

## Comparison of the Inhibitory Effect of Some Antibiotics with Silver Nanoparticles Against *Proteus Mirabilis* Isolated from Urinary Tract Infection

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**Annotation:** Urinary tract infections (UTIs) are caused by different types of *Proteus* and are one of the most common diseases. This disease is endemic in many influenza outbreaks, including Iraq. The aim of this study was to determine the prevalence of bacterial infections in females and males and compare their sensitivity to antibiotics and silver nanoparticles in infected individuals. This prospective cross-sectional study was conducted in the laboratory of the College of Basic Education, Sumer University, Iraq, during the period from January 2, 2025 to April 2025. Fifty urine samples were collected from all age groups, 25 males and 25 females. All isolates were confirmed to be *Proteus* by biochemical tests. Twenty (40%) tested positive for *Proteus* bacteria. Of these cases, 7 (35%) were males and 17 (65%) were females. The antibiotic resistance of these isolates was studied. The resistance pattern against different antimicrobials was as follows: ampicillin (100%), gentamicin and cefepime (50%), norfloxacin (40%) and amikacin (35%). The sensitivity of bacteria against silver nanoparticles was also studied

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at five different concentrations and was sensitive to the highest concentration and decreased at lower concentrations as follows: 0.1 (20 mm), 0.75 (19 mm), 0.05 (15 mm), 0.025 (12 mm), 0.001 (5 mm).

**Keywords:** Proteus mirabilis, Urinary Tract Infection, Antimicrobial susceptibility, Silver Nanoparticles.

## 1. Introduction

*Proteus mirabilis* (*P. mirabilis*) is a Gram - negative bacterium, *P. mirabilis* has facultative anaerobic characteristics, and a rod- shaped morphology. Classified within the Gamma proteo bacteria class [1] (Adeolu et al., 2016). It is actively motile with peritrichous flagella, and surrounded by capsules. Additionally, they exhibit dimorphism as "swimming" and "swarming" forms [2, 3], *P. mirabilis* facultative anaerobe bacterium with an inability to ferment lactose and ability to ferment maltose, produces urease enzyme that converts urea into ammonia. Infection by *P. mirabilis* can be detected by the alkaline urine sample (pH 8 and up) with large amount of ammonia [4]. *Proteus mirabilis* is known in clinical laboratories and microbiology survey course as the species that swarms across agar surface. Urease production and robust swarming motility are the two hallmarks of this organism, overtaking any other species present in the process [5]. These bacteria are highly motile, which can make it to recover pure culture of other organisms. they form colorless non lactose fermenter colonies On MacConkey agar. *Proteus mirabilis* tests negative for indole but positive for urease, in addition they produce H<sub>2</sub>S, which Convert the TSI agar's butt to turn black [6]. This motile bacterium has been identified by Gustav Hauser in 1885 [7] Its production was small of urease, distinctive "swarming" behavior on agar plates, and rapid and harmonious multicellular activity were the first characteristics Show it by Gustav Hauser, very important for its movement across surface and resulting in the distinctive "bull's-eye" pattern [8]. The sequencing of the bacterium's genome in the early 21st century, It has provided insights into their metabolic adaptability in different environments. Notably, *P. mirabilis* can be found in a variety of environments, including soil, water, and wastewater, where it plays a major role in the decomposition of organic matter [3].

*P. mirabilis* can lead to range of human infections encompassing wounds, gastrointestinal tract, ocular regions, and the urinary system [9]. Patients harboring a *P. mirabilis* infection may manifest clinical symptoms consistent with urethritis, prostatitis, cystitis, or pyelonephritis. A medical record revealing recurrent nephrolithiasis could suggest a persistent *P. mirabilis* infection, given the fact that a stone formation history may indicate chronic *Proteus* infection [10]. *Proteus mirabilis*-caused urinary tract infection UTIs can further progress into sepsis and bacteremia, whether by damage the renal parenchyma or by utilizing catheters as "mediators" allowing them to spread across tissues and move along surfaces [11].

Antibiotics are naturally occurring organic substances produce as secondary metabolites by many microorganisms that have the ability to inhibit the growth of other organisms. They may be partially synthetic or artificially produced for their biological activity against many other organisms by preventing the growth or killing of these organisms, such as bacteria, parasites, and fungi [12]. Beta-lactam antibiotics are effective and numerous, that can be considered most important groups of effective antibiotics that work to inhibit the functions of the cell wall, while There is a group of the aminoglycoside includes two antibiotics, Amikacin and Gentamycin, that work to inhibit protein synthesis, and the quinolones group works to stimulate the synthesis of

DNA, [13]. Carbapenem antibiotics inhibit the peptidoglycan-binding protein (PBP) that assembles the bacterial cell wall. These antibiotics can penetrate the outer membrane of bacteria because they are small and hydrophilic. [14].

In nanotechnology, a particle (molecule) is defined as extremely small unit that has the chemical and physical properties of a volumetric (large) substance, and the dimensions of these particles range from 1 to 100 nanometers [15]. Silver (Ag) nanoparticles (AgNPs) are one of the most important products of the nanoindustry [16]. Researchers have given silver nanoparticles most attention due to their properties such as electrical conductivity and high thermal, chemical stability, high catalytic activity, and antimicrobial activities [17]. Silver is known for its antimicrobial. Very effective against a wide range of pathogenic microorganisms [18]. Silver has been used since ancient times for its medicinal properties. The applications of silver nanoparticles (AgNPs) are being explored in medical research. antimicrobial ointments and creams containing silver prevent bacterial infections of burns and burns  $\epsilon$ . Silver is also used in medical devices and implants made from silver-impregnated polymers [19]. Silver nanoparticles have a much lower effect on Gram positive - bacteria than Gram negative - bacteria this is due to the composition of the cell membrane [20]. Gram-negative bacteria have a thin cell membrane and are therefore more susceptible to physical degradation. The antimicrobial effect depends on the concentration of silver particles and the formation of holes in the cell membrane [21]. There are several different methods for synthesizing silver nanoparticles, including chemical and physical methods. The disadvantages of physical and chemical methods are that they take a very long time and require the use of harmful substances and hazardous solvents, which remain in the environment and may be difficult to dispose of. They also require a lot of energy [22]. Using the metabolites of microorganisms, including bacteria, fungi, viruses, algae, or plant extracts [23], silver nanoparticles are biosynthesized. The most important feature of this method is that it is environmentally friendly, does not require energy, and is fast and cheap. Plants also contain many organic compounds such as carboxylic acids, flavonoids, amino acids, phenols, and proteins, these materials work to reduce mineral salts and produce nanoparticles in safe, easy, and fast ways [24].

## 2. Materials and Method

### 2.1 Sample collection:

Fifty samples were collected from individuals with urinary tract infections of all age groups (25 males and 25 females). The isolates were grown on MacConkey agar, Nutrient agar, and xylose-lysine-dextrose agar. 20 isolates were identified as bacteria, while the rest were neglected and diagnosed based on morphological, cultural, and biochemical characteristics.

### 2.2 Antibiotic Susceptibility Test.

This test was conducted via the Kirby-Bauer technique on Muller Hinton agar (Bauer, 1966). Isolates of inoculums have been created by emulsifying colonies grown overnight in sterile normal saline to a turbidity comparable to 0.5 McFarland solution standard. Using of sterile swab, a homogenous bacterial suspension was streaked and allowed to dry on the Muller Hinton agar. Utilizing of sterile forceps, the antibiotic discs have been left on the plate. After maintaining the cultures at 37°C for 24 hours, the size of the inhibitory zone was measured, and the outcomes were interpreted using the [25].

### 2.3 Silver Nanoparticle Testing

The etching method was used to test silver nanoparticles as follows: The bacterial suspension was prepared by transferring 5-6 colonies to tubes containing nutrient broth medium. The tubes were incubated at 37°C for 18-24 hours. The growth of the bacterial suspension in the tubes was measured by comparing it to the turbidity of a standard McFarland tube ( $1.5 \times 10^8$  cells/ml). Mueller-Hinton medium was prepared according to the company's instructions, and the medium was sterilized using an autoclave. After sterilization, the flask was allowed to cool to 37°C to be inoculated with the prepared bacterial suspension (Proteus bacteria). The medium inoculated with

Proteus was poured into Petri dishes with a thickness ranging from 12-17 ml, the medium was left to solidify, then holes were made using a cork drill to inject concentrations of silver nanoparticles (200) and transfer 50 microliters of them to each hole, then the dishes were incubated at 37°C [26].

### 3. Result and Discussion

#### 3.1 Study sample

Isolated 50 sample from patients with UTI infection, the study noted 20 (40%) positive for *P. mirabilis*, while 30 (60%) negative for *P. mirabilis*, as shown in the figure 1, Among 20 (40%) *P. mirabilis* isolated from patients with UTI infection, the study noted 13 (65%) infection in male with UTI, while 7 (35%) infected in female with UTI, the results also noted a non-significant difference in availability of *P. mirabilis* according to sex at p. value < 0.05, are shown in the Table 1. coincides with mentioned by [27,28]. They were diagnosed based on cultural, microscopic, and biochemical characteristics and grown on Neutrino Agar, McCoy Agar, and Blood Agar. The prevalence of *Proteus mirabilis* was 40%, which contrasts with the results of [29] which reported a prevalence of 10.7% in human urine samples, and also with [30], which reported a prevalence of 19.3%. Variation in prevalence is attributed to several factors, including geographic region and antibiotic use [29].

#### 3.2 Bacterial Diagnosis

All *Proteus* isolates appeared on MacConkey agar. *Proteus* colonies typically appeared pale yellow or colorless. On blood agar, *Proteus* colonies appeared characteristically as concentric rings that resembled a rippling motion Microscopic examination of *Proteus* bacteria shows the bacteria as motile Gram-negative rods

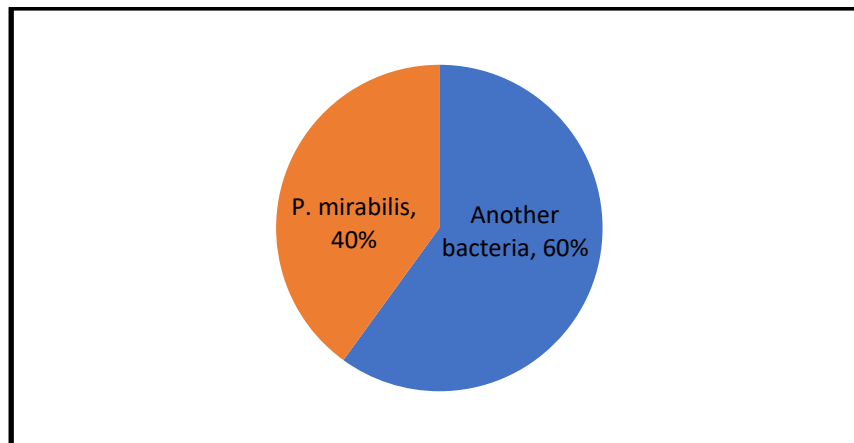
#### 3.3 Antimicrobial Susceptibility of the Bacterial Isolates

The majority of the *P. mirabilis* isolated from UTI infection study were resistant to Ampicillin, amikacin, gentamicin, cefprime, and norfloxacin that used in this study as shown in the figure 2 Table 2, which produced difficulties with the treatment of UTIs caused by *Proteus mirabilis*. The increasing prevalence of antibiotic resistance mechanisms, particularly beta lactamase and efflux pumps, makes treating UTIs more difficult [31]. The findings of study about resistance to (Ampicillin) was absolute compatibility with [32], but disagree with [28], who found the resistance rate (69, 88,2.7) respectively. The results show that these antibiotics are being used inappropriately to treat UTI infection. [32]. The study indicate the finding of gentamicin cefepime resistance rate was (50%) differs with other studies such as [28], their results were (83.8%, 91%) respectively. But, corresponds to previous investigations by [33], their results were (72.3%, 53.2%, 67.7%) respectively.

#### 3.4 Silver Nanoparticle Testing

The results of the current study are shown in the figure3 Table 3: the inhibitory activity of silver nanoparticles against *Proteus* at different concentrations (0.10, 0.075, 0.05, 0.025, 0.001 mg/L). Using the diffusion method, the inhibitory activity of silver nanoparticles was observed by measuring the inhibition diameters determined around the pitted areas on the Muller-Hinton medium. The results of the current study showed a significant effect on inhibition, as the inhibitory activity is directly proportional to the increase in concentration. The inhibitory diameter was 20 mm at a concentration of 0.10, the inhibitory diameter was 19 mm at a concentration of 0.075, the inhibitory diameter was 15 mm at a concentration of 0.05, the inhibitory diameter was 12 mm at a concentration of 0.025, and the inhibitory diameter was 5 mm at a concentration of 0.001 as shown in Table 3. The results of the current study showed similarity to what was reached by the researcher [35]. who determined the inhibitor diameter of the secondary silver particles at about 14 mm, which is a result similar to the current study, which stated that the inhibitory diameters increase with increasing concentrations used against *proteus*. It was also similar to the

results of the researcher [36], who determined the inhibitory diameter at 17 mm. The effectiveness of silver nanoparticles is due to their physical, chemical and biological properties. They differ from regular silver ions in that they concentrate on the bacterial cell wall after adhering to the cell wall. They can penetrate the cytoplasm and enter the bacteria. Therefore, the antibacterial effect depends on the size of the secondary silver particles. That is, smaller silver nanoparticles have a large surface area that attaches to bacterial cells and can reach the cytoplasm more than larger secondary particles, which leads to damage to the cell membrane, which [20].



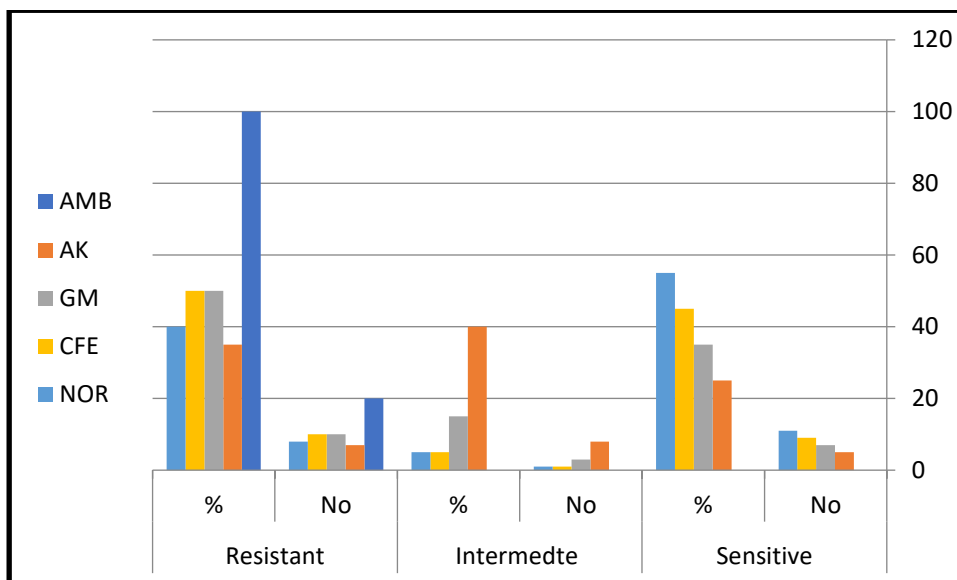
**Figure -1** Percentage of *Proteus mirabilis* in cultured samples from UTI cases for males and females of patients

**Table -1** Percentage of *Proteus mirabilis* in cultured samples from UTI cases for males and females of patients

Sex	Examined No.		<i>P. mirabilis</i> infected	
	No.	%	No	%
<b>Male</b>	16	36%	13	65%
<b>Female</b>	32	64%	7	35%
<b>Total</b>	50	100	20	100

**Table 2-** . Number and percentge of *Proteus mirabilis* Resistant, Intermediate and Sensitive to Antibiotic.

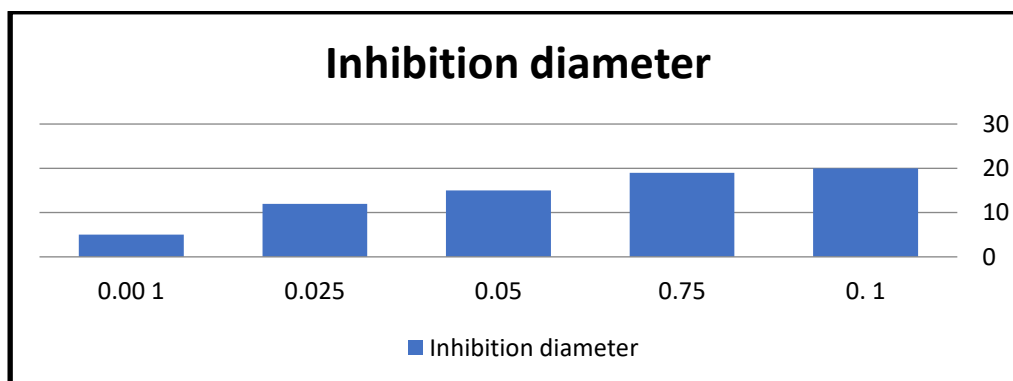
	<i>Antimicrobial Proteus sp. (n=20)</i>					
	Sensitive		Intermedte		Resistant	
	No	%	No	%	No	%
<b>AMB</b>	0	0	0	0	20	100
<b>AK</b>	5	25	8	40	7	35
<b>GM</b>	7	35	3	15	10	50
<b>CFE</b>	9	45	1	5	10	50
<b>NOR</b>	11	55	1	5	8	40



**Figure -2** Number and percentge of *Proteus mirabilis* Resistant, Intermediate and Sensitive to Antibiotic.

**Table 3- *Proteus mirabilis* sensitivity to silver nanoparticles**

concentration of silver nanoparticles	0. 1	0.075	0.05	0.025	0.001
Inhibition diameter	20	19	15	12	5



**Figure -3** *Proteus mirabilis* sensitivity to Silver Nanoparticles

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