Mechanisms of Development of Multidrug Resistance (MDR) in Intestinal *E. coli* Bacteria Causing Urinary Tract Infections

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Abstract
Background: Urinary tract infections (UTIs) are among the most common bacterial infections worldwide. Studies indicate that *E.coli* bacteria are responsible for the majority of these infections, especially in women. The danger of these infections lies in their recurrence and rapid progression if there is no response to treatment.
Methods: 30 samples were collected for the period from December 2025 to March 2026,from patients of both sexes and different ages whom suffering from urinary tract infections and cultured using appropriate media such as MacConkey agar and EMB agar, in addition to microscopic and biochemical examinations.
The results: The *E. coli* bacteria were among the most common pathogens. The colonies appeared pink on MacConkey agar and metallic green sheen on EMB agar. Microscopic examinations showed that they were Gram-negative bacilli, and biochemical tests confirmed their positivity for the catalase test and their negativity for the oxidase test.
The results of antibiotic susceptibility testing of the isolates using Mueller Hinton agar showed high resistance rates were recorded for ceftriaxone and trimethoprim/SXT. While high susceptibility to imipenem and amikacin,. A clear prevalence of multidrug resistance (MDR) was also observed, in addition to co-resistance among several drug classes.

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Keywords: Escherichia coli, Urinary Tract Infection, Multidrug Resistance (MDR), Antibiotic Susceptibility, β-lactam Resistance

1. Introduction

Scientific interest in *Escherichia coli (E.coli)* began in the late 19th century when it was first isolated from the human intestine by the German scientist Theodor Escherich (1885). Initially, it was considered a non-pathogenic bacterium and an indicator of fecal contamination in water and food (Tenaillon, 2020) [24].

With the development of microbiology during the 20th century, it became clear that *E. coli* was not simply a harmless intestinal bacterium, but included strains capable of causing diseases both within and outside the digestive tract. This led to its classification into intestinal pathogenic strains and extraintestinal pathogenic strains (Croxen, 2020) [9].

In the mid-20th century, following the widespread discovery of antibiotics such as penicillin and streptomycin, the treatment of bacterial infections became more effective, and high cure rates for urinary tract infections were recorded. However, the first signs of *E.coli* resistance to some antibiotics began to appear as a result of the intensive and unregulated use of these drugs (Hutchings, 2019) [11]. During the following decades, a clear increase in the ability of *E. coli* to resist several types of antibiotics simultaneously was observed as a result of acquiring resistance genes carried on plasmids and mobile genetic elements, which formed the basis for the emergence of the concept of multidrug resistance (MDR) (Ullah,2012).

With the beginning of the 21st century, antibiotic resistance has become an increasingly global health problem, with multidrug-resistant strains of *E.coli* becoming particularly common in cases of urinary tract infections, both community-acquired and

hospital-acquired. This has necessitated a review of treatment policies and the strengthening of drug surveillance programs (Prestinaci.2021) [22].

In recent years, modern studies have focused on understanding the molecular mechanisms of resistance, such as the production of antibiotic-degrading enzymes and changes in outer membrane permeability, as well as the role of modern technologies in detecting MDR, reflecting a significant advance in understanding and controlling this phenomenon (Bonomo,2021) [3].

1.2. Characteristics of Escherichia coli :

E.coli is widespread in nature and possesses a set of morphological, physiological and genetic characteristics that distinguish it from other bacteria and contribute to its ability to adapt and cause disease in humans (Willey, 2020) [29].

1.2.1. Morphological Characteristics :

E.coli is characterized as a rod-shaped bacterium ranging in length from 1–3 micrometers and is Gram-negative. It has a thin cell wall of peptidoglycan surrounded by an outer membrane containing lipopolysaccharide, which is an important factor in generating the inflammatory response. Most strains of E.coli are non-spore-forming, and some have flagella that give them the ability to move, a characteristic that helps them move within the urinary tract (Brooks,2019) [4].

1.2.2. Physiological Characteristics:

E.coli is a facultative anaerobe, meaning it can grow in the presence or absence of oxygen, giving it a high degree of adaptability to living both inside and outside the human body and it has the ability to ferment lactose producing acid and gas. This characteristic is used in laboratory diagnosis using culture media such as MacConkey agar, where colonies appear pink (Klein,2020) [14].

1.2.3. Genetic Characteristics:

E.coli possesses a single circular DNA chromosome and can carry plasmids containing genes responsible for virulence factors or antibiotic resistance making it one of the most adaptable bacteria for acquiring new traits. Furthermore, E. coli exhibits a high capacity for horizontal gene transfer through conjugation or transformation, contributing to the spread of multidrug resistance traits among different strains (Thomas & Nielsen, 2005) [25].

1.2.4. Pathogenic Characteristics:

Some strains of E. coli possess specific virulence factors, such as cilia and toxins, which help them adhere to cells of the urinary tract and cause inflammation. These characteristics are more pronounced in urinary tract pathogens, making them a leading cause of urinary tract infections (Johnson & Russo,2005) [12].

2. The materials and methods:

2.1. The instruments and the materials used in the experiments:

- Refrigerator
- Incubator
- Laminar air flow hood
- Autoclave
- Sensitive electronic balanced
- petri dishes
- Tubes, beakers, flask
- Loop
- Oven
- UV- transilluminator
- EMB agar
- Macconkey agar
- Muller hinton agar
- Blood base agar
- Urea Agar
- Antibiotics
- Nutrient agar

2.2. Preparation the cultural media:

The variety selective and differential media were prepared for isolating and cultivating E.coli.

2.2.1. Preparation of the prepared media:

The culture media were prepared according to the manufacturer's instructions and their pH was adjusted as needed.

2.2.2. Synthetic Culture Media

Blood Agar : Use sterile blood agar prepared according to the manufacturer's instructions. Add (5%) human blood type AB from a healthy donor in sterile Petri dishes only, under suitable conditions until use (Macfaddin, 2000) [16].

Urea Agar : Use sterile urea agar prepared according to the manufacturer's instructions. Add (5) milliliters of 20% urea solution to the medium after cooling it in a water bath to a temperature of (50) then distribute the medium onto tubes and place them at an angle to form a (Deep slop) it was used to detect the ability of bacteria to produce the urease enzyme (Benson, 2002) [2].

2.3. Sterilization Methods:

Moist Heat Sterilization :The culture media were sterilized by an autoclave at a temperature of (121)°C and a pressure of (15) pounds/inches for (15) minutes.

2.4. Preparation of Solutions and Reagents:

2.4.1. Solutions

Physiological saline solution: Prepared by dissolving (0.85) g of sodium chloride (NaCl) in (90) ml of distilled water, then bringing the volume up to (100) ml, then sterilizing with autoclave for 15 minutes, and storing it at a temperature of (4) °C. This solution was used to dilute the suspension and prepare the direct bacterial inoculum during laboratory experiments (Macfaddin,2000) [16].

2.4.2. Reagents:

Oxidase Reagent: The reagent was prepared for use by dissolving (0.1) g of Tetramethyl-P-Phenyl Diamine Dihydro chloride in (10) ml of distilled water. The reagent was placed in an opaque bottle. This reagent was used to investigate the

ability of bacteria to produce the oxidase enzyme (Forbes,2007) [10].

Catalase reagent: A 3% hydrogen peroxide solution was used, with 3 ml of H_2O_2 added to 100 ml of water, to detect the bacteria's ability to produce the catalase enzyme. (Cappuccino,2020) [6].

2.5. Sample Collection

The study included collecting (30) clinical samples from cases of urinary tract infections of both sexes and different ages, during the period from December 2025 to March 2026, from Marjan Medical Hospital and Al-Jumhouri Hospital. Isolation and laboratory diagnosis of the bacterial isolates were carried out and their characteristics were studied, with a focus on E coli.

2.6. Bacterial Diagnosis of Isolates:

Culture Characteristics :The isolates were initially identified as E.coli based on their morphological characteristics, specifically the shape, size, and color of the colonies.

Microscopic Characteristics :The characteristics were studied using Gram staining. A single, pure colony growing on nutrient agar was taken using a sterile loop carrier and placed on a glass slide with a few drops of sterile water to dilute the cells. The slide was fixed by passing it over a flame and then stained with Gram stain. The shape and aggregation of the cells were observed using an oil-lens light microscope (Forbes, 2007) [10]

2.7. Biochemical Tests

Catalase Test: A portion of a 24-hour pure colony was transferred from its growing medium using sterile wooden sticks to a clean glass slide. Drops of 3% hydrogen peroxide (H_2O_2) solution were then added. The result is positive if bubbles of oxygen gas appear (Cappuccino, 2017) [5].

Oxidase Test :A portion of a 24-hour pure colony was transferred using sterile wooden sticks to filter paper saturated with oxidase reagent. The result is positive if the color turns purple within 10 seconds of the test (Forbes, 2007) [10].

2.8. Sensitivity Testing

Two to four purified colonies of 18–24 hours-old E.coli were collected and mixed in sterile physiological saline to a turbidity equivalent to the McFarland standard of 0.5.The surface of Mueller-Hinton agar was inoculated using a sterile cotton swab, spreading the bacteria in a striped pattern and in multiple directions to ensure a uniform distribution of bacterial cells. The plates were left to stand for 5 minutes at room temperature to allow for moisture absorption. Antibiotic tablets were then placed on the surface of the medium using sterile forceps, ensuring adequate spacing between the tablets.The plates were incubated at 37°C for 18–24 hours. After incubation, the diameters of the zones of inhibition were measured using a graduated ruler. This method relies on the principle of antibiotic diffusion in the culture medium, where it inhibits the growth of susceptible bacteria around the disc, forming a growth-free zone known as the inhibition zone. This zone is used to determine the bacteria's sensitivity to antibiotics. The results were interpreted according to the standard criteria adopted by CLSI (2023).

2.9. Co-Resistance to Other Antibiotic Classes:

The Co-resistance pattern of E.coli isolates was determined based on the results of antibiotic susceptibility testing using the disk diffusion method. After measuring the diameters of the inhibition zones and interpreting the results according to the CLSI,2023 criteria, the resistance of each isolate to the antibiotics used in the study was recorded. Subsequently, the results were analyzed to identify concurrent resistance to more than one class of drugs. An isolate was considered cross-resistant when it exhibited resistance to antibiotics belonging to different drug classes, such as β-lactams, fluoroquinolones, aminoglycosides, and tetracyclines. The results were organized in tables to illustrate the co-resistance patterns and to compare the number of antibiotics to which the isolates showed resistance, in order to determine the prevalence of this phenomenon among the studied E.coli isolates.

3. Results

Cultural characteristics of E.coli: E.coli isolates showed clear growth on the culture media used. On MacConkey agar, the colonies appeared with a distinct pink color due to the bacteria's ability to ferment lactose and produce acids, which leads to a change in the indicator's color. This result is consistent with (Tortora.2021) [26]. While On Eosin Methylene Blue (EMB) agar, the colonies appeared dark with a metallic green sheen, a characteristic appearance of E.coli bacteria resulting from vigorous lactose fermentation and reaction with the medium's pigments. This result is consistent with (Madigan2018) [17].

And also consistent with the study (Cheesbrough.2019) [7], which confirmed that these characteristics are important diagnostic markers in differentiating between E.coli and other intestinal bacteria.

3.1. Distribution of Isolations by Age Group and Sex

Table (1) shows the distribution of patients by age group and sex. The total number of samples was (30), with females outnumbering males.

Table 1: distribution of patients by age group and sex.

Age Group	Female (n)	Male (n)	Total (n)	Percentage (%)
10–19	2	1	3	10.0
20–30	9	3	12	40.0
31–40	5	2	7	23.3
41–50	3	2	5	16.7
Above 51	1	2	3	10.0
Total	20	10	30	100.0

The total number of samples was (30). It was observed that the infection rate was higher in females compared to males, and the highest infection rate was recorded in the 20–30 year age group. The higher infection rate in females is attributed to the anatomical structure of the urinary tract, where the urethra is shorter and closer to the anus, facilitating the transfer of bacteria, especially E.coli into the urinary system. Behavioral and hormonal factors also play a significant role in increasing the likelihood of infection.

Regarding age group, the results showed that the 20–30 year age group was the most susceptible. This can be explained by increased daily activity and exposure to infectious agents during this age range, in addition to certain lifestyle factors. These results are consistent with several recent studies, as the study (Assafi.2022) [1] indicated that the age group (20–39 years) recorded the highest rates of infection among females, and the study (Kumwenda, 2025) [15] showed that the infection is largely concentrated within the age group (15–45 years).

The 2024 study by Mouanga also indicated a higher infection rate among women compared to men, supporting the findings of the current study regarding gender distribution. Furthermore, the 2023 study by Walsh suggested that women aged 15–44 years are the most susceptible to urinary tract infections caused by E.coli which aligns with the results of this study. Therefore, the current findings are consistent with the general trend of recent studies, which confirm that females, particularly younger women, are the most vulnerable group to E. coli infections.

3.2. Biochemical Tests of E.coli

Biochemical tests showed that E.coli isolates were positive for the catalase test and negative for the oxidase test. These results are considered key diagnostic characteristics of this bacterium. The positive catalase test result is attributed to the bacterium's ability to produce the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. This is evident in the form of gas bubbles upon the addition of the reagent. The negative oxidase test result indicates the absence of the cytochrome oxidase enzyme in the bacterium, distinguishing it from some other bacteria. These results are consistent with the known biochemical characteristics of E.coli, which are positive for the catalase test and negative for the oxidase test, thus helping to differentiate it from other Gram-negative bacteria. These results are also consistent with the study by (Cappuccino & Welsh 2020) [6], which confirmed that E.coli exhibits this pattern of reaction in biochemical assays, and this is an important indicator in laboratory diagnosis.

3.3. Antibiotic Sensitivity Test

Carbapenems (imipenem, meropenem) are among the most effective antibiotics against Gram- bacteria due to their high resistance to the β-lactamases produced by these bacteria. These antibiotics are characterized by their ability to penetrate the bacterial cell membrane and bind to penicillin-binding proteins, thereby inhibiting bacterial cell wall synthesis and ultimately eliminating the bacteria. The results of the current study demonstrated a high sensitivity of E.coli isolates to these antibiotics, indicating their effectiveness in treating severe infections, particularly those associated with multidrug-resistant bacteria (MDR). This finding is clinically significant, as carbapenems are often used as a last resort when other antibiotics have failed. These results are consistent with the study by (Papp-Wallace.2020) [21] which demonstrated that carbapenems remain among the most effective antibiotics against Gram- bacteria, despite the global increase in antibiotic resistance.

The results of the current study showed high rates of resistance to certain antibiotics, such as ceftriaxone and trimethoprim. This is attributed to the overuse and unregulated use of these drugs, leading to the development of bacterial strains capable of adapting and surviving despite antibiotic exposure. These findings are consistent with the study by (Murray.2022) [19] which confirmed a significant increase in antibiotic resistance in E.coli bacteria globally, particularly to commonly used antibiotics, due to continuous selective pressure resulting from misuse.

Table 2: The Results of Antibiotics Sensitivity.

Antibiotic	Resistant (R) n (%)	Intermediate (I) n (%)	Sensitive (S) n (%)
Imipenem	4 (13.3)	0 (0.0)	26 (86.7)
Meropenem	5 (16.7)	0 (0.0)	25 (83.3)
Amikacin	4 (13.3)	0 (0.0)	26 (86.7)
Ciprofloxacin	15 (50.0)	0 (0.0)	15 (50.0)
Ceftriaxone	27 (90.0)	0 (0.0)	3 (10.0)
Ceftazidime	16 (53.3)	0 (0.0)	14 (46.7)
Trimethoprim/Sulfamethoxazole (SXT)	28 (93.3)	0 (0.0)	2 (6.7)
Colistin	5 (16.7)	5 (16.7)	20 (66.7)

The high levels of resistance also indicate that the bacteria possess several advanced defense mechanisms, most notably:

1. Production of β-lactamases, which break down and inactivate the antibiotic.
2. The presence of efflux pumps, which reduce the concentration of the antibiotic inside the bacterial cell.
3. Target modification, which reduces the antibiotic's effectiveness. These results were consistent with a study by (Kakoullis.2021) [13] which showed that these mechanisms are among the most important reasons responsible for the development of resistance in Gram-bacteria, including E.coli.

3.4. Co-resistance

Co-resistance has been observed among several drug classes, particularly between β-lactams and fluoroquinolones, indicating that E.coli isolates possess multiple resistance mechanisms simultaneously. This is often attributed to the presence of multiple resistance genes carried on plasmids or mobile genetic elements, which can readily transfer between bacterial cells. This phenomenon is of great importance because it leads to the emergence of bacterial strains capable of resisting more than one type of antibiotic concurrently, complicating treatment and reducing the effectiveness of available therapeutic options. The presence of these genes on plasmids also contributes to the rapid spread of resistance among bacteria in the clinical setting. These findings are consistent with the study by (Partridge,2018) [20] which demonstrated that plasmids and mobile genetic elements play a key role in the transfer of resistance genes between bacteria, leading to the emergence of co-resistance and multi-resistance patterns as(Rodríguez-Baño.2021) [23] also pointed out, the association between β-lactam resistance and fluoroquinolones is common in E. coli isolates causing urinary tract infections.

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