Prevalence and Risk Factors of Urinary Tract Infections Caused by Multidrug-Resistant Enterobacteriaceae in Nasiriyah, Iraq

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Abstract

Background: Any area of the urinary system—the kidneys, ureters, bladder, or urethra—is susceptible to infection when a UTI occurs. The urethra and bladder are the most common sites of infection in the lower urinary tract. When microorganisms including bacteria, viruses, fungi, and parasites evolve to resist antibiotics, a phenomenon known as antimicrobial resistance (AMR) happens. This makes infections more difficult to cure and raises the likelihood of disease transmission, serious illness, and death. The researchers in this study set out to quantify the prevalence of urinary tract infections (UTIs) in Iraqi residents and to determine which bacterial strains were resistant to antibiotics.

Methods: The study included 100 patients who were classified into 75 that were diagnosed with UTI patients, their ages ranged between (16) to (67) years, (45) females and (30) males. In addition to 25 uninfected individuals as controls, their ages ranged between (15) to (59) years, (15) females and (10) males. All participants in the study underwent identical laboratory tests to diagnose UTIs, identify the types of bacterial isolates, and determine their antibiotic resistance patterns

Results: The result of the present study shows that *E.coli, Klebsiella pneumonia* and *pseudomonas aeruginosa* were the most common types of UTI-associated bacteria with the percentage of 44.71%, 38.57% and 15.71% respectively.

Conclusion: pseudomonas aeruginosa had been developed its resistance to several antibiotics and had great predisposition to be MDR.

Keywords: Urinary tract infections; Escherichea coli; Klebsiella pneumonia; Pseudomonas aeruginosa; drug resistance.

Introduction: A urinary tract infection (UTI) is an infection that effect in any part of the urinary system like kidneys, ureters, and most infections involve the lower urinary tract, the

bladder and the urethra (1). UTIs are some of the most common bacterial infections, affecting 150 million people each year worldwide (2). Enterobacteriaceae are the most common cause of urinary tract infections in both community and healthcare settings (3) The aim of the study was to detect UTI infection among Iraqi population and to estimate antibiotic resistance of the bacterial isolates.

The location of infection in the urinary system determines the unique symptoms that may accompany each kind of UTI (4).

Part Of Urinary Tract Affected	Signs And Symptoms
Kidneys (Acute Pyelonephritis)	Back Pain Or Side (Flank) Pain High Fever Shaking And Chills Nausea Vomiting
Bladder (Cystitis)	Pelvic Pressure Lower Abdomen Discomfort Frequent, Painful UrinationBlood In Urine
Urethra (Urethritis)	Burning With Urination Discharge

Most occurrences of urinary tract infections are caused by bacteria. These infections usually start in the bladder after the germs enter the urinary tract through the urethra (5). There are instances when the urinary system's defenses, which are meant to ward off such tiny intruders, aren't enough. Bacteria may then colonize the urinary tract and cause an infection if that occurs. The urethra and bladder are the most prevalent sites of infection in women. (6).

Only in extremely rare cases can viruses like enterovirus, coxsackievirus, adenovirus, or echovirus cause a urinary tract infection (7). Although pyelonephritis can be caused by fungi, specifically Candida spp., Aspergillus spp., and Cryptococcus neoformans, this infection is extremely rare and typically affects children who have a history of urinary tract medical implants, a severe immune deficiency, or are on long-term antibiotic treatment (8).

Given that they may account for as much as 80% of clinically relevant Gram-negative bacteria, the Enterobacteriaceae family is quantitatively highly important to medical microbiologists (9).

For example, swarming for Proteus spp. and the so-called mucoid capsule for Klebsiella-Enterobacter spp. are two examples of how certain species display themselves on solid media that could lead to identification (10).

The short, straight rods that do not produce spores and are Gramme negative are members of the family Enterobacteriaceae. The motility of several genera is facilitated by peritrichous flagella, with the exception of the non-motile species of Shigella and Klebsiella. Although some species do better around 25-30°C, the majority of these plants thrive at 37°C and are facultatively anaerobic. They thrive in environments with peptone and meat extract. Different members of the family Enterobacteriaceae have different catalase reactions and are oxidase negative. A small number of Erwinia strains are capable of reducing nitrates to nitrites. Many different things, including soil, water, plants, people, and animals, can harbor them (11). Curiously, multiple investigations have shown that although Escherichia coli is the most prevalent bacteria that causes bladder cystitis, it is more commonly found in females than in males (12), (13).

All five species are recognized to be harmful to humans. Their cells can develop in both aerobic and anaerobic environments; they are rod-shaped, do not produce spores, and can be motile with peritrichous flagella or not. At 37°C, growth is at its peak. Colonies on MacConkey agar are pink in color. They test positive for catalase but negative for oxidase. A decrease in nitrates is also noted (11). Escherichia coli is the most often seen type; it has many serotypes, some of which cause particular illnesses. Enterotoxins and other virulence factors, including invasiveness-associated ones, can be produced by some E. coli strains. An antigen known as K encapsulates certain strains (14).

Six species and three subspecies make up the genus Klebsiella. The four species of Klebsiella that are connected to humans are: oxytoca, ozaenae, pneumoniae, and subspecies pneumoniae. There are many serogroups that result from the genus's more than seventy-seven capsular antigens (K antigens). The colonies get their distinctive mucoid look from these mature polysaccharide capsules. The Enterobacteriaceae family includes the non-motile, rod-shaped, mostly encapsulated Klebsiella species (15). Indole and urease assays, in addition to the Voges-Proskauer test, typically yield positive results for these bacteria. They may sometimes function without oxygen. On regular media, all strains grow easily. When cultured on MacConkey agar, massive, mucoid colonies that diffuse red color into the surrounding agar suggest acid generation and lactose fermentation (11).

This bacterium is a member of the Enterobacteriaceae family, which is defined by a set of shared traits. Every every strain is mobile. On blood agar, they might form a film or concentric zones if they swarm. Colonies of Proteus vulgaris and Proteus mirabilis grown on MacConkey agar will be colorless, flat, slightly swarmy, and 2-3 mm in diameter. Some animals do not swarm at all. The ability of certain Proteus species to ferment lactose has been demonstrated in a triple sugar iron (TSI) test, however this ability varies from species to species. Additionally, they do not

produce oxidase, but they do produce catalase and nitrate. One of the most important ways to tell Proteus from Salmonella is via the urease test (11).

Two of the fifteen species in the genus Serratia—Serratia liquefaciens and Serratia marcescens—are frequently found in clinical samples. Serratia marcescens is known to produce a pigment called prodigiosin, which can be anywhere from dark red to pale pink depending on the age of the colonies grown at 20°C. Species type and incubation period are two of several variables that affect pigment production, which varies greatly among them. Similar to other Enterobacteriaceae species, non-pigmented colonies look like this (16). While 37°C is ideal, they may thrive in temperatures anywhere from 5 to 40°C. Some facultative anaerobes are these. With peritrichous flagella, the majority of these organisms are able to move about. Cells take the form of a rod. There are three enzymes that are typically produced by members of this genus: lipase, DNase, and gelatinase. Additionally, they can have a target-zone look due to their resistance, which can be heterogeneous, to polymyxin B and colistin. They had a negative indole, urease, and oxidase test, but a positive glucose and sucrose (gas producing) fermentation and nitrate test (11).

Gessard (17) was the first to find the genus Pseudomonas in 1882. The rod-shaped, Gram-negative, aerobic bacteria belonging to the Pseudomonas genus are catalase, oxidase, citrate-positive, and motile. Tested negative for indole and urease. The pathogenicity of Pseudomonas is greatly influenced by the fact that it is non-capsulated, non-sporing, and has only one flagellum.Pyocyanine, a green-blue pigment, pyorubrin, a yellow-green pigment, and too fluorescence are among the pigments that these non-spore producing organisms can make (18).

We still have a serious challenge in effectively treating common illnesses due to the rise and spread of drug-resistant bacteria and viruses that have developed new ways to evade antibiotics (19). Because of the rise in cases of multidrug-resistant Enterobacteriaceae (MDRE) infections, choosing antibiotics for UTIs has grown increasingly difficult (20). Antibiotic resistance for UTI-related bacterial infections is continuously increasing, rendering guidelines outdated and making it increasingly difficult to define the appropriate empiric antibiotic therapy (22). This is true despite the fact that inpatients and outpatients are treated differently (21). The development of antibiotic-resistant bacteria, viruses, fungi, and parasites is a leading cause of antimicrobial resistance (AMR), which in turn makes it more difficult, if not impossible, to treat infections with these types of medications and raises the likelihood of disease transmission, serious illness, and death (4). There is a growing concern about the availability of effective antibiotics due to the high rates of resistance against commonly used drugs for common bacterial infections. These infections include UTIs, sepsis, STDs, and certain types of diarrhea (23). One example is the range of antibiotic resistance rates reported by different countries in the Global Antimicrobial Resistance and Use Surveillance System (GLASS) (23). For instance, ciprofloxacin resistance ranged from 8.4% to 92.9% for Escherichia coli and 4.1% to 79.4% for Klebsiella pneumoniae.

Material and methods

Patients and ethical approval: The study included 100 patients who were classified into 75 that were diagnosed with UTIs patients, their ages ranged between (16) to (67) years, (45) females and (30) males. In addition to 25 uninfected people as controls, their ages ranged between (15) to (59) years, (15) females and (10) males. All. All participants in the study underwent identical laboratory tests to diagnose UTIs, identify the types of bacterial isolates, and determine their antibiotic resistance patterns

Culture media: Culture media were prepared according to the manufacturer's instructions and sterilized using moist heat sterilization in an autoclave at 121°C and 15 pounds for 15 minutes. The sterilized media were then dispensed into sterile Petri dishes or test tubes, as required. To confirm sterility, the media were incubated at 37°C for 24 hours. The laboratory-prepared media, including Human Blood Agar, MacConkey Agar, and Mueller-Hinton Agar, were prepared following the protocol outlined by Magiorakos,et al (24).

Identification of bacteria: The identification of bacterial isolates was performed based on their morphological characteristics on culture media, microscopic examination, and a series of biochemical tests (24).

Morphological Identification: Some distinctive features of bacterial isolates were detected using different mediums. We looked at the size, colour, form, and pattern of blood haemolysis of the colonies that had grown on the blood agar plate. Consequently, the present investigation relied on cultivating all bacterial isolates on MacConkey agar to assess their capacity for lactose sugar growth and fermentation.

Microscopic Identification: Fixing and staining an isolated colony using Gramme stain allowed for its transfer onto a microscopic slide. Under the microscope, we examined the dye reaction, the morphology of the cells, and their organization.

Biochemical Identification: The method outlined by Harely (2019) was adhered to for all biochemical analyses. There are a number of biochemical tests that can be done on bacteria to determine their ability to convert tryptophan into indole, a bacteriological laboratory technique called Gramme staining, which sorts bacteria into two broad groups called gram-positive and gram-negative, and bacteria that produce hydrogen sulphide gas, which is a byproduct of metabolizing sulfur-containing compounds (mostly sodium thiosulphate) in the culture media. Following this, the ferric ions or lead acetate combine with the hydrogen gas to create ferrous sulphide or lead sulphide, two substances that are insoluble in water. An organism's capacity to use citrate as an energy source can be determined using citrate agar. The oxidase test is a phenotypic characteristic for bacterial strain identification; it can help identify Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella, and Pasteurella, all of which produce the enzyme cytochrome oxidase. A biochemical assay known as the Urease test can identify bacteria capable of producing the enzyme urease, which hydrolyses urea, by

detecting the alkaline fermentation of urine (urea) and the subsequent formation of ammonia. The triple sugar iron (TSI) test is a microbiological assay that can distinguish urease-positive Proteus from other Enterobacteriaceae and other gram-negative rods. It is mainly used to distinguish members of the Enterobacteriaceae family from other gram-negative rods and to differentiate among Enterobacteriaceae based on their sugar fermentation patterns.

Bacterial Antibiotics Susceptibility Estimation

Principle: In vitro measurement of the ability of an antibiotic to inhibit bacterial growth is performed using the diffusion test which include that the paper discs that should be impregnated with antibiotic are placed on agar medium uniformly seeded with the tested bacteria, and according to the sensitive or resistance of the tested bacteria, the area around each antibiotic disc suggested to be clear as a sign to bacterial inhibiting via the antibiotic , commonly termed as (IZ) which their diameters are differ according to the bacterial susceptibility for the certain antibiotic.

At first five ml of normal saline was inoculated with the tested bacterial isolate to form diluted suspension for each tested isolates. Then by using Sterilized swabs, bacterial suspensions were cultivated on Mueller Hinton agar plates. Antibiotic discs of 8 types were placed onto the cultivated plates using a pair of tweezers. The plates were incubated at 37 Co under aerobic condition for 24-48 hours. Then after incubation, the results were estimated as Susceptible (S), Intermediate (I) and Resistant (R) by their relation to the universal antibiotics manual.

Results: The present study shows that *E.coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were the most common types of UTI- associated bacteria with the percentage of 45%, 30% and 25% respectively, as shown in Table 1-1.

Table (1-1) Subjects of the present study

Total Samples	No. Of Samples	No Growth	Growth	No Of Isolates(%)	Type OfIsolate
Patients	7 5	5	7 0	32 (44.71 %)	*Escherichea Coli*
				27 (38.57 %)	*Klebsiella Pneumonia*
				11 (15.71 %)	*Pseudomonas Aeruginosa*
Control	2 5	2 5	0	-	-

Table (1-2) Result of Biochemical Test

Feature		*Escherichea Coli*	*Klebsiella Pneumonia*	*Pseudomonas Aeruginosa*
No. Of Isolates (%)		32 (44.71%)	27 (38.57%)	11 (15.71%)
Morphology	Motility	Positive	Negative	Positive
	Shape	Rods	Rods	Rods
Biochemical Tests	Gram Stain	Negative	Negative	Negative
	H2S Production	Negative	Negative	Negative
	Indole	Positive	Negative	Negative
	Citrate	Negative	Positive	Positive
	Oxidase	Negative	Negative	Positive
	Coagulase	Negative	Negative	Negative
	Urase	Positive	Positive	Negative
	TSIT	A/A	A/A	A/A

Table 1-2 discloses biochemical test to Enterobacteriaceae, E.coli, Klebsiella pneumonia and Pseudomonas aeruginosa. Where E. coli showed positive results for indole test and urase test. While pseudomonas aeruginosa showed positive results for citrate and oxidase tests. Moreover, klebsiella pneumonia showed positive results for citrate and urase tests.

Table (1-3): Bacterial isolates of the study and their antibiotics resistance classification

	Name Of Antibiotic	No. Of Resist *Pseudomonas Aeruginosa* (11)	No. Of Resist *Klebsiella Pneumonia* (27)	No. Of Resist *Escherichia Coli* (32)
1	Ampicillin	11 (100%)	27 (100%)	32 (100%)
2	Ceftriaxone	11 (100%)	23 (85.1%)	28 (87.5%)
3	Cefoxitin	11 (100%)	23 (85.1%)	24 (75%)
4	Ciprofloxacin	11 (100%)	19 (70.37.5%)	24 (75%)
5	Levofloxacin	11 (100%)	17(62.9%)	27 (84.37%)
6	Clarithromycin	11 (100%)	27 (100%)	32 (100%)
7	Azithromycin	11 (100%)	27 (100%)	32 (100%)
8	Erythromycin	11 (100%)	27 (100%)	32 (100%)

Discussion : Twenty studies were considered for this meta-analysis, all of which reported antibiotic resistance in UTI patients. The prevalence of antibiotic resistance was elevated in cases of UTIs, with P. aeruginosa, E. coli, and K. pneumoniae being the most often documented Gram-negative bacteria with this resistance.

Some 26% of Iraqis have had a urinary tract infection. Furthermore, around 30% of patients encounter recurrent UTIs (25). The Enterobacteriaceae family includes the common bacteria Escherichia coli and Klebsiella pneumoniae, which are common in the gut microbiome of healthy people and animals. Accounting for approximately one third of all Gram-negative infections globally, it is a prevalent opportunistic pathogen linked with healthcare facilities (26). Urinary tract infections (UTIs), cystitis, pneumonia, surgical wound infections, and potentially fatal infections (septicaemia, endocarditis, and endocarditis) all include it. Serious community-onset infections, including pyogenic liver abscesses, endogenous endophthalmitis, and necrotising pneumonia, are also caused by it (27).

It is well-known that antibiotics have a hard time controlling Pseudomonas aeruginosa. The issue of antibiotic resistance in respiratory disease has been highlighted in multiple papers on P. aeruginosa drug sensitivity patterns. Due to the selection pressure caused by the widespread use of antibiotics, P. aeruginosa has developed resistance to these drugs (28). Pseudomonas aeruginosa possesses a broad range of resistance mechanisms encoded in its genome or an innate resistance to antimicrobial drugs resulting from its cell wall's limited permeability; both

contribute to its overall resistance (29). In addition, it can develop resistance by altering chromosomal genes that control resistance genes or by acquiring extra resistance genes from other species through plasmids, transposons, or bacteriophages (30).

It is quite challenging to understand the mechanisms of antibiotic resistance due to differences in the resistance levels of microorganisms. Antimicrobial resistance against several antibiotics is revealed in Tables 1-3. Unlike E. coli and K. pneumonia, Pseudomonas aeruginosa showed remarkable resistance to the drugs that were tested. In terms of antibiotic resistance, Pseudomonas aeruginosa bacteria exhibited the highest level of resistance at 100% to ampicillin, ceftriaxone, cefoxitin, ciprofloxacin, levofloxacin, clarithromycin, azithromycin, and erythromycin; in contrast, Escherichia coli and Klebsiella pneumonia bacteria exhibited resistance to cefoxitin and ciprofloxacin at 75% each. Klebsiella pneumoniae had the lowest resistance rates to ciprofloxacin and levofloxacin (62%). Additionally, ceftriaxone and ecfoxitin were highly resistant to K. pneumonia (85%).

According to a 12-year study that tracked antibiotic resistance in China, the percentage of Escherichia coli strains that were resistant to ciprofloxacin did not rise above 60% (31). In a similar vein, Ahmed et al. demonstrated that E. coli was resistant to levofloxacin (62%), ceftriaxone (59%), and ciprofloxacin (55%). A systematic analysis conducted in 2022 found that of the medications examined, 70% of K. pneumoniae isolates were resistant to ceftriaxone, 50% to levofloxacin, 21% to ciprofloxacin, and 39% to ampicillin (33).

Ampicillin, ceftriaxone, cefoxitin, azithromycin, ciprofloxacin, and levofloxacin were all shown to have little resistance in P. aeruginosa in 2018, according to a study that included 18 nations across the globe (34).

Conclusion: Finally, we discovered that pseudomonas aeruginosa has evolved into multidrug-resistant strains, which poses a significant risk of biological calamity. Therefore, UTIs in Iraq are most commonly caused by resistant and extremely dangerous strains of Escherichia coli, Klebsiella pneumonia, and pseudomonas aeruginosa. The pathogenicity of strains may be increased when antimicrobial resistance and virulence factors are present at the same time. The results show that treatment and control of UTIs in Iraq with prescription medications such as ciprofloxacin, clarithromycin, ampicillin, azithromycin, and erythromycin is not possible. Still, other studies need to look at other Enterobacteriaceae epidemiological traits in UTIs.
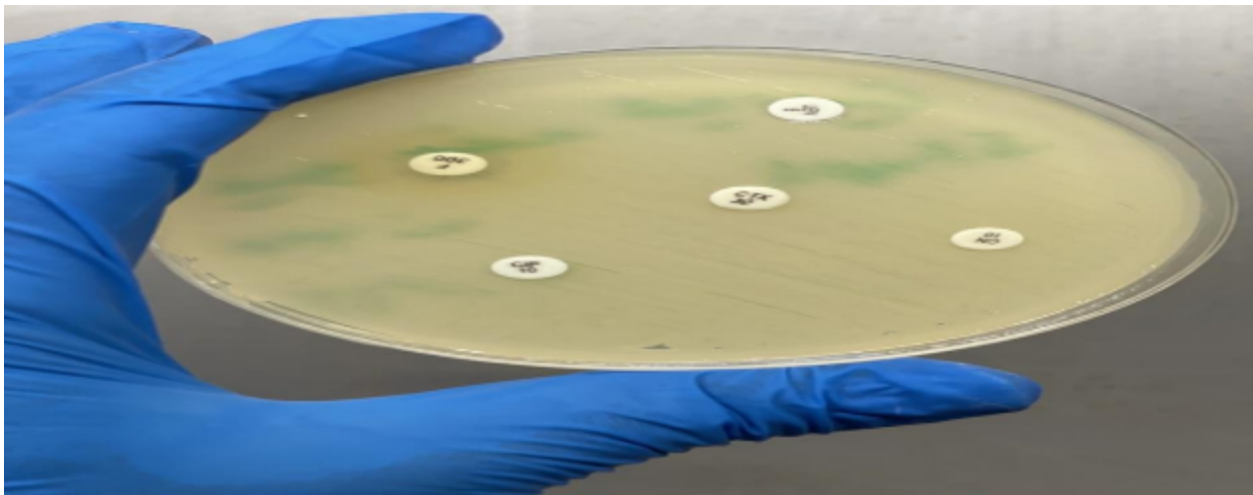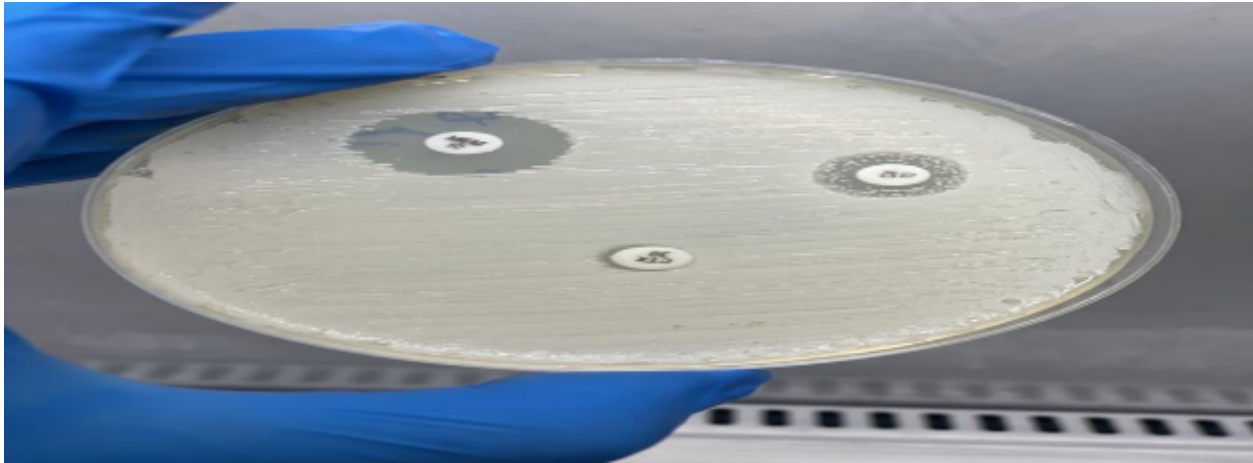
Figure (1) *Klebsiella pneumonia* on blood agar
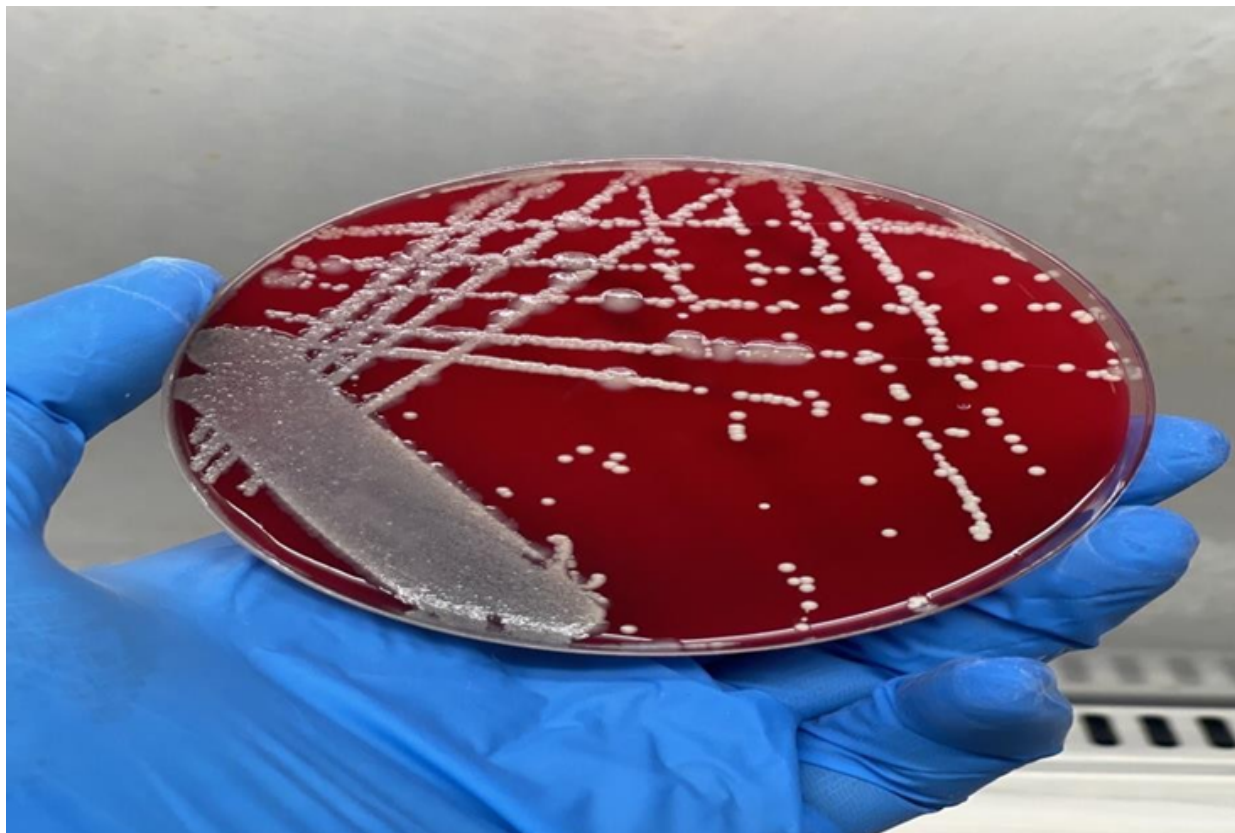
Figure (2) *Escherichia coli* on blood agar
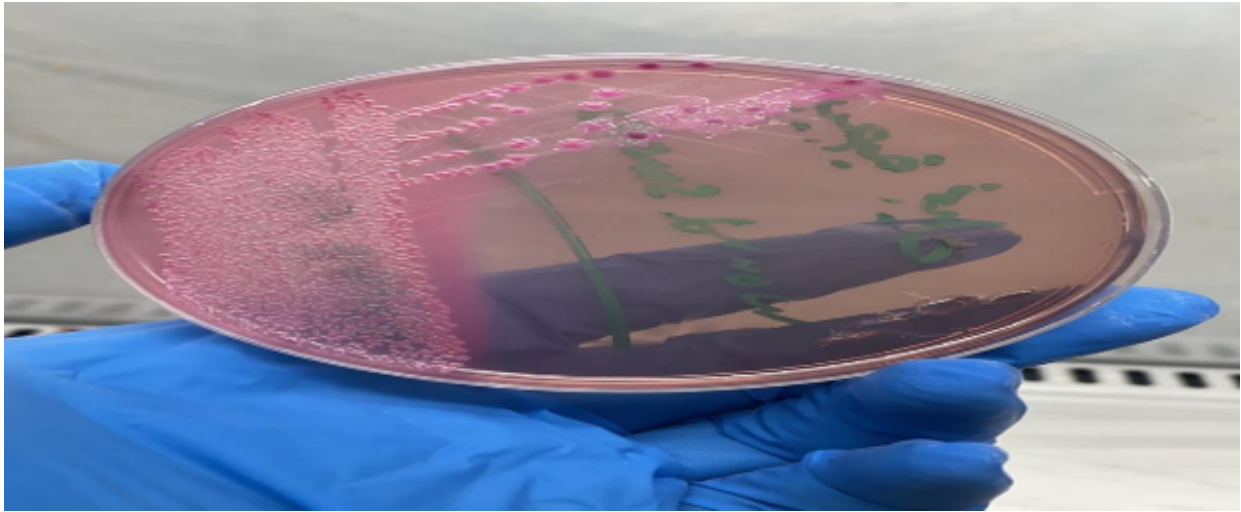
Figure (3) Escherichia coli on MacConkey agar

Figure (4) *pseudomonas aeruginosa* **on Meuller hinton**

Figure (5) Escherichia coli on Meuller hinton agar

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