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ANTI-BACTERIAL ACTION OF SILVER NANOPARTICLES AGAINST MDR BACTERIA ISOLATED FROM HOSPITAL

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ABSTRACT

Hospital-associated infections (HAIs) are considered to be a major source of infections in patients, especially in patients with permanently impaired immunity. There is alarming increase of multi drug resistant (MDR) bacteria and Antibacterial medication resistance has been deemed a serious hazard to public health by the World Health Organisation (WHO). The study aimed to isolate and identify main bacteria caused nosocomial infection, and trying to treatments by using nanoparticles. By measuring the antibacterial activity of the synthesised AgNPs using the agar disc diffusion technique, AgNPs demonstrated antibacterial properties against the Pseudomonas aeruginosa and Staphylococcus aureus MDR.

KEYWORDS: Hospital-Associated Infections, MDR, Nanotechnology and Anti-bacterial.

INTRODUCTION

Among hospitalised patients, hospital-associated infections (HAIs) are a leading cause of morbidity and death, with disturbing clinical outcomes occurring after admission and hospitalization, a major global problem.[1] Infections occur during or before treatment, and sometimes occur asymptomatically or in an incubation period until the patient is admitted to another health facility.[2,3] Hospital-associated infections are a major problem for patients with serious illnesses due to decreased host defense mechanisms, random reuse of antibiotics, reuse of contaminated medical equipment, cross-contamination between hospitalized patients and health care workers, and lack of proper hygiene and occupational safety measures to reduce the incidence of HAIs. [4,5] Hospital-acquired infections are caused by live microorganisms that the development of infectious illnesses is caused by and that are quickly acquired from the hospital setting. Bacteria are the main source of infection due to contamination in the hospital environment. [6] These organisms stay on surfaces for several days and weeks after contamination occurs, depending on different environmental and incident conditions. However, most of these microbial pathogens have change and become resistant to antibiotics, which is currently a major global problem. Studies have confirmed the presence of bacteria on various health surfaces such as floor surfaces, sanitary facilities, doors, and offices, which can easily be transmitted to patients and hospital workers, especially individuals with weak immunity. [7-9]

Nanotechnology is defined as one of the latest and distinctive technologies in the field of modern science and technology, which contributes to the production of new materials with high and distinctive specifications due to its extremely small size, which ranges between 1-100nm. [9,10] The precise mechanism by which nanoparticles act as antibacterial agents has been explained by these hypotheses, the most likely being their accumulation on the outer wall of bacteria, leading to the formation of holes in the surface wall, which leads to holes in the bacterial membrane, then damage to its structural structure, and a series of other changes that end with the death of the bacterial cell. [11-13]

In this study, we isolated MDR bacteria from different hospital locations and tested drug susceptibility, and we determined the antibacterial activity of AgNPs against MDR bacterial isolates alternated therapies.

MATERIALS AND WORKING METHODOLOGY

Isolation and Identification of Bacteria

The research was conducted from May to June 2023 on different days, chosen periodically from various areas (Intensive Care Unit, Obstetrics and Gynecology Department, Operation Theatre, General Surgery Department, Out Patient Department of a hospital, Male Department of Surgery, Female Department of Surgery, Department of Pediatric, Outpatient Clinics, Intensive Care Unit for Newborns, and Laboratory) of AL-Hilla Education Hospital, Babylon. Using sterile spatulas, all samples were gathered and simultaneously placed into a closed, clean class container before being sent to the laboratory for additional operations. A 2 gm. of material was adding and suspended in two millilitres of sterile normal saline. To isolate and purify the sample, 50 µl of the supernatant was taken and spread-plated onto recently made blood, nutrient, and Mac Conkey's agar dish (made in India, Himedia) for 24 to 48 hours at 37°C. The tiny Vitek-2 system verified the isolates (Biomérieux) [14].

Susceptibility test for different antibiotics

Using disk diffusion test [Kirby-Bauer], the CLSI (2021) [14]. Rules were followed to determine antibiotic disc susceptibility. The different antibiotics utilized to determine the drugs resistance of bacterial under study. The results were expressed as a proportion of resistant to different isolates among all bacterial isolates that were found. Antimicrobial resistance exhibiting three or more classes is known as multidrug resistance (MDR). The bacterial strains stand in for MDR.

Antibacterial Activity of AgNPs

The antibacterial action of AgNPs was determined against the many bacterial isolates, after cultured on nutrient agar slants (NAS). A 5- different dilution concentrations of AgNPs (500; 250; 125; 62.5; and 31.25 µg/ml) in adding on sterile deionized water is used to determine the effects of the nanoparticles against the bacteria study. The isolates were firstly incubated at [20- 25°C] room temperature for 15 min.; then ALL plates incubated at 37°Cover night. After period of incubation, results were recording as a positive when noted the inhibition zone appered around each well. By digital Vernier caliper can quantify the diameter of each inhibition zone in millimeters. [14]

RESULTS AND DISCUSSION

MDR Bacteria Isolated from Different Sources in Hospital

In Hillah, Iraq, at the Teaching Hospital, thirty isolates of G-ve (Pseudomonas aeruginosa) and thirty isolates of G+ve (Staphylococcus aureas) were extracted and identified from various sources. Biochemical assays and the Vitek-2 system (Biomérieux) method were used to identify each isolate. The result shown that the probability of bacteria ranging from (96–98 percent), as in Table 1, the Vitek-2 technique was utilized to confirm earlier identification findings for isolates obtained from diverse materials.

Table 1 *(The likelihood that G- and G-ve bacterial isolates from various sources will be diagnostic using the Vitek-2 system.)*

MDR Bacterial Isolates	Gram stain bacteria	Probability%
Pseudomonas aeruginosa	G^{-ve}	98%
Staphylococcus aureas	G^{+ve}	96%

Isolation of Multiple Drug-Resistant Bacteria

Lists of antibiotic susceptibility tests were created to all bacterial isolates, to determine the MDR bacteria to complete the AgNPs examination. A bacterial isolate We used the publications and breakpoints provided by the Clinical Laboratory Standards Institute (CLSI). the European Committee on Susceptibility Testing to Antimicrobials [EUCAST], Drug Administration [FDA] and US Food. The antibiotic sensitivity down on the samples of MDR bacteria was investigated, and sixteen antibiotics were chosen for susceptibility testing of the isolated bacteria Ampicillin, Amoxicillin-clavulanate, Ceftazidime, Cefotaxime, Ceftriaxone, Meropenem, Amikacin, Streptomycin, Azithromycin, Doxycycline, Ciprofloxacin, Levofloxacin, Chloramphenicol, Nitrofurantoin, Trimethoprim, Aztreonam, 5- isolate of P. aeruginosa and 5- isolate of S. aureus determine as MDR bacteria isolats , Table 2.

Table 2 *(Antibiotic susceptibility of bacterial isolates from various origins.)*

NO.	Antibiotic disc	*P. aeruginosa* Isolates					*S. aureus* Isolates				
		1	2	3	4	5	1	2	3	4	5
1	AMP 10 µg	R	R	R	R	S	S	R	I	R	R
2	AMC20/10 µg	R	I	S	R	R	R	I	S	R	R
3	CAZ 30 µg	I	R	R	S	R	R	S	I	S	R
4	CTX 30 µg	R	R	I	R	I	R	S	R	I	R
5	CRO 30 µg	S	R	S	R	R	S	R	R	R	I
6	MEM 10 µg	R	R	S	I	R	R	R	I	R	R
7	AMK 30 µg	I	R	R	R	R	R	R	R	R	R
8	STR 30 µg	R	S	R	R	R	R	R	S	R	R
9	AZM 15 µg	R	R	R	R	R	R	R	R	R	R
10	DOX 30 µg	I	R	R	I	R	S	R	I	R	R
11	CIP 5 µg	R	R	R	R	R	R	R	R	R	R

12	LVX 5 µg	R	R	I	R	I	R	R	I	I	R
13	CHL 30 µg	R	S	R	R	I	I	R	R	R	R
14	NIT 300 µg	R	R	R	R	I	S	R	R	I	R
15	TMP 5 µg	R	I	R	S	I	R	I	S	R	R
16	ATM 30 µg	S	R	R	S	R	R	S	R	R	S

AMP= Ampicillin; AMC= Amoxicillin-clavulanate, CAZ= Ceftazidime, CTX= Cefotaxime, CRO=Ceftriaxone, MEM= Meropenem, AMK=Amikacin, STR= Streptomycin, AZM= Azithromycin, DOX = Doxycycline, CIP=Ciprofloxacin, LVX= Levofloxacin, CHL= Chloramphenicol, NIT=Nitrofurantoin, TMP=Trimethoprim, ATM=Aztreonam.

Results in the Table 2, checked the 5-isolates susceptibility pattern of P. aeruginosa and showed were 100% sensitivity to Ciprofloxacin and Azithromycin, 66.6% to Ampicillin, Amikacin, Streptomycina and Nitrofurantoin ,33.3% to Amoxicillin-clavulanate, Ceftazidime, Ceftriaxone, Meropenem, Doxycycline, Levofloxacin, Trimethoprim and Aztreonam. From 5- isolates of Staph. aureus showed 100% susceptibility to Amikacin, Ciprofloxacin and Azithromycin, 66.6% susceptibility to Meropenem, Streptomycin and Chloramphenicol, 33.3% to Ampicillin, Amoxicillin, Ceftazidime , Cefotaxime, Ceftriaxone, Doxycycline, Levofloxacin, Chloramphenicol, Nitrofurantoin, Trimethoprim, Aztreonam.

Given that resistance to several antibiotic classes is characterised as acquired resistance to one or more antimicrobial agents in three or more classes, P. aeruginosa and Staph. aureus was categorised as multidrug resistant bacteria (MDR) in this investigation. Gene transposons and resistance R plasmids, which each code for a distinct agent, can aggregate to form multidrug resistant bacteria, or multidrug efflux pumps, which can individually release several drugs, can also cause multidrug resistance in bacteria [15-17].

Antibiotic sensitivity of MDR Bacteria

When AgNPs are tested for their antibacterial activity against multidrug-resistant bacteria, 5-isolates of P. aeruginosa and 5-isolates of Staph. aureas bacteria, the results of the agar disc diffusion test demonstrate a potent, broad-spectrum antibacterial activity. These findings are then compared to the activity of various antibiotics on those isolates. All five of the bacterial isolates were not successfully treated by the antibiotics that were chosen, as seen in Table 2. The inhibitory zone width of AgNPs was evidently greater than the activity of specific antibiotics as the concentration of NPs increased. The largest zone of inhibition of 24 mm was seen against Staph. aureas, which exhibited excellent sensitivity even at 31.25 µg/ml. The 500 µg/ml dose demonstrated the highest zone of inhibition against all 10 bacterial isolates. Comparing P. aeruginosa to other bacterial isolates, it was the least susceptible to AgNPs. lead to the (Figs. 1 and 2) apparent activity of AgNPs as a dose-dependent suppression of the growth of all microorganisms under observation. [18] AgNPs' antibacterial action is dependent on several processes. The first mechanism is dependent on ROS generation, followed by oxidative stress, which causes chemical damage to proteins and the genetic material (DNA) present in bacteria, Secondly, another study revealed that the tiny size of the nanoparticles may enhance AgNPs' antibacterial efficacy. Electrostatic interactions between AgNPs and the proteins in bacterial cell membranes can cause physical harm to the cells, which in turn results in bacterial cell death. [19-23]

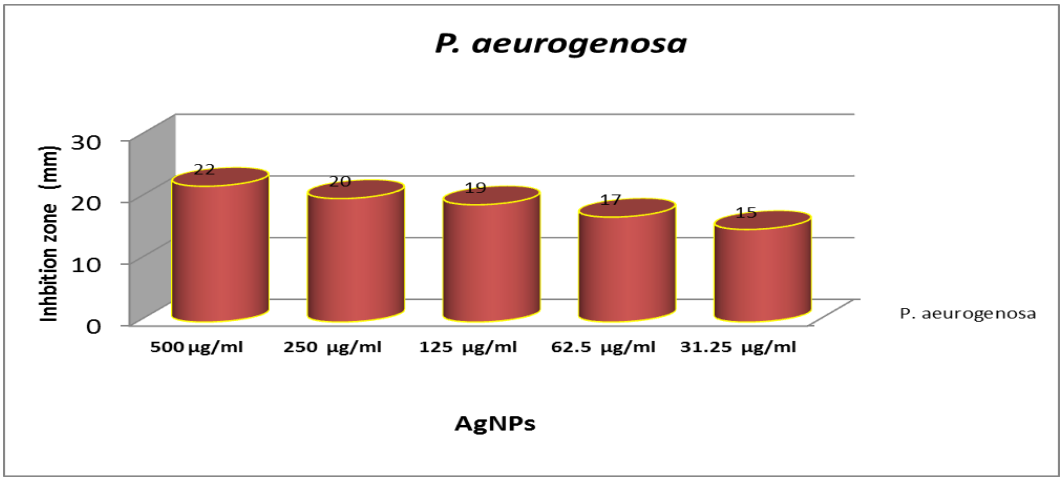

Figure 1. *(Antibacterial action of AgNPs on P. aeruginosa.)*

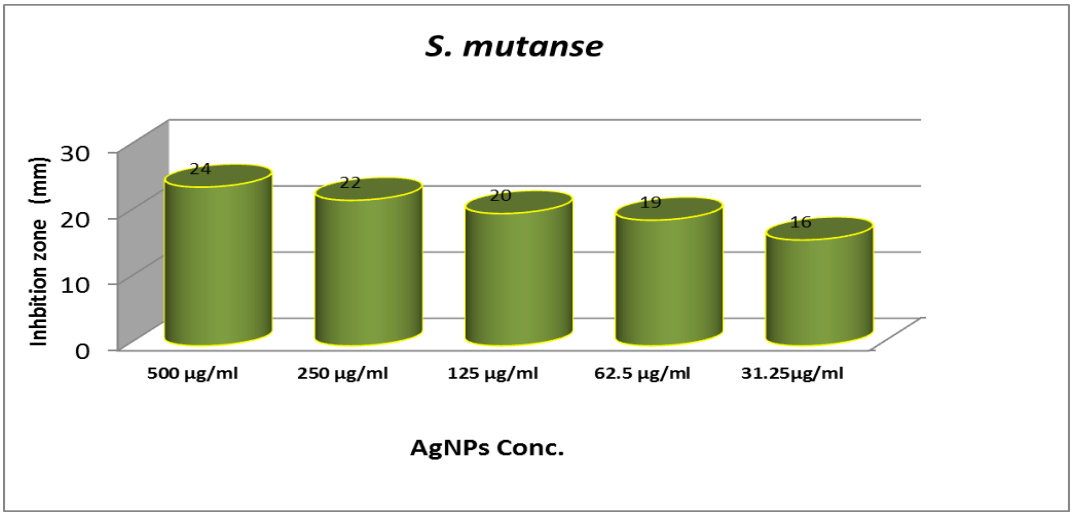

Figure 2. *(Antibacterial action of AgNPs on Staph. Aureas.)*

CONCLUSION

Numerous multi-drug resistance bacteria have been identified from patient-risk situations, such as operating rooms and critical care units, even when their microbial load is within advised bounds. the management and mitigation of nosocomial infections, mostly via surfaces, air and medical equipment. As an overall view, the current study proposed more antibacterial effects for AgNPs. Moreover, it revealed a dose-dependent biocompatibility along with slightly more antibacterial effects for AgNPs compared with traditional antibiotics. The production of alternative tretment AgNPs may be an efficient substitution for the traditional antibiotics-based medicines with fewer or non-side effects.

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