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Research Article



## Phenotypic characterization of *Escherichia coli* bacteria and determination of their sensitivity to antibiotics

Hiba Sahib Sadeq<sup>1</sup> and Mohammed Qasim Waheeb<sup>2</sup>

<sup>1</sup>Department of Medical Laboratories, College of Health and Medical Technology, Sawa University, Samawah, 66001, Al-Muthana, Iraq

<sup>2</sup>Department of Biology, College of Science, Al-Muthanna University, Samawah, 66001, Al-Muthana, Iraq

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(✉) Corresponding Author: [mhmdkas@mu.edu.iq](mailto:mhmdkas@mu.edu.iq)

### Abstract

The primary focus of this research is to analyze the appearance, characteristics, and relationship between antibiotics and *E. coli* bacteria isolated from urinary tract infections (UTIs). Conducted at Al-Muthanna Governorate Hospital and educational laboratories, 100 clinical samples were collected from the urine of UTI patients between March 5, 2024, and June 6, 2024, all under the supervision of a specialist physician. The antibiotic susceptibility test revealed that the *E. coli* bacteria were multi-drug resistant (MDR). All 50 isolates (100%) exhibited antibiotic resistance. Specifically, 47 isolates (94%) were resistant to erythromycin, 49 isolates (98%) to carbenicillin, 44 isolates (88%) to rifampin, 38 isolates (76%) to ceftazidime, 37 isolates (74%) to cefotaxime, 26 isolates (52%) to tetracycline, and 33 isolates (66%) to novobiocin.

**Keywords:** Phenotypic, *Escherichia coli*, sensitivity, antibiotics.

### Introduction

*Escherichia coli* bacteria are a member of the Enterobacteriaceae family, gram-negative, rod-shaped, motile or non-motile, aerobic or facultatively anaerobic, fermenting the sugar lactose, most of which are fermenting the sugar rhamnose and sorbitole, producing the enzyme glucuronidase - the optimum temperature for its growth is (36-37) M (Wanger et al., 2017) oxidase test, negative for catalase test, positive for catalase test), Jawetz et al., 2016, producer of indole and not consuming citrate, positive for methyl red test (Hemraj et al., 2013) Vogase-Proskauer. And negative for the Fox-Proskauer test. They live naturally in the intestines of humans and animals, and at the same time they are opportunistic pathogens that cause many diseases, such as diarrhea, meningitis, sepsis, and bacteremia. They are among the most common bacterial types causing urinary tract infections, as they cause about (90%) of urinary tract infections worldwide, and they are most common in childhood (Hadi et al., 2014).

The pathogenicity of this bacteria is due to the possession of many virulence factors, and among these factors is the possession of colicin, cytotoxic necrotizing factor, and iron-chelating siderophores. They possess surface structures such as flagella, capsules, and lipopolysaccharides (LPS), which give bacteria antigenic properties by producing somatic antigen (O) and flagellar antigen (capsular antigen K). It also possesses cilia (fimbriae) or (pili) that help it adhere to host tissues, giving it the ability to form biofilm (Terlizz et al., 2017; Zowawi et al., 2015) Due to their possession of multidrug-resistant (MDR) enzymes like beta-lactamases, which confer resistance to beta-lactams, and enzymes that confer resistance to aminoglycosides, *E. Coli* bacteria are known for their high level of antibiotic resistance. In addition, these bacteria possess efflux pumps, alter the target site, modify the permeability of the cell membrane, and prevent protein production, among other defense mechanisms that make them resistant to antibiotics. The efflux pumps in these bacteria are classified into five types, and they are rendered

resistant to antibiotics, including classes of macrolides. Antibiotics, rifamicin, and novobiocin (Kapoor et al., 2017). Toxic Efflux Family (MATE) Resistance and Multidrug Resistance Characterize Small Multidrug Family Major Facilitator Super Family (MFS) Families.

The study aimed to

1. Isolation and identification of *Escherichia coli* germs from urinary tract infection patients' urine
2. *Escherichia coli* under the microscope.
3. Make sure you test for sensitivity to several antibiotic classes.

## Materials and Methods

### Methods

#### Sterilization

Brown and Smith, (2017) The culture media and solutions were sterilized using two methods according to My Agencies.

#### Heat sterilization

All ready-made and synthetic culture media and most of the solutions used in this study, which are not affected by heat, were sterilized in an autoclave at a temperature of 121°C and under a pressure of 15 pounds per ang for 15 minutes. As for the glassware, they were sterilized in an electric oven at a temperature of 180°C for two hours

#### Sterilization by filtration

Some of the solutions whose nature is affected by heat, such as sugars, urea, and some dyes, were sterilized using millipore filters with a diameter of 0.22 micrometers.

#### Reparation of culture media

#### Preparation of ready-made cutler media preparation

I have prepared ready-made growing media, which are chromagar Orientation Medium, Eosine Methyiene Blue Medium, Eosine Methyiene Blue Medium, MacConkey Agar, Methyl Red-Voges Proskauer Agar, Simmon's Citrate Agar Medium, and Peptone Water Medium. Nutrient Agar Medium, Brain and Heart Broth, Tryptone Agar Medium, Nutrient Agar, according to the instructions (Müller-Hinton Agar) and Tryptone Soyagar, the manufacturer of which is indicated on the packaging of each medium. The autoclave was sterilized at a temperature of 121°C and a pressure of 15 psi for 15 minutes, then the culture medium was poured into sterile Petri dishes and tubes and incubated at 37° for 24 hours to ensure that it was not contaminated, then it was stored in the refrigerator at a temperature of 4° Until use.

#### Laboratory culture media composition

#### Urea agar medium

This medium was prepared according to the manufacturer's instructions by dissolving 24 grams of urea agar base medium in 950 milliliters of sterile distilled water, then sterilizing the medium in an autoclave, then leaving it to cool to a temperatur50 m, then add 50 milliliters of 40% urea solution (prepared by dissolving 40 grams of urea in 100 milliliters of distilled water and then sterilize using Millipore filter units with a diameter of 0.22 microliters), then pour the medium into sterile tubes and store at 4 until use. Use this the middle To detect the ability of bacteria to produce the urea enzyme (Tille, 2017).

#### Hekton enteric agar medium

This medium was prepared after sterilizing distilled water in an autoclave, then cooled to a temperature of 50°C, after which it was added. Dilute the medium under sterile conditions, mix well, then pour into sterile Petri dishes and leave to solidify. use This medium is used to differentiate between *E.coli* bacteria and the rest of the species of the Enterobacteriaceae family (Brown and Smith, 2017).

#### Blood agar medium:

Prepare blood agar base medium according to the manufacturer's instructions, sterilize it in an incubator, then cool it to a temperature of 50°C. Then blood was added to it at a rate of 5%, mixed gently, then poured into sterile Petri dishes and left to solidify. This medium was used to investigate the production of the enzyme hemolysin before the isolates under study (Forbes et al., 2007).

#### Bacterial isolates

#### Bacterial sample collection

100 urine samples were collected from patients suffering from symptoms of urinary tract infections, under the supervision of a specialist doctor, from Al-Hussein Teaching Hospital in Al-Muthanna Governorate, and educational laboratories. For the period from 5/3/2024 until 6/6/2024 Containers were used to collect the mid-urine sample in the early morning for all patients. The samples were then cultured by taking 100 microliters of urine, cultured in several differentiation media, and then incubated at a temperature of 37 degrees Celsius for a period of time. 24 hours. The samples were then centrifuged in a centrifuge at 200 rpm for 5 minutes, then the sediment was mixed with a mixer for half a minute, after which a drop of sediment was placed on the glass slide and examined under an optical microscope using a centrifuge. Magnification 40 after 10 or more white blood cells are present. In the microscopic field, the presence of between 50-200 pure colonies growing in the dish is considered a positive result.

### Cultural identification

The phenotypic characteristics of the isolated colonies were studied after cultivation and purification of the bacterial isolates on different culture media: Macconkey agar medium, eosin-methylene blue medium, intestinal hecnone agar medium, blood agar medium, and chromium orientation agar medium. The study included shape, size, texture, color, rims, and heights (Wanger et al., 2017) for isolated bacterial colonies

### Microscopic identification

Smears were prepared from bacterial isolates that were grown on MacConkey agar medium, aged 18-24 hours, using Gram technology, and then examined under an optical microscope to see the shape, arrangement, and colors of the cells. According to its interaction with Gram stain (Levinson, 2016).

### Biochemical identification

#### Catalase test

A 24-hour-old colony growing on agar medium was transferred onto a sterile, dry glass slide using sterile wooden sticks, then one drop of catalase reagent at a concentration of 3% was added to it. The appearance of bubbles on the surface of the glass slide is evidence of the production of the catalase enzyme (Hemraj et al., 2013).

#### Oxidase test

A 24-hour-old growing colony was transferred to a filter paper using sterilized wooden sticks, then a drop of the oxidase reagent prepared in advance was added according to paragraph (1.4.2.3). The color change to purple in 30-60 seconds is evidence of the production of the oxidase enzyme (Hemraj et al., 2013).

#### Antibiotic resistance test

The sensitivity of *E.coli* bacteria to antibiotics was tested using the Kirby-Baure method as stated Agencies: Vandepitte et al. (2003).

1 - (3-5) colonies cultured on Macaroni media aged for 24 hours were moved to Five milliliters of regular saline in a tube was confiscated. Turbidity of the solution at 0.5 Coverland solution, which is equivalent to  $(1.5 \times 10)$  cells/ml and was prepared beforehand in accordance with paragraph (1.3.2.3). The solution's turbidity was compared to that of the Cover land solution, which was earlier made in compliance with paragraph (1.3.2.3). This is the same as  $1.5 \times 10^9$  cells/ml.

2- To eliminate extra inoculum, the sterile cotton swab was placed into the tube holding the bacterial suspension, turned, and pressed up against the tube's inner wall. Subsequently, it

was repeatedly run across dishes with Acar Mueller-Hinton media in various directions to ensure homogenous development.

3. Equal intervals between antibiotic tablets were placed on the culture medium's surface. After using sterile forceps to gently press the tablets, the plates were incubated at room temperature.37 meters in a day.

4- The results of the diameters of the inhibition zones around the discs were recorded in millimeters using a ruler, then the results were compared to standard tables (CLSI 2017).

### Statistical Analysis:

The collected data was statistically analyzed using SPSS with significance values of P 0.05 statistical package for (Social Sciences) is a ready-made program for analyzing data using statistical methods. This program is considered one of the most important programs used, as it is used to analyze all types of statistical data from various types of sciences (White et al., 1990).

### Results and discussion

#### Bacterial isolation

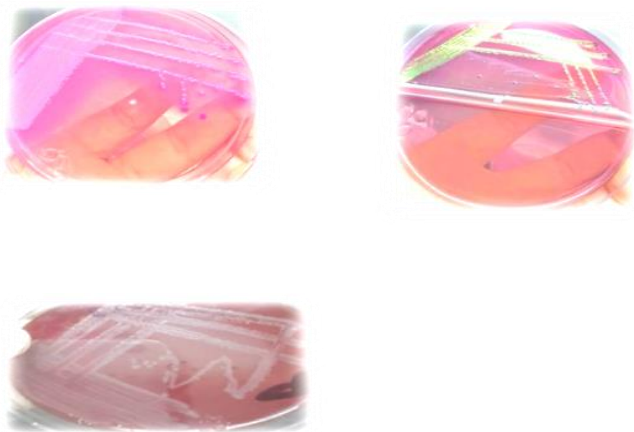
100 urine samples were collected from patients with urinary tract infections from Al-Hussein Teaching Hospital and educational laboratories. At the Medical City for the period from 5-3-2024 to 6-6-2024, and after conducting phenotypic, microscopic, biochemical and molecular tests, 50 isolates belonging to the *E.coli* bacteria were obtained.

#### Morphological identification

*E.coli* bacterial isolates were initially identified based on their phenotypic characteristics, after growing them on MacConkey agar medium, Eosin Methylene Chromagar medium, Chromagen blood agar medium, Blue agar (EMB) Orientation medium, and Hekton intestinal agar medium. Enteric agar: The results showed that the bacteria were fermenting the sugar lactose, and produced smooth, shiny, pink colonies with sharp edges on the differential agar medium, which contains bile salts and crystal violet dye, which allows the growth of gram-negative bacteria, including the enteric agar family, and inhibits the growth of gram-positive bacteria. The isolates are shiny, metallic green colonies on the blue eosin-methylene agar medium. This characteristic is one of the distinguishing characteristics of the *E.coli* bacteria from other members of the Enterobacteriaceae family, as a result of the medium containing the dyes eosin and methylene. The blue color that precipitates in the acidic environment after bonding together gives a metallic green sheen, which indicates that the bacteria produced organic acids as a result of fermenting the sugars lactose and sucrose. As for the chromagen agar medium, the bacteria produce B-glucuronidases enzymes that work to decompose the conjugated chromogen and release the

glucuronidchromagenic substrate, or what is called the chromagenic conjugate, the chromophore, thus giving the pink color to the bacterial colonies. The colonies that were grown on hemolytic medium were mostly non-hemolytic, except for three isolates (6%) that were completely hemolytic, beta B-hemolysis, which indicates the production of the enzyme hemolysin. When bacterial colonies were grown on intestinal hectone agar medium, it was found that they showed yellow colonies. It turns orange in color as a result of it being bacteria that ferment the sugar lactose, as this medium is one of the differentiation media that Differentiate between gram-negative bacteria that ferment lactose and non-lactose fermenters, and also differentiate between [Wanger et al., 2017](#).

This is consistent with what was reported in both *Shigella* and *Salmonella* bacteria. The results reached by researcher [Bhattacharyya et al. \(2015\)](#) in India showed that the percentage of *E.coli* isolates isolated from hemolytic urinary tract infections was (10%). He also indicated that the bacteria were heterogeneous in producing the enzyme hemolysin, so they were Weak virulence. The study conducted by researcher [Sharmin et al. \(2010\)](#) showed that most of the bacterial isolates showed colonies Pink on the chromagen agar medium and small white colonies with sharp edges on the blood agar medium, while (72%) of them showed pink colonies on the medium of the Macconkey agar.



**Fig. (3-1) The phenotypic appearance of *E.coli* bacteria colonies on several diagnostic media: (1) pink colonies on MacConkey agar 2 Bright metallic green colonies on Eosin Methylene Blue agar - Milky white, non-hemolytic colonies on Blood agar medium**

### Microscopic identification

Staining was performed with Gram's stain by preparing a smear of the colony growing on Macan agar medium, aged

(18-24) hours. The bacteria cells, *Bacillus brevis*, appeared to be Gram-negative and non-spore forming. These results are consistent with what was stated in [Levinson, 2016](#).

### Biochemical identification

Biochemical tests were performed on all isolates, and they were all positive for the catalase test, as the *E.coli* bacteria had decomposed the hydrogen peroxide reagent into water and oxygen. The bacteria were negative for the oxidase test because the color of the colonies did not turn purple when the reagent was added, as the bacteria do not possess the enzyme cytochrom oxidase as a hydrogen acceptor, and negative for the urease test because the color of the medium did not change, which confirms that Bacteria do not consume urea because they do not possess the enzyme urease.

### Antimicrobial resistance of *E. coli* bacteria

The sensitivity of the bacterial isolates was tested to ten different antibiotics. The bacteria varied in their resistance to these tested antibiotics, as all the bacterial isolates were multi-drug resistance (MDR). The results showed that 50 (100%) bacterial isolates were resistant to the antibiotic carbenicillin, 49 (98%) isolates were resistant to the antibiotic erythromycin, 47 (94%) isolates were resistant to the antibiotic 4 rifampin, 444 (88%) isolates were resistant to the antibiotic ceftazidime, and 38 (76%) isolates were resistant to the antibiotic. Bio cefotaxime 37 (74%) isolates resistant to the antibiotic noobiocin 33 (66%) isolates resistant to the antibiotic 15 (30%) isolates ciprofloxacin 26 (52%) isolates resistant to the antibiotic tetracycline Bio resistant to the antibiotic gentamicin and two (4%) isolates resistant to the antibiotic nitrofurantoin, The results of this study regarding the antibiotic carbenicillin are consistent with previous studies, including the results [Ibrahim et al. \(2014\)](#).

In which the percentage of bacterial isolates reached Resistance to the antibiotic carbenicillin (100), as this study showed that many enzymes Beta-lactamases are controlled by genes carried on the plasmid Gram-negative bacteria can degrade the antibiotics carbenicillin, ampicillin, cephalothin, and other antibiotics belonging to the same group. The results of the current study are also consistent with the results reached by the researcher [Hinthong et al. \(2017\)](#), in which the percentage of resistant bacterial isolates was for this antibiotic (92.2%). Growing it on Acar Müller-Hinton medium While the results of this study did not agree with the results reached by researchers [Kafilzadeh and Farsimadan, \(2016\)](#).

the percentage of resistance of bacterial isolates to this antibiotic was (42.8%), and they indicated that the bacteria Many mechanisms have been used in resistance to this antibiotic, and they also indicated that the causes of high resistance are more Antibiotics are due to excessive use of antibiotics, mutations that encode enzymes as well Plasmid-mediated transmission of resistance. The results of the current study regarding third-generation antibiotics from the group of



cephalosporins, which include the antibiotics ceftazidime and cefotaxime, are consistent with the study conducted by researcher Harran, (2012), who found that *E.coli* bacteria were highly resistant to antibiotics belonging to the group of beta-lactams, as they were The rate of bacterial resistance to these two antibiotics was (78.6%) and (76.6%), respectively. It also agrees with the study concluded by researcher Sures et al. (2016) in India, in which the percentage of bacterial isolates resistant to these two antibiotics reached (98%) and (100%), respectively, and they indicated that the main reason for this high resistance of bacteria is their possession of pumps. An efficient flow of antibiotics out of the cell and removing their harmful effect on the cell, and this study came close to the findings of the researcher Hegazy et al. (2018) in Egypt, whose study showed that the percentage of *E. coli* bacteria isolates resistant to the antibiotic (2018) Hegazy et al 74.4%), while the results of this study did not agree with the researcher's study (cefotaxime) regarding the antibiotic ceftazidime, as the rate of bacterial resistance to it reached (64.3%).The results of the current study differed from the results of the study concluded by the researcher Lee et al. (2015) in North Korea, the study concluded by the researchers Kafilzadeh and Farsimadan, (2016) and the study concluded by the researcher Maleki et al. (2017) in Iran, as the percentage Bacterial resistance (6.8%), (38.9%), and (26.1%) to the antibiotic ceftazidime, respectively, is (15.5%) and (42.2%).The results of the current study regarding the antibiotic ciprofloxacin, which belongs to the quinolones group, agree with the results reached by the researcher Tajbakhsh et al. (2016) in Iran, as the rate of bacterial resistance to this antibiotic was (56.25%). It was shown that the rate of resistance to antibiotics includes this. The antibiotic resistance is higher in bacterial isolates that produce biofilm than in bacterial isolates that do not form it, and it also agrees with the results reached by researcher (47.3%) (Rameriz, 2018) in Mexico, where the rate of bacterial resistance to this antibiotic was It has been shown that the high resistance to ciprofloxacin is due to the fact that this group of antibiotics was the first choice for treating urinary tract infections in Mexico.Using it excessively.This study also agrees with the results of the study concluded by researcher Malekzadegan et al. (2018) in Iran, which showed that a percentage (55.6%) of bacterial isolates were resistant to the antibiotic ciprofloxacin, in addition to showing that the high percentage of resistance is higher in bacterial isolates. This antibiotic is the most widely used antibiotic from the group of quinolones in the treatment of urinary tract infections, and its excessive use has led to a significant increase in the rate of resistance over the years. The latter is also consistent with the results of the study conducted by researcher Abdu et al (2018) in Nigeria, where the rate of bacterial resistance to this antibiotic was (46), and it was shown that the bacteria were multi-resistant to antibiotics. While the results of the current study did not agree with the results reached by researcher Asadi (2014), as the rate of bacterial resistance to this antibiotic was (21.7%).One of the reasons for the resistance of *E.coli* bacteria to antibiotics belonging to the quinolones group is a change in the target site, a decrease in the permeability of the outer membrane of

the bacteria, its possession of efflux systems, and the inhibition of the synthesis of AcrAB-TOIC, MdfAYhi (Paltansing, 2015). By inhibiting the enzyme DNA gyrase or by causing genetic mutations in it (Zaman et al., 2017).

### Conflict of Interest

The author hereby declares no conflict of interest.

### Consent for publication

The author declares that the work has consent for publication.

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