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Isolation and Diagnoses *Escherichia coli* from Patients at Hospital in Baghdad

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Abstract
The study included collecting (100) pathological bacterial samples from patients with urinary tract infections, bacteremia, and others from Yarmouk Teaching Hospital and Medical City hospitals. Escherichia coli isolates were diagnosed using morphological, microscopic, and biochemical methods. Tests of locally isolated bacterial samples proved that all isolates used in laboratory experiments were Escherichia coli. The sensitivity and resistance of the identified bacterial isolates to 10 different antibiotics were tested using the disc diffusion method. The results showed that all bacterial isolates were sensitive to the antibiotic Ciprofloxacin, which gave the highest rate of inhibition diameter for E. coli compared to other bacterial isolates through sensitivity testing. A significant increase in the diameter of inhibition was observed for the gold nanoparticles concentration (4.0 mg/ml), which recorded the highest average diameter of inhibition (11.6 mg/ml) compared to the other concentrations of the mixed extract, the aqueous extract, distilled water, and the standard antibiotic. The results of the experiment with three replicates also showed that all isolates had the ability to form biofilms, but to varying degrees under the same experimental conditions.

Keywords: Bacteria; Antibiotics; Pathogen; Biochemical tests

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1. Introduction

The identity of E. coli bacteria was first proven by Theodore Escherich in 1885 through his analysis and examination of the stool of newborn babies, as the results reached were proven again by the scientist Adam in 1933 through analysis where he found that strains of "dyspepsiekoli" can be infective in newborn babies [1]. E. coli belongs to the Enterobacteriaceae family, which includes a large group of different bacterial species that naturally live in the digestive tract of humans and animals (Meng et al., 2012). This type of bacteria is one of the most common types in humans, causing diseases such as diarrhea, meningitis, and bacteremia. It is also one of the most common bacterial species that infect the urinary tract [2]. *E.coli* is one of the types of pathogenic bacteria that inhabit the intestinal tract of humans and animals and cause diseases. It is a Gram-negative, aerobic or facultative anaerobic rod-shaped bacterium, Motile by flagella or non-motile and non-spore producing. Its colonies are smooth, soft, and slightly convex in front. They are characterized by having a mucous structure when forming a capsule. They are also characterized by their shiny pink color when placed on MacConkey agar and metallic green when placed on Eosin Methylene Blue (EMB) agar. They also give a shiny pink color when placed on Cromagen Orientation agar [3]. The percentage of species that ferment rhamnose exceeds 80%, while the percentage of species that ferment sorbitol exceeds 90%. They do not ferment cellulobiose, and they do not produce hydrogen sulfide gas (H_2S) in the triple iron sugar agar medium, and they do not have the ability to decompose gelatin. In addition, most of them produce glucoronidase-β (GUD) [4]. On the other hand, with regard to the conditions suitable for growth, it was found that this species cannot grow in the presence of potassium cyanide (KCN), but it grows well in a moist medium with a wide pH range (4.49-7.25) and a temperature between (36-37°) [5]. *E. coli* are characterized by having a wide range of factors that increase their ability to resist antibiotics, which gives them the ability to invade the organism and infect it with diseases, the most important of which are urinary tract infections and blood poisoning [6]. One of the most prominent pathological factors that distinguishes this type of bacteria is its ability to lyse red blood cells. This is due to its possession of the cytotoxic necrotizing factor, in addition to the hemolytic enzyme hemolysin, which comes in three types according to the shape of the lysed cell (alpha, beta, gamma). The first type, α-hemolysin, gives the bacteria the ability to partially lyse human red blood cells, while the second type, β-hemolysin, causes complete lysis of red blood cells. The breakdown of blood cells in other organisms occurs by the third type

called hemolysin-γ [7]. What also increases the epidemic of bacteria is that they possess internal surface structures such as endotoxins, which consist of lipopolysaccharide (LPS), which is characterized by containing the somatic antigen (O), which enables the bacteria to take over the host cells, especially the cells of the inner lining of the bladder, and also enables them to overcome the host's immune system [8]. This bacteria have a support system even from members of their own genera, including the bactericide Bacteriocin, which is called Colicin, which kills other bacterial genera and even protects bacteria. In addition, they have Cyclomodulins toxins, which are considered to be DNA-inhibiting fighters in other bacterial genera, which partially protects the genus. Bacteria are also characterized by their possession of a number of toxins, such as enteroaggregative heat-stable toxin, heat-labile enterotoxin, and plasmid-encoded toxin, which are toxic to the intestinal cells and red blood cells of the host [9]. In addition to the ability of bacteria to grow in an environment containing a small amount of iron (the essential element for growth), this is due to their possession of iron chelators (siderophores) that help them compensate for the deficiency of iron and thus increase their colonization in the host tissues, which makes their study very important [10]. So, the aim of this study is that isolation and identification of E. coli bacteria and testing for antibiotic resistance.

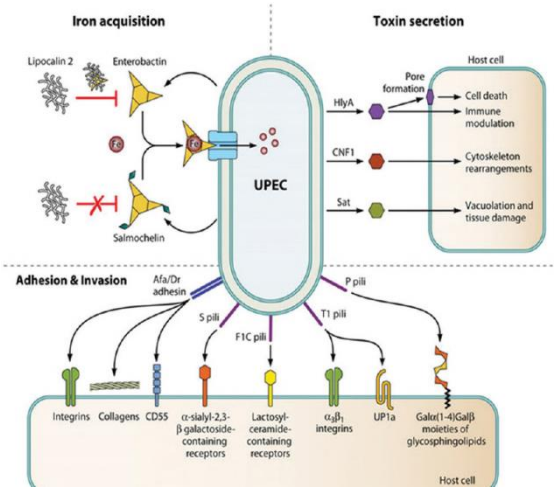

Fig. (1) Virulence factors in E. coli [11]

2. Preparation of Solutions and Dyes

Crystal violet dye solution (0.2%) is used as a component of the Gram stain for the initial diagnosis of bacterial isolates, as well as to detect the ability of bacteria to form biofilms. It was used for staining by dissolving 0.2 g of crystal violet in 100 ml of distilled water and stirring well until dissolved. The solution was then filtered through filter paper and placed in a sealed tube [12].

Resazurin Blue Dye Solution

A 0.015% resazurin dye was prepared by dissolving 0.015 g of the dye powder, mixing it with an electric mixer, sterilizing it by filtration (0.22 lm filter), and storing it at 4° [12].

Physiological saline solution (normal saline)

Prepared by dissolving 8.5 g of sodium chloride (NaCl) in distilled water. Bring the volume to 1 liter, then sterilize in an autoclave and store at 4°C until use. It is used to dilute and prepare bacterial suspensions [13].

Macfarland Standard Solution 0.5%

The standard turbidity constant solution was prepared by dissolving 1.175 g of barium chloride in 100 ml of distilled water (solution A), then adding 1 ml of concentrated sulfuric acid to 100 ml of distilled water (solution B). 0.5 ml of solution A was taken and added to 99.5 ml of solution B. The solution was then placed in glass tubes with tight caps to prevent evaporation. Stored in the dark until use at room temperature [14].

Ready-made culture media

The culture media were prepared according to the manufacturer's instructions on the packages, then sterilized in an autoclave at 121°C under a pressure of 15 psi for 15 minutes. After pouring, the media were incubated at 37°C for 24 hours to ensure they were not contaminated. They were then refrigerated at 4°C until use. The media included, Brain Heart infusion broth, Brain Heart infusion agar, Nutrient broth, Nutrient agar, Muller Hinton agar, MacConkey agar.

Collection of bacterial isolates

Approximately 100 clinical urine samples were collected from patients with urinary tract infections, sepsis, and diarrhea of various ages and genders from Al-Yarmouk and Al-Karamah Teaching Hospitals, the Central Children's Hospital in Baghdad, and the Medical City Hospital during the months of October and November, and were examined.

Diagnosis of bacterial samples

Fifty isolates were initially diagnosed as Escherichia coli, based on morphological and anatomical characteristics, through microscopic, diagnostic, and biochemical examinations.

Microscopic Examination

Bacterial isolates were subjected to microscopic examination by taking a small smear from the colony, transferring it to a microscopic slide, staining it with Gram stain, and examining it under an oil-based lens to distinguish the shape of the cells, their arrangement and arrangement, and their positivity or negativity to the stain [15].

Cultural characteristics

Colonies were initially characterized based on their cultural characteristics, including colony shape, color, texture, odor, and size, on MacConkey agar and blood agar media.

Biochemical Tests

The following biochemical tests are approved for initial diagnosis [16].

3. Results and Discussion

Isolates of *E. coli*

The isolation results of the samples under study showed that 50 *E. coli* isolates were obtained through diagnostic tests, as shown in table (1).

Table (1) Diagnostic tests of *E. coli* isolates

Test	Result
Growth on MacConkey agar	Growth was pink colonies fermenting lactose
Growth on EMB agar	Growth was Green metallic sheen
Growth on Blood agar	It shows a variable ability to produce the enzyme Hemolysin
Growth on Chrom agar orientation	Growth was small pink colonies
Growth on Hakton Enteric agar	Growth was yellow to orange colonies fermenting lactose
Gram stain test	-
Oxidase	-
Catalase	+
Urease	-
Indole	+
Methyl red	+
Citrate	-
Voges-proskauer	-

Morphological identification

The results showed that *E. coli* bacteria were lactose fermenters, forming smooth, shiny, pink colonies with sharp edges on McConnell's differential agar medium containing bile salts and crystal violet (which allows the growth of Gram-negative bacteria and inhibits Gram-positive bacteria), including the Enterobacteriaceae family (Fig. 2). While the metallic isolates appeared as healthy, shiny colonies on a medium distinguished by eosin blue monoculture agar, this characteristic of heat has distinguished these bacteria from other members of the Enterobacteriaceae family, a name given to the medium containing the dyes eosin and methylene blue, which, when linked to each other, precipitate in the acidic medium, giving it a bright metallic green color. It was clear that they are susceptible to different acidic conditions, except for the fermentation of lactose sugar.

When grown on chromagen agar medium, bacteria produce enzymes called β-glucuronidases, which have the ability to analyze the chromagenic conjugate, or what is called β-glucuronidases, which release the chromaphore, which gives the colony a pink color. On the other hand, the results showed that the colonies that were grown on blood agar medium were mostly non-hemolytic, with the exception of five isolates (10%) that were hemolytic, as they completely lyse blood of the beta hemolysim type - β. When the bacterial colonies were grown on the intestinal hectone agar medium, it was found that they were yellow colonies tending to orange in colour as a result of them being lactose fermenting bacteria. This medium is one of the differential mediums that distinguish between Gram-negative bacteria that ferment lactose and those that do not [17].

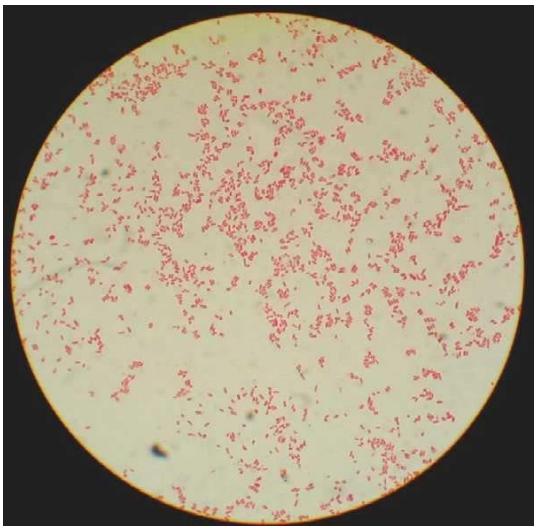

Fig. (2) Microscopic identification of *E. coli*

The results obtained from the 50 diagnosed isolates (table 2) showed that the monthly rate of *E. coli* bacteria among females was 68% and among males 32%, which indicates that females are more susceptible to infection with this type of bacteria. As for the half of the age groups, the largest percentage (65%) was for juveniles, while it did not decrease to 35% among those who improved (18 years old). The current study results similar to those of another study, which showed that the incidence of urinary tract infections among females was 62% and among males 38%, with the incidence rate concentrated in the age group under 17 years. The reason for the higher incidence rate among females was attributed to the short urethra and the close proximity of the anus and vagina [18].

Table (2) Distribution of *E. coli* by gender and age

Sample	Rate %			
	Gender		Age	
	Male	Female	<18	>18
Urine of patients	%32	%68	%65	%35
No. of samples	20	30	25	25

E. coli is the main type of bacteria among the Enterobacteriaceae family that invades the digestive system in early childhood and coexists naturally in the intestines of infected individuals. However, some members have the potential to cause disease in the host if the conditions are suitable for their growth. These bacteria are characterized by their resistance to many antibiotics due to their possession of resistance enzymes, in addition to their possession of other mechanisms such as changing the permeability of the

cell membrane, changing the target site, inhibiting protein synthesis, and others [19].

4. Conclusion

The study included collecting 100 pathological bacterial samples from patients with urinary tract infections, bacteremia, and others from Yarmouk Teaching Hospital and Medical City hospitals. Escherichia coli isolates were diagnosed using morphological, microscopic, and biochemical methods. Tests of locally isolated bacterial samples proved that all isolates used in laboratory experiments were Escherichia coli. The sensitivity and resistance of the identified bacterial isolates to 10 different antibiotics were tested using the disc diffusion method. The results showed that all bacterial isolates were sensitive to the antibiotic Ciprofloxacin, which gave the highest rate of inhibition diameter for E. coli compared to other bacterial isolates through sensitivity testing. A significant increase in the diameter of inhibition was observed for the gold nanoparticles concentration 3.0 mg/ml, which recorded the highest average diameter of inhibition 11.6 mg/ml compared to the other concentrations of the mixed extract, the aqueous extract, distilled water, and the standard antibiotic. The results of the experiment with three replicates also showed that all isolates had the ability to form biofilms, but to varying degrees under the same experimental conditions.

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