

Prevalence of Uropathogenic *Escherichia coli* Among Different Age Groups

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ABSTRACT

Escherichia coli is a significant reason for urinary tract infections (UTIs), especially in immunocompromised individuals such as pregnant women, elderly people, and youngster. Therefore, this cross-sectional research initiative to detect the prevalence of uropathogenic *E. coli* (UPEC) to understand its impact and spread among patients with UTIs across different age groups. The current study was conducted between October 2024 and March 2025, with 155 urine samples collected from outpatients. Microscopical, cultural, and biochemical examinations revealed that 78 out of 155 samples (50.3%) were *E. coli*. All UPEC isolates showed a green metallic sheen on Endo agar and Eosin Methylene Blue (EMB) agar, and a blue colour on Chrome agar. Of the 78 UPEC isolates, 60 (76.9%) were female, and 18 (23.0%) were male. The percentage of UPEC isolates in females was greater in the 21-30-year age group 18 (24.3%), while in males it was greater in the 51-60-year age group 4 (5.1%). Official health institutions must assume responsibility for raising awareness of the importance of hygiene and rationalizing the consumption and use of antibiotics.

Keywords: Prevalence, UPEC, *E. coli*, Age groups, UTI.

Article Information

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INTRODUCTION

Out of the most observed model bacteria in molecular biology or microbiology studies is *E. coli* [1]. Extraintestinal pathogenic *E. coli* are participants in UTIs, nosocomial pneumonia, surgical infection, sepsis, gastrointestinal tract infections, and meningitis [2]. The main causal agent for UTI is UPEC and the primary reservoir for UPEC strains is the human gastrointestinal tract. Variances in the genome of UPEC deliver developmental traits, allowing them to survive in specific regions inside the urinary system [3]. While many lineages of *E. coli* have gained several virulence features, providing them the ability to achieve success in certain niches and

generate various diseases [4]. UTIs course, ranging from mild cystitis and urethritis to serious bacteremia, septic shock, and pyelonephritis [5]. UPEC is the most commonly isolated species from outpatients and inpatients with UTIs [6]. A significant medical issue is the increasing incidence of community-acquired UTIs induced via UPEC, and such isolates show developed resistance to a lot of antibiotics [7]. Multidrug resistance (MDR) has developed into a significant public health and clinical concern in the treatment of patients with UTIs, limiting available therapeutic options [6]. Gram-negative bacteria's integrons, transposons, and plasmids

are among the genetic components that make a serious contribution to the evolution of antibiotic resistance [8].

The targets of the current investigation, were to isolate, identify, and detect the spreading of UPEC among outpatients suffering from UTI and to highlight the age groups most affected by local UPEC in Al-Najaf City, Iraq.

MATERIALS AND METHODS

Bacterial Isolation

The study was continued from October 2024 to April 2025, with 155 midurine samples obtained randomly from outpatients at Al-Furat General and Al-Sadder Medical City Hospital. Each urine sample was collected from the patient, transferred into a sterile container, and separated between two parts. One part was for direct microscopical examination. A specimen was identified as positive for UTI based on the number of colonies $\geq 10^5$ colony forming unite (CFU)/ml and polymorphonuclear leukocytes (PMNs) ≥ 8 leukocytes/mm³ where the second was streaked on the medium using the standard loop method [9].

Bacterial Identification

Microscopic Examination

Gram stain was applied to all test isolate smears, and the staining reaction, size, shape, and arrangement of bacterial cells were assessed using an oil immersion lens at 100X magnification under a light microscope [10].

Macroscopic Identification

Following that, every swab specimen was cultivated on many types of media, included enriched media such as Nutrient agar (NA) and Blood agar (BA) (Himedia, India), then differential and selective media including EMB, Endo agar, MacConkey agar (MAC) (Himedia), and Chrome agar (ChromAgarTM ECC, France)

incubated at 37°C for 24 hours and purified isolates several times until the colonies became purified [8]. The edges, shape, size, and color of the colony are among the general morphological features that help in the identification of isolates. Suspected *E. coli* colonies were also inoculated into Chrome agar medium for *E. coli* and coliforms and colonies that gave a blue color have been recognized as *E. coli* [8, 9].

Biochemical Identification

Several of common biochemical testing methods were used to identify the test isolates to ensure precision. According to MacFaddin [9], these tests include the oxidase test, catalase test, IMVC (Indole test, Methyl red test, Voges-Proskauer test, and Citrate utilization test), gelatinase test, urease test, H₂S production, and motility test.

Data Analysis

Chi-square and *P*-value analysis were performed on the results using a website (<https://www.standarddeviationcalculator.io/chi-square-calculator>).

Research Ethics

This research was approved by the Iraqi Ministry of Health according to Ethics Approval No. 33394.

Conflict of Interest

The authors declare no conflict of interest.

RESULTS

Distribution of Isolated Bacteria

In the present investigation, 155 midurine samples were obtained from outpatients with UTI. Based on microscopic,

cultural, and biochemical properties, 19 (12.2%) specimens did not display bacterial growth, while 78 (50.3%) were UPEC, and 35 (22.5%) isolates were other Gram-negative and 23 (14.8%) isolates were Gram positive (**Figure 1**).

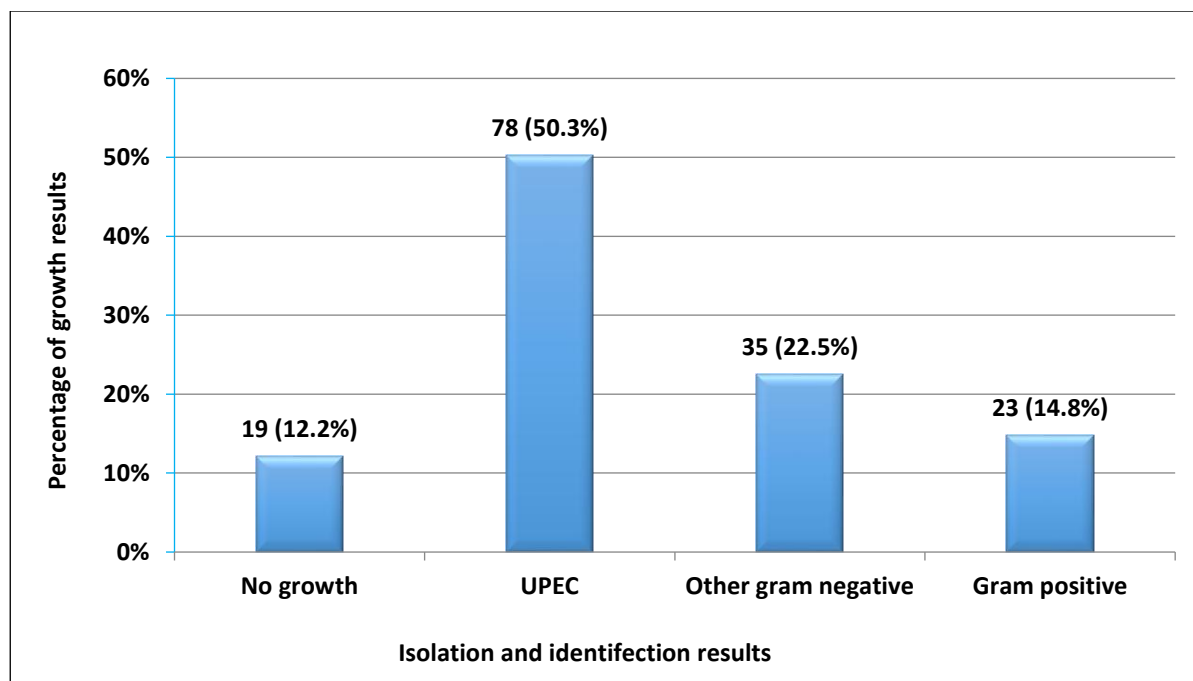


Figure 1. Results of bacterial isolation of 155 urine samples collected from patients with suspected UTI.

Macroscopic Features of *Escherichia coli*

Cultural and Biochemical Features

Colonies of the test isolates on NA medium were large, opaque, grayish-white, thick, moist, and smooth. On enriched media such as BA, they appeared round, large, gray, and moist. Meanwhile, *E. coli* colonies on MAC differential medium appeared pink because they were lactose fermenters. The *E. coli* colonies under study also gave a metallic green sheen on EMB agar medium, and this response is due to the specific properties of the dyes (methylene blue and eosin) and the lactose fermentation property of *E. coli*.

On Endo agar, *E. coli* exhibits a strong reaction, causing fuchsin to crystallize and giving the colonies a green metallic appearance. Moreover, colonies of *E. coli* on Chrome agar, develop with an intense blue color (**Figure 2**).

In parallel with the microscopic and cultural tests, the isolates under test were subjected to many available biochemical tests and were positive for the catalase and motility tests and negative for oxidase, urease and gelatinase and had a distinct profile for the IMVC test, which is : positive, positive, negative, and negative result. Some *E. coli* cultural and biochemical tests are listed in **Table (1), and Figure (3)**.

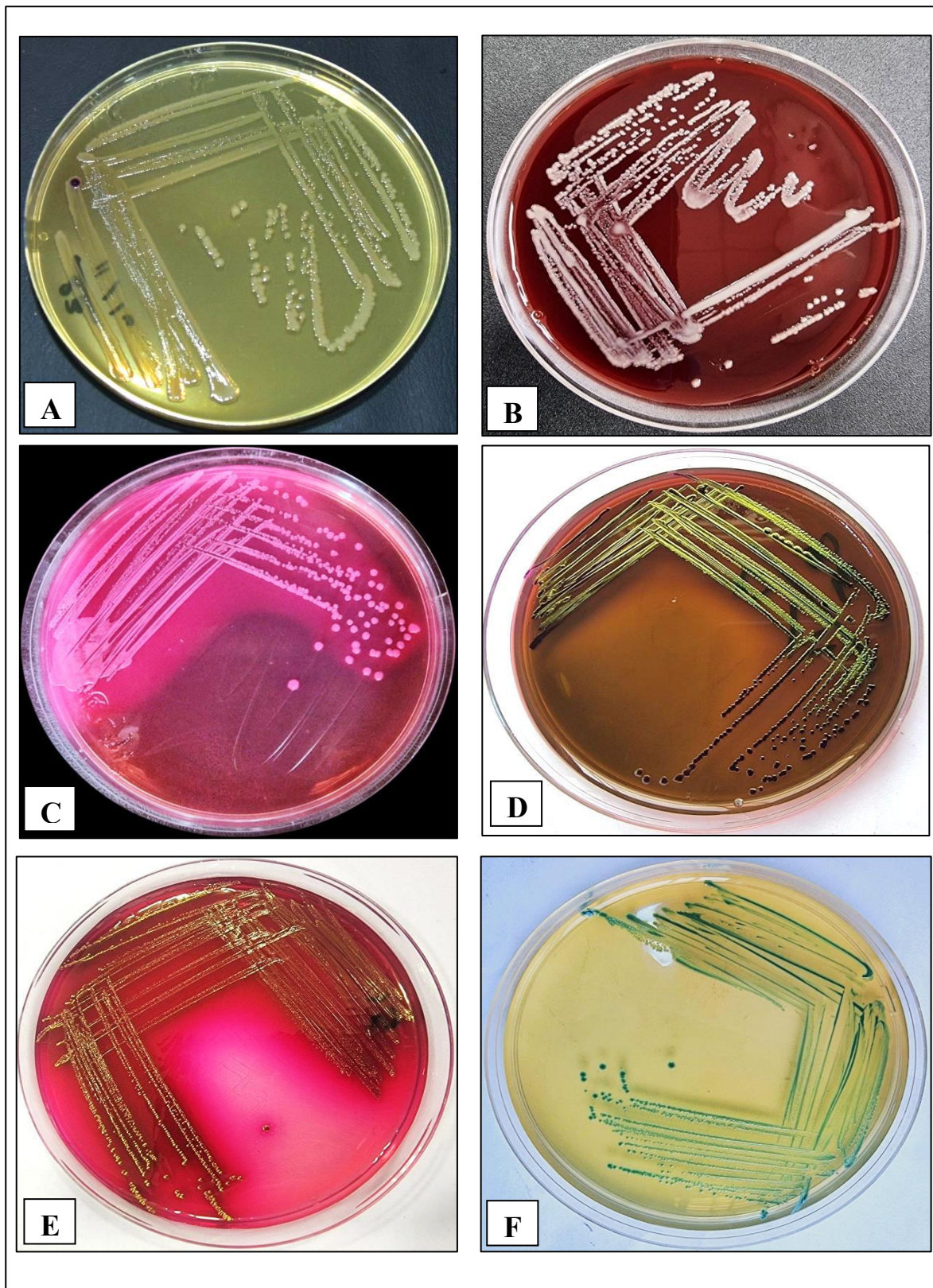


Figure 2. Characteristics of *E. coli* colonies on some enriched, differential, and selective media.

(A) Colonies on nutrient agar media showed large, greyish white, smooth, moist, and opaque, (B) Colonies were big, circular, grey, and moist on blood agar, (C) On MacConkey agar colonies showed pink due to lactose fermentation. (D) *E. coli* colonies gave a green metallic shine when grown on Eosin Methylene Blue agar media, (E) *E. coli* colonies on Endo agar produced a green or golden metallic sheen colour, (F) Colonies on Chrome agar appeared in an intense blue colour.

Table 1. Some biochemical tests and cultural assays were performed on 78 *E. coli* isolates.

Test	Result	Test	Result
Oxidase	Negative	Citrate	Negative
Catalase	Positive	Urease	Negative
Indole	Positive	KIA	A/A no black precipitate
Methyl red	Positive	Motility	Positive
Voges-Proskauer	Negative	Gelatinase	Negative

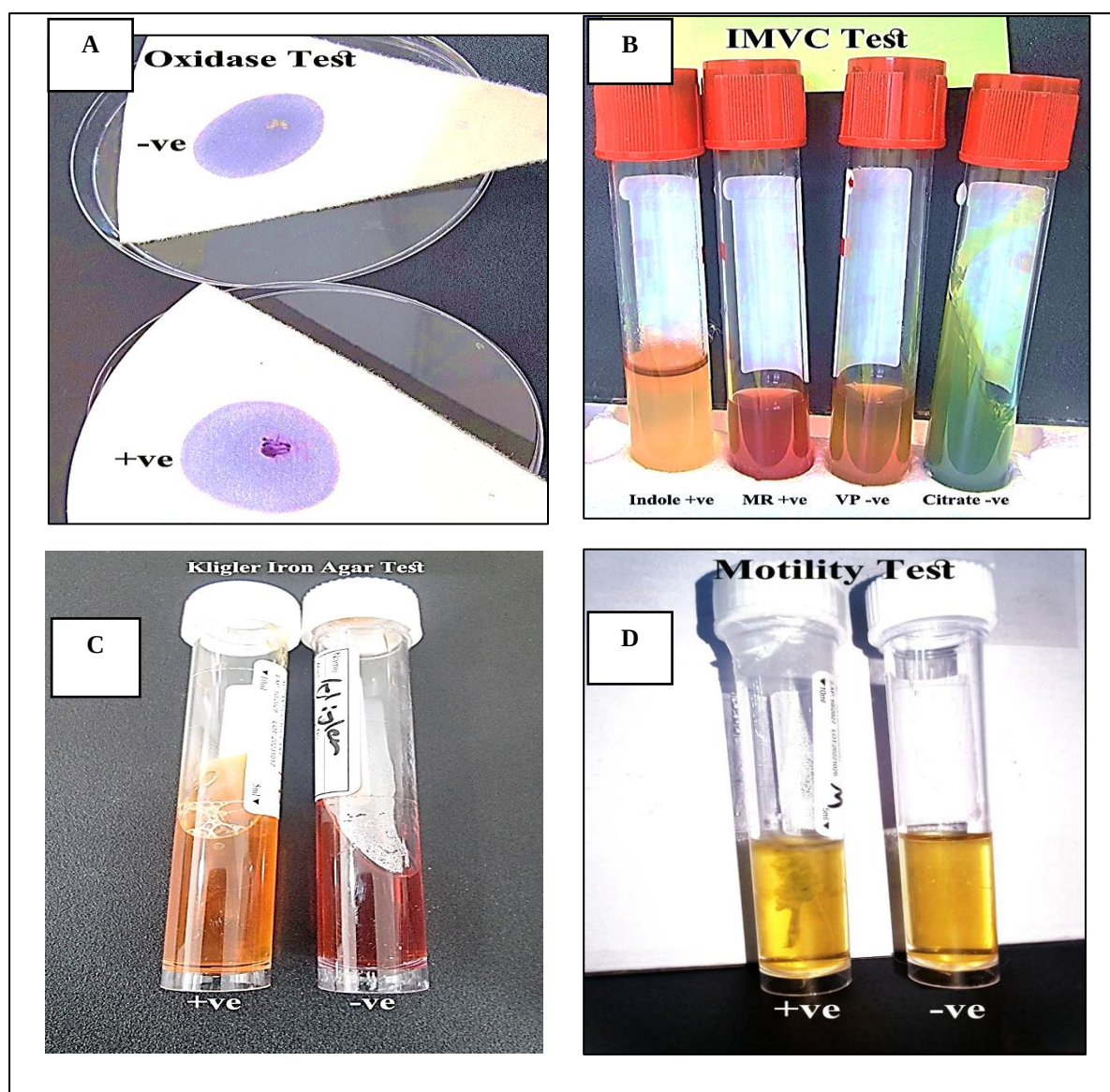


Figure 3. Some cultural and biochemical tests of *E. coli*. (A) *E. coli* is known to be negative for the oxidase test, which is colorless, (B) *E. coli* showed a response to the AMVC test as follows: Indole +ve with a bright red ring, Methyl red +ve gave red color, Voges-proskauer -ve gave no color change, Citrate -ve result in a green color, (C) Kligler Iron Agar test showed Acid/Acid (Yellow/Yellow), (D) The motility test showed a turbidity (+ve) result.

Demographics of Outpatients with Urinary Tract Infection

Generally, this study revealed that UPEC was more prevalent in females 60/78 (76.9%) than in males 18/78 (23%). Also, this study

demonstrated that a large percentage of UPEC was collected from females 18/78 (24.3%) in the age group 21 to 30 years and from males 4/78 (5.1%) among those aged 51 to 60, followed those aged 31 to 40 (12.8%) of females and (61-70) years (3.8%) of males (**Figure 4 and 5**).

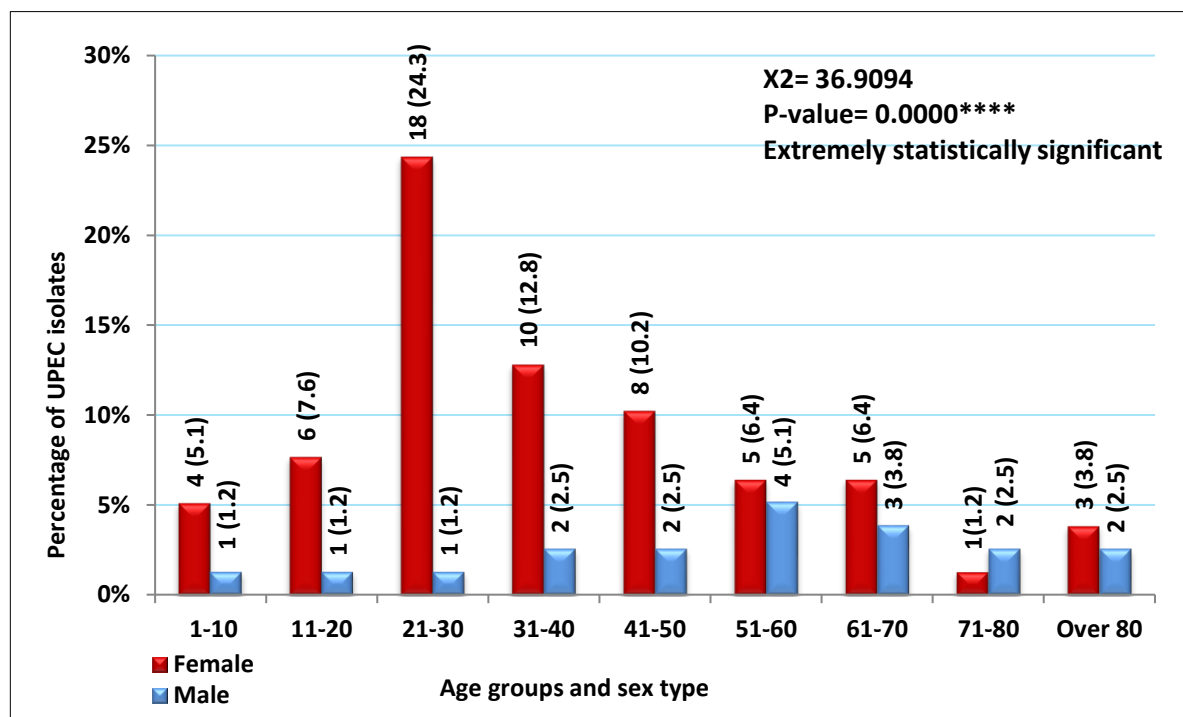


Figure 4. Distribution of 78 UPEC isolates across different age groups and sex type.

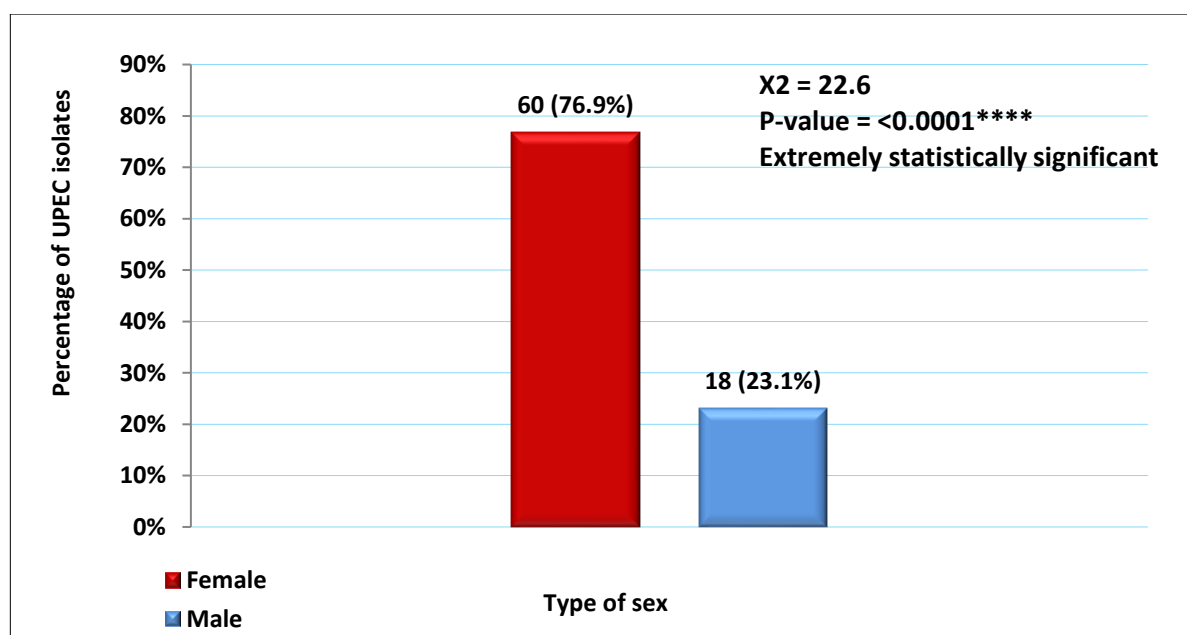


Figure 5. Distribution of 78 UPEC isolates among sex type.

Table 2. Frequency of 78 UPEC isolates in nine different age groups in both sexes.

Age groups	N= 60 no.	Female (76.9) (%)	N= 18 no.	Male (23.0) (%)
≥1-10	4	6.6	1	5.5
11-20	6	10	1	5.5
21-30	18	30	1	5.5
31-40	10	16.6	2	11.1
41-50	8	13.3	2	11.1
51-60	5	8.3	4	22.2
61-70	5	8.3	3	16.6
71-80	1	1.6	2	11.1
<81	3	5	2	11.1

DISCUSSION

Urinary tract infections represent a considerable health concern, ranking second following respiratory tract infections as the most frequent bacterial infection in people. These infections can be challenging to treat and are frequently recurring. Additionally, UTIs can lead to damage in the kidney tissues, which may lead to renal insufficiency and other complications [11]. Each year approximately 150 million individuals worldwide develop UTIs [12]. Recurrent infections pose a significant challenge in treating patients suffering from UTIs. Certain groups, such as women, children, the elderly, people with urinary catheters or stones, and diabetic individuals, are at greater risk of developing these infections [13]. In pregnant women, the main risk factor for several acute diseases is asymptomatic bacteriuria [14]. Accurate and accurate pathogen identification makes a difference in the progression or eradication of bacterial infections. Many media were developed to ease the identification of

bacteria; in this study, we found that the Chrome agar was the better media than EMB and Endo agar in the purity of colonies and effective identification of *E. coli*. This agrees with Antony et al. [15], who revealed that the Hichrome *E. coli* and other chromogenic media are more costly than EMB agar; whenever all aspects are taken into account, it is a significantly efficient, quick, and cost-effective approach that can save both money and time. A previous study by Mhana and Aljanaby [16] reported that 90% of patient samples showed positive bacterial growth, while 10% of urine samples had no bacterial growth. From positive bacterial growths, there were 15% Gram positive bacteria and 85% Gram negative bacteria.

In this investigation, the most common bacteria was *E. coli* (47%), and other Gram negative were 29.7%; there were 26.4% men and 73.6% women. While this study revealed that 12.2% of specimens showed no bacterial growth, 50.3% of isolates were Gram-negative *E. coli*,

22.5% were other Gram-negative, and 14.8% Gram positive. Additionally, 76.9% of *E. coli* isolates were females, and 23.1% were males. This study agrees with Zhan et al. [17] and Mohsenzadeh et al. [18], who found that UTIs are more prevalent in women contrasted to men. The women are much more likely to have UTIs than males, primarily due to specific physiological and anatomical differences. The risk of infection is higher in women for anatomical reasons because the urethral opening is closer to the anus and the urethra is shorter than in men. Additionally, factors such as changes during menopause and pregnancy contribute to this disparity, and the difference in UTI prevalence tends to increase with age [17].

In this study, we found that the highest prevalence of UTI in women was among the 21-30 age group 30%, followed by 16.6% among the 31-40 age group and 13.3% among 41-50 years of the total female population (Table 2). Recent findings from the Global Burden of Disease (GBD) data reveal critical insights into UTIs among women. In 2019, the highest incidence of UTIs was predominantly reported in women aged 25 to 35, a demographic particularly vulnerable due to factors like lifestyle choices, pregnancy, and sexual activity. The 2021 GBD statistics indicate an alteration in the age distribution, with women between the ages of 40 and 49 having a high prevalence. This change may be associated with decreased oestrogen levels that occur around menopause, a reduction in keratinocyte numbers, thinning of the urethral tissue, as well as a decrease in the urethra and vagina's capacity to clean themselves. All of these factors can contribute to an increase in bacterial growth [19]. While Ibrahim et al. [20] showed that the highest infection rate in males was reported among those aged 51 years and older, which was 16.6%. In this study, we observed that the greatest incidence of UTI was among 51-60 years (22.2%), followed by those aged 61-70 years (16.6%) of the total male population Table 2. UTI infections in males are mainly due to prostate infections, urinary

stones, and the use of catheters in the age group of 41-80 years [21], and prostatic hypertrophy in older men can increase the risk of urinary tract infections due to difficulty urinating [20].

In Baghdad City, UTI caused by UPEC was the most dominant bacteria, with 33.33% [22]; in Al-Diwaniya, *E. coli* demonstrated 35.2% [23]; in Tikrit, 31.1% [24]; in Duhok, 51.7% [25]; in Babylon, 68.3% [26]; in Kirkuk, 50.4% [27]; in Basrah, 49.0% [28]; in Wasit, 42.2% [29]; and in Mosul, 51.94% [30]. Meanwhile, in different countries nearest to Iraq, the incidence ratios were Iran 62% [31], Kuwait 67.3% [32], Turkey 79.9% [33], Saudi Arabia 51% [34], Syria 58.4% [35], and Jordan 29.5% [36]. When compared to previous research, the prevalence proportion in this study was lower than in other studies, such as 88% in Nigeria [37], 71% in Pakistan [38], and 75.7% in China [39]. In contrast, the percentage of prevalence was higher than the rates conducted in Uganda 10% [40].

CONCLUSIONS

The high predominance of UTIs, especially via UPEC, is due to the living of *E. coli* as microbiota inside the human body and the adaptable development of UPEC, which has been documented in multiple modes, including modifications in intracellular replication, colonization, invasion, and adhesion to infiltrate the urothelium and persist inside the cells. The possibility that women are more likely to develop UTIs than men has been revealed in various studies for many reasons. Females 21 to 40 years old have higher UTIs than other age groups, who may be sexually active or pregnant or may be in the time following menopause; the risk rises mainly due to reduced levels of oestrogen. Males aged 51 to 70 years have higher infections with UPEC than younger males due to factors like prostatitis, diabetes mellitus, and prostate hypertrophy.

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