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Isolation and inhibition of antibiotic-resistant bacteria using silver nanoparticles

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This study evaluates the antibacterial effect of silver nanoparticles against two clinically significant bacterial species: Escherichia coli and Klebsiella pneumoniae. Bacterial isolates are collected from Al-Shaheed Ghazi Al-Hariri Hospital for Specialized Surgeries in Baghdad, and activated using Brain Heart Infusion (BHI) broth. They are then cultured on MacConkey and Nutrient agar media. Both species have demonstrated lactose fermentation, as evidenced by the change in the MacConkey medium to a pink color. Silver nanoparticles are prepared in four concentrations (0.0100 g, 0.0150 g, 0.0200 g, and 0.0500 g) dissolved in 100 ml of dimethyl sulfoxide (DMSO), and their antibacterial activity is tested by using the well diffusion method. After 24 hours of incubation, E. coli has showed a clear inhibition zone of 4 cm at the highest concentration, indicating strong sensitivity to the nanoparticles. In contrast, K. pneumoniae has exhibited no inhibition zones at any concentration, suggesting resistance under the same conditions. These findings highlight the species-specific antibacterial activity of silver nanoparticles. The effective inhibition observed against E. coli but not against K. pneumoniae emphasizes the need for further research to optimize nanoparticle formulations or to explore their combined use with antibiotics to enhance antimicrobial efficacy.

Keywords: Silver Nanoparticles; Escherichia coli; Klebsiella pneumoniae; DMSO Dimethyl Sulfoxide.

1. INTRODUCTION

Nanotechnology is a rapidly evolving interdisciplinary science that focuses on manipulating materials at the Nano scale, typically ranging from 1 to 100 nanometers. The term "nano" originates from the Greek word for "dwarf," indicating extremely small objects that are invisible to the naked eye and can only be observed using advanced tools like electron microscopes [1]. At this scale, materials exhibit unique physical, chemical, and biological properties that differ significantly from their bulk counterparts. One of the most promising applications of nanotechnology is in the medical field, particularly in combating antibiotic-resistant bacteria. Due to their high surface area-to-volume ratio and enhanced reactivity, nanoparticles (NPs) can interact with bacterial cell membranes, generate reactive oxygen species (ROS), and disrupt vital cellular functions, ultimately leading to bacterial death [2]. These features make nanoparticles highly effective even against multidrug-resistant (MDR) strains such as Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa, which are responsible for many hospital-acquired infections and are challenging to treat. Among the most effective antimicrobial nanoparticles are those made of metals, including silver (AgNPs). These can be synthesized through physical, chemical, or biological (green) methods, with the green synthesis being environmentally friendly and biocompatible [3-4]. Studies have shown that combining nanoparticles with conventional antibiotics results in synergistic effects, improving efficacy and reducing required dosages.

Numerous recent studies have explored the antimicrobial activity of nanoparticles against drug-resistant bacteria. For instance, [5] demonstrated that silver nanoparticles synthesized using plant extracts effectively inhibited the growth of MDR strains such as Pseudomonas aeruginosa and Klebsiella pneumoniae. Similarly, [6] showed that combining nanoparticles with antibiotics significantly enhanced bacterial inhibition zones compared to using antibiotics alone. Magnesium oxide nanoparticles (MgO NPs) have also shown strong antimicrobial activity and relative safety. It has been reported, according to [7], that MgO NPs effectively inhibited the growth and biofilm formation of bacteria such as Escherichia coli and Salmonella typhi. Other studies, such as [8], have highlighted the potential of nanoparticles in targeted drug delivery, reducing side effects while increasing therapeutic efficacy—an important step toward clinical applications.

The primary objective of this work is to highlight the potential of nanotechnology as a promising solution to address the growing challenge of antibiotic-resistant bacterial strains. By developing nanoparticles with strong antibacterial properties—either used alone or in combination with conventional therapies-treatment efficacy can be enhanced, and the emergence of resistance can be reduced. This work also emphasizes the importance of green synthesis of nanoparticles using biological agents such as plant extracts or microorganisms, due to their sustainability and higher biocompatibility. Additionally, it aims to stress the need for standardized protocols to characterize the physicochemical and biological properties of nanoparticles, along with the necessity of conducting in vivo studies and clinical trials to evaluate biocompatibility, pharmacokinetics, and therapeutic effectiveness, with careful consideration of long-term toxicity and bio distribution. The study further proposes expanding the use of nanoparticles in hospital infection control by incorporating them into surface coatings, wound dressings, and medical devices. Ultimately, the goal is to promote strong collaboration between researchers, healthcare institutions, and regulatory bodies to translate laboratory findings into effective therapeutic applications that have a meaningful impact on public health.

2. EXPERIMENTAL

2.1 Equipment and Instruments

The following laboratory instruments are utilized in this study:

1. Autoclave Charles Chamberland (1884, France)
2. Incubator Evolved over time; no single inventor
3. Electric Oven Thomas Ahearn (1892, Canada)
4. Zone of Inhibition Measuring Ruler Standard lab tool; no specific inventor
5. Hot Plate Industrial development; no specific inventor
6 .Sensitive Balance Concept refined by Joseph Black (18th century)
7. Filter Paper Carl Friedrich Schönbein (c. 1860, Germany)
8. Compound Light Microscope Zacharias Janssen (1590, Netherlands)
9. Micropipette Heinrich Schnitger (1957, Germany)
10. Orbital Shaker Industrial laboratory invention; no single inventor
11. Refrigerator Carl von Linde (1876, Germany)
12. Bunsen Burner Robert Bunsen (1855, Germany)
13. Micropipette Tips Related to micropipette development (Schnitger)
14. Test Tubes Widely used laboratory glassware; no specific inventor
15. Spatula Basic lab tool; inventor unknown
16. Gloves (Medical) William Stewart Halsted (1890, USA)
17. Sterile Cotton Swabs Leo Gerstenzang (1923, USA – modern version)
18. Cork Borer Traditional lab tool; origin unknown

2.2 Culture Media

The following culture media are used:

1. Nutrient agar
2. Nutrient broth
3. Blood agar
4. MacConkey agar
5. Pseudomonas agar base
6. Mueller-Hinton agar
7. Methyl red-Voges Proskauer (MR-VP) agar

All media are prepared according to the manufacturers' instructions, sterilized by autoclaving at 121 °C for 15 minutes under 15 psi pressure, and cooled to 40-45 °C before use.

2.3 Chemicals and Reagents

The following chemicals and reagents are applied:

1. Silver Nanoparticles (Nanosilver) not attributed to a single inventor; nanotechnology research began in the late 20th century. Nanosilver has been studied extensively since the 1990s.
2. Dimethyl Sulfoxide (DMSO). Discovered by Alexander Zaytsev in 1866 (Russia).

3. Absolute Ethanol Ethanol. It is known since antiquity; absolute ethanol (pure, 100%) is first produced by Johann Tobias Lowitz in the late 18th century using chemical drying agents.
4. Kovac's Reagent Developed by Helmut Kovac, used for detecting indole production in microbiology.
5. Oxidase Reagent Based on the oxidase test developed by Gordon and McLeod in 1928 to detect cytochrome c oxidase.
6. Gram Stain Reagents Developed by Hans Christian Gram in 1884 (Denmark); includes crystal violet, iodine, alcohol (decolorizer), and safranin.

2.4 Sample Collection and Bacterial Isolation

Clinical samples from burns and wounds are collected using sterile cotton swabs from Ghazi Hariri Hospital and the Specialized Burns Hospital in Medicine City, Baghdad. Samples are activated in nutrient broth and incubated at 37 °C for 24 hours, then subcultured on nutrient agar and MacConkey agar. Bacterial identification is performed based on morphological, biochemical, and physiological characteristics according to updated diagnostic protocols [9-10].

Isolated bacteria included:
- Escherichia coli
- Klebsiella pneumoniae

(Pseudomonas aeruginosa is excluded from this study).

2.5 Preparation of Culture Media

Nutrient Agar
28 g of nutrient agar powder is dissolved in 1000 ml of distilled water, then heated to dissolve completely. After that, it is sterilized by autoclaving at 121 °C for 15 minutes at 15 psi, then cooled to 40-45 °C before pouring into sterile Petri dishes.

Nutrient Broth
25 g of nutrient broth powder is dissolved in 1000 ml of distilled water, sterilized as above step, cooled, and dispensed into sterile test tubes.

2.6 Preparation of Silver Nanoparticle Solutions

Different weights of silver nanoparticles are accurately weighed using a sensitive balance:
- 0.050 g
- 0.0100 g
- 0.0150 g
- 0.0200 g

Each weighed sample is dissolved in 100 µL of DMSO, transferred to sterile tubes, and mixed on an orbital shaker for 24 hours to ensure complete dissolution. Prepared solutions are stored at 4 °C until use.

2.7 Antibacterial Activity Testing (Agar Well Diffusion Method)

Bacterial suspensions are evenly spread on Mueller-Hinton agar plates using sterile cotton swabs. Wells are bored into the agar using a cork borer. 100 µL of each silver nanoparticle concentration is added to the wells. Plates are incubated at 37 °C for 24 hours. Zones of inhibition are measured with a special ruler to assess antibacterial activity.

3. RESULTS AND DISCUSSION

3.1 Bacterial Response

Silver nanoparticles are applied to different types of bacteria at different concentrations to obtain and measure the highest Zon value. After preparing the nanoparticles, adding DMSO, and after 24 hours of bacterial multiplication, the silver nanoparticles are placed in the holes. The results show that Zon is not obtained for Klebsiella bacteria, but the colony counting method is demonstrated. Escherichia coli bacteria are quantified and assessed using a specialized Zon ruler.

Table 1 Effect of Silver Nanoparticles at Different Concentrations on the Growth of Escherichia coli and Klebsiella pneumoniae Using Zone of Inhibition and Colony Count Methods.

Measurement of microbial inhibition zones using a ruler (cm)	DMSO (Dimethyl Sulfoxide) (ml)	Concentrations of silver nanoparticles (gm)
4	100	0.050
10	100	0.0100
10	100	0.0150
8	100	0.0200

3.2 Silver Nanoparticles (AgNPs)

Silver nanoparticles (AgNPs) are nanoscale materials ranging from 1 to 100 nanometers in size, possessing unique physical, chemical, and biological properties that distinguish them from bulk silver. Their exceptional antimicrobial potency, combined with high electrical conductivity and remarkable chemical stability, makes AgNPs highly versatile and effective in numerous medical applications, including wound healing, burn treatment, infection control, and enhancing antibiotic efficacy [11-12]. One of the most significant advantages of AgNPs is their broad-spectrum activity against a wide range of pathogens, including multidrug-resistant bacteria, which positions them as a powerful alternative or adjunct to conventional antibiotics.

AgNPs exert their antibacterial effects through multiple complementary mechanisms: they adhere to bacterial cell surfaces, accumulate, and penetrate the cell wall and cytoplasmic membrane. Resulting in irreversible structural damage and leakage of vital cellular components. Furthermore, silver ions (Ag^{+}) released from the nanoparticles bind strongly to thiol groups in essential proteins, disrupting respiratory enzymes and halting ATP synthesis, thereby inducing bacterial cell death [13-14]. Additionally, AgNPs interfere with bacterial DNA by binding to its phosphate backbone, inhibiting replication and transcription processes, which further contributes to their antimicrobial efficacy [15]. Their small size and high surface area enable efficient interactions with bacterial cells, enhancing their potency while minimizing required dosages and reducing side effects.

Moreover, the differential sensitivity of bacteria, with Gram-negative strains generally more susceptible than Gram-positive ones due to variations in cell wall composition and thickness, highlights the tailored effectiveness of AgNPs across diverse microbial targets. These advantages collectively underscore the hopeful role of silver nanoparticles as potent, multifaceted antimicrobial agents in modern biomedical applications.

3.3 Escherichia coli

Escherichia coli (E. coli) is a Gram-negative, facultative anaerobic bacillus, typically measuring about 0.7–0.4 µm, found singly or in pairs. Motile strains possess peritrichous flagella, while others are non-motile. Optimal growth occurs at 37°C with a pH of 7.2, although it can grow within a range of 10–40°C [16]. On MacConkey agar, E. coli forms pink colonies due to lactose fermentation, and it produces colonies with a metallic green sheen on EMB (eosin methylene blue) agar. Some strains produce hemolysin, which causes hemolytic zones on blood agar. Biochemically, E. coli is positive for catalase, indole, and methyl red tests, and negative for Voges-Proskauer, oxidase, and hydrogen sulfide production. It ferments multiple sugars including glucose, lactose, sucrose, mannitol, and maltose with acid and gas production [17-18]. According to this study, when E. coli is exposed to silver nanoparticles, it displays a detectable zone of inhibition (ZOI), suggesting that AgNPs have strong antimicrobial activity and high sensitivity against this species.

3.4 Klebsiella pneumoniae

Klebsiella pneumoniae is a Gram-negative, non-motile, short bacillus that does not form spores. It grows between 12–43°C, with optimal growth at 37°C. The colonies are large, moist, and mucoid due to the production of a polysaccharide capsule, which is a major virulence factor [19-20-21]. Biochemically, it is positive for catalase, Voges-Proskauer, citrate utilization, and urease, and negative for indole, oxidase, and gelatin hydrolysis. The organism ferments glucose and sucrose, producing acid and gas, but does not produce hydrogen sulfide and is non-hemolytic on blood agar [22-23]. Silver nanoparticle treatment of K. pneumoniae in the current investigation does not result in a zone of inhibition, indicating a higher level of resistance. Its strong capsule structure and cell wall composition may be the cause of this, since they may restrict AgNPs' ability to penetrate and interact with cellular components.

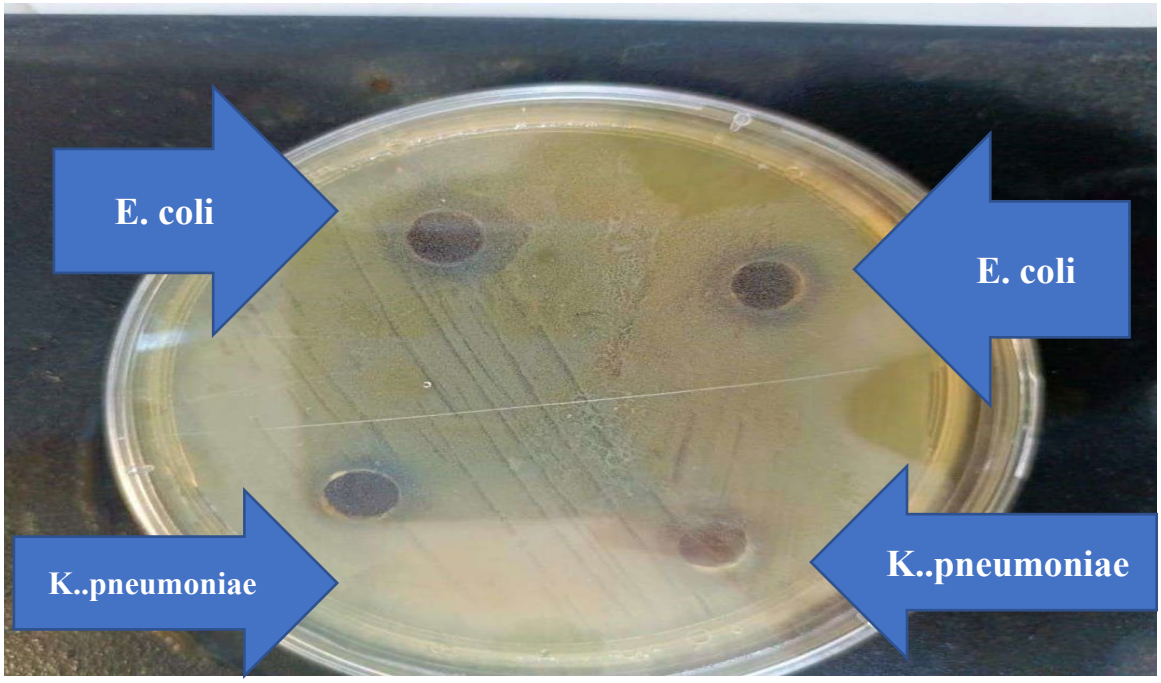

Figure 1 Effect of Silver Nanoparticles on Bacterial Growth: No Inhibition Zone for Klebsiella, Clear Zone Formation for Escherichia coli.

4. CONCLUSIONS

The study has revealed that silver nanoparticles exhibit significant antibacterial activity against Escherichia coli, as evidenced by a clear inhibition zone of 4 cm in diameter at the highest tested concentration (0.0500 g). This finding indicates that E. coli is highly sensitive to the effects of silver nanoparticles. In contrast, Klebsiella pneumoniae shows complete resistance under the same experimental conditions, with no observable inhibition zones at any tested concentration. These results highlight that the antibacterial efficacy of silver nanoparticles is dependent on the bacterial species involved. Suggesting that not all Gram-negative bacteria respond similarly to a nanoparticle treatment. The well diffusion method has been proved to be an effective preliminary screening tool for assessing the antibacterial properties of nanoparticles. While silver nanoparticles show promise as alternative antimicrobial agents against certain drug-resistant bacteria like E. coli, their ineffectiveness against others, such as K. pneumonia, indicates the need for further research. Future studies should focus on optimizing nanoparticle formulations and investigating potential synergistic effects when combined with conventional antibiotics to enhance their antimicrobial efficacy, particularly against resistant bacterial strains.

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