Prevalence of *E. coli* among pregnant women:

Out of 70 *E. coli* isolates from urinary tract infections in women 50 (75%) isolates were obtained from pregnant and Observation in this study on the distribution of UTI infection according to pregnancy age (trimesters) showed that the 3rd trimester had the highest prevalence rate patients as it is described in (table3-5). This study is compatible with a study done by Al-mukhtar (2018) in Kirkuk, Iraq from 450 urine samples found that *E. coli* accounted for 11.7% for pregnant women while it accounted for 21% and 44% for married and single women, respectively. Another study done by Al-Nasrawi and Al-Hashimy (2020) in Al-Najaf, Iraq from 500 urine samples, 27.82% of it define as *E. coli*. Al-Kashif (2019) detected in a study in Saudi Arabia on 303 pregnant women, *E. coli* accounted for 37% while Azami (2020) in

Iran found that *E. coli* was present in 63.3% in pregnant women. It is apparent from the studies mentioned above and this study that *E. coli*'s presence in pregnant women is high and this could be due to physiological reasons, pregnancy's effects on the woman's body which could make her more susceptible to infectious microorganisms, or the high presence of albumin and other amino acids in a pregnant woman's body which makes her a suitable environment for microorganisms capable of causing UTI [9] The weakening of the immune system and the incapability of a pregnant woman to take antibiotics during the first trimester increase a pregnant woman's risk of getting a UTI [10] Also the current study agree with Al-Nasrawi (2020) [11] and who explains that pregnant women in the 3rd trimester of pregnancy have suppressed immune system than those in the 2nd and 1st trimesters which steps up the risk of uti infection. This is due to the emotional stress, which increases as one is expecting a child. At this trimester, an increased level of estrogen and corticoids hormones decreases the level of vaginal defense mechanisms against such infections as *E. coli*. These factors contributed to the highest prevalence of uti infection in the 3rd trimester of pregnancy.

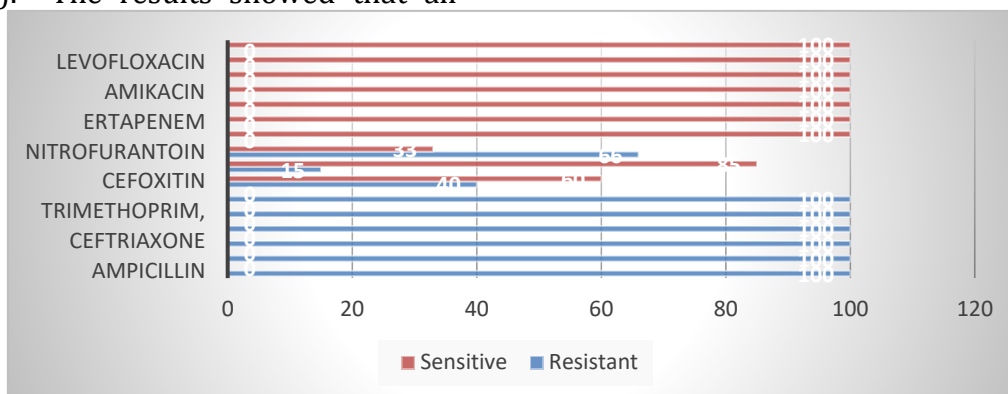
Table:-Prevalence of *E. coli* among pregnant women

Patient group	No. (%) of isolate			
	Total	First trimester	Second trimester	Third trimester
Pregnant	50(71.42)	10 (20)	16(32)	24 (48)
Nonpregnant	20(28.57)			
Total	70(100)			
X2	25.71	8.88		
P value	0*	0.012*		

3.6 . Antibiotic susceptibility of *E.coli* isolates:

The Antibiotic susceptibility test was done by Vitek YST system .The all *E.coli* (70) isolates susceptibility to 16 different antibiotics (Ampicillin, Piperacillin, Cefazolin, Cefoxitin, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Levofloxacin, Tigecycline, Nitrofurantoin, and Trimethoprim). The results showed that all

pathogenic *E.coli* isolates resistant to (Ampicillin, Cefazolin, Ceftriaxone, Cefepime, Trimethoprim, Ceftazidime)100%, Cefoxitin was 40%, Gentamicin was 15%, Nitrofurantoin was 66%, while 100% of isolates were sensitive to Piperacillin, Ertapenem, Imipenem, Amikacin, Ciprofloxacin, Levofloxacin, Tigecycline, as shown in (Figure 3-8),appendices(3)



Figure(3-4):Percentage of Antibiotics use ptibility for *E.coli* isolates.

The antimicrobial resistant tests showed a high resistant of *E.coli* isolates to the Ampicillin, and Trimethoprim. This was also reported by Vranic and Uzunovic (2016), which described a major antimicrobial resistance of *E. coli* to ampicillin and trimethoprim-sulfamethoxazole, as well as the study showed the high resistant of *E.coli* isolates to Cefazolin, Ceftriaxone, Cefepime, Ceftazidime,100%.These compatible with results of Raeispour and Ranjbar (2018) [12] and with Kader and Kumar (2004). AL- Dulaimi et al. (2015) [13] refer to diarrheagenic *E.coli* from children also showed a resistance to these antimicrobial agents.Other study refers that the resistance to ceftazidime and ceftriaxone were both 90% in *E.coli* causing diarrhea [14] Study in north of Iraq revealed that *E.coli* isolates were resistance to Cefazolin, Ceftriaxone, Cefepime, Ceftazidime, Trimethoprim 100% [15] The study showed that the Nitrofurantoin resistance was 66%, this result agree with Tulara (2018) [15] who refer to resistance of isolates about 57%, and with Aghemwenhio et al. (2017) [16],while Cefoxitin resistance was 40%, and this percentage compatible with AbdelRahim et al. (2015) [17] and disagree with Mataseje et al. (2009) [18],

this showed 100% of isolates are resistance to Cefoxitin .In addition, the results demonstrated the resistant of *E.coli* isolates to Gentamicin as well, which reached up to 15%. This result is compatible with results of Hasvold et al. (2013) [19], who refereed that all the *E. coli* cultures at their institution had shown a 12.9 % incidence of gentamicin-resistance and despite being a relatively uncommon cause for neonatal sepsis. The results showed that none of the isolates were resistant to imipenem, ciprofloxacin and amikacin, etrapenem, levofloxacin, piperacillin, tigecycline, this compatible with results of Marejková et al (2013) [17].

Genetically, the microorganisms develop resistance either through spontaneous mutation in existing genes, or by extraneous gene acquisition. On the other hand, the cost of fitness is a crucial factor to determine the success and survival of the resistant mutation. The *E. coli* resistance is almost always plasmidic, however, the porin and efflux-mediated mechanisms are negligible [20]An antibiotic therapy is required to treat traveler's diarrhea, prolonged diarrhea, and antibiotic-associated diarrhea. While awaiting the results of microbiological test, an empiric therapy may

be started depending on the symptom severity or the risk of spreading. Suspected agents, host conditions, and local epidemiology are the main factors that determine the choice of antibiotic. Oral metronidazole could be used as the first choice of empiric therapy and in severe cases; parenteral treatment with ceftriaxone might be considered [19] Through observation as researchers, the correct use of antibiotics and development of newer antibiotic may offer solution to the problem of resistance among diarrheagenic bacteria with pathogenic E.coli .

Conclusions:

The current study provides a basic database for UPEC's spread, virulence factors and pathogenesis in wasit Province and shows that an epidemiological program is necessary to monitor the spread of UPEC and its virulence genes in the local area.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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