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ORIGINAL ARTICLE

Prevalence of Carbapenem Resistance and Antibacterial Potential of Nanoparticles in *Pseudomonas aeruginosa* Isolated from Burn Units in Egypt

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ABSTRACT

Key words:
P. aeruginosa, Carbapenemase production, AgNPs

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Background: *Pseudomonas aeruginosa is an opportunistic microorganism and quite frequently associated with skin infections. Biosynthesized silver nanoparticles can be a promising key to eliminate these microbes.* ***Objectives:*** *The current work aims to identify P. aeruginosa carrying metallo-β-lactamase from burn infections and assess the impact of silver nanoparticles and antibiotics on them.* ***Methods****: A total of 120 samples collected from patients suffering from burn wound infections were subjected to conventional microbiological techniques to identify P. aeruginosa isolates. Testing for antimicrobial susceptibility was conducted utilizing the Kirby Bauer disc diffusion method. Carbapenemase-producing strains were detected both phenotypically by detecting metallo-beta-lactamases through performing modified carbapenem inactivation method (mCIM) and genotypically using PCR. Using the agar well diffusion method, AgNPs' antibacterial activity was assessed. The synergistic effect of a combination of antimicrobials (colistin, gentamicin, and ciprofloxacin) and AgNPs was estimated by the checkerboard method.* ***Results****:Out of 120 isolates, 46 (38%) P. aeruginosa isolates were identified and confirmed by PCR assay. Total 25 isolates (54%) were multidrug resistant isolates. Modified carbapenem inactivation method showed that 39 isolates (84.78%) were producing carbapenemases whereas 35 isolates (76%) were confirmed for carbapenemases by performing PCR. The prevalence of carbapenemase encoding genes was as follows blaSPM (14%), blaVIM (25.7%), blaNDM (40%), blaKPC (2.85%) and blaIMP (17%).* ***Conclusions****: P. aeruginosa isolates showed high-level carbapenem resistance. The majority of the isolates were multi drug resistant (MDR), indicating a concerning spread of resistant isolates. AgNPs exhibited considerable antibacterial effects, so they should be considered as an effective means of combating MDR.*

INTRODUCTION

Patients with burn wounds are easily susceptible to microorganism infection due to weaker immune systems caused by skin loss and burning injuries [1]**.** *Pseudomonas aeruginosa* is a gram-negative microbe that is considered the third nosocomial pathogen after *Staphylococcus aureus* and *Escherichia coli*. It is a main pathogen, particularly in patients with cystic fibrosis (CF), burns, immunosuppression, and intubation[2]. Carbapenems have emerged as important antibacterial agents for the clinical management of severe infections triggered by *P. aeruginosa*. Isolates of *P. aeruginosa* resistant to carbapenem constitute a developing global issue[3] and it is a great challenge to treat such “Superbugs” because of their increasing tendency to develop resistance towards almost every drug used[4]. Intrinsic resistance pathways are significantly more common, and carbapenem-resistant *P. aeruginosa* frequently exhibits diminished expression or deletion of *OprD* outer membrane protein, by losing prions i.e. *OprD* as well as by producing enzymes that inactivate antibiotics e.g. β-lactamase, AmpC β-lactamase, extended-spectrum β-lactamase and metallo-β-lactamase and hyperexpression of efflux pump systems, which quickly pumps antibiotics outside the cell[5,6]. Beta lactamases trigger the hydrolysis of antibiotics by

cleaving an amide bond present in their β-lactam ring i.e. penicillin, carbapenem, cephalosporins, etc. [7].

Metallo-β-lactamases are extracellular or periplasmic enzymes generated by bacteria. Metal binding sites are preserved in all known representatives and zinc ions are essential as enzyme cofactors. Except for monobactams, these enzymes are capable of degrading every β-lactam antibiotic and have a particular carbapenemase activity that is continuous and efficient. Furthermore, therapeutic β-lactamase inhibitors have little effect on metallo-β-lactamases [8]. Carbapenemases have been identified using a variety of phenotypic and genotypic methods. For detecting carbapenemase synthesis, the mCIM exhibits higher sensitivity than the CIM in enterobacteriaceae and is a simple procedure, especially in a laboratory with low resources. The objectives of the current study include isolation, identification, molecular characterization, and antibiogram analysis of *P. aeruginosa* as well as phenotypic detection of carbapenem-resistant *P. aeruginosa* that can produce metallo-beta lactamase by utilizing mCIM technique [9].

Nanotechnology is an innovative and modern method to develop novel formulations based on antimicrobial activity of nanoparticles. Few studies have demonstrated that adding nanoparticles to antimicrobial drugs can increase their effectiveness against *P. aeruginosa* [10]. Nanoparticles are biomaterials that are valuable substitutes or supplements to existing antimicrobials. Nanoparticles can be either organic or inorganic. The most experimented nanoparticles are Silver, gold, Zinc Oxide, and copper oxide nanoparticles [11]. Synthesis of AgNPs was confirmed through transmission electron microscopy (TEM) and UV analysis. The nanoparticles had 40 nm size. The activity of AgNPs against the test organism was estimated by using the disc diffusion method, serial dilution turbidity assay, and agar cup assay. Results indicate that green synthesized AgNPs, from leaf extract of guava can inhibit the growth of bacteria. Numerous research assessed the antibacterial activity of biosynthesized silver nanoparticles (AgNPs) against the gram-negative bacteria *P. aeruginosa* and *E. coli*. Results indicated that AgNPs have antibacterial activity that is dependent on concentration, and these nanoparticles cause 100% death of both bacteria [12]**.** The reactive oxygen species ROS release weakens the antioxidant defense mechanism and causes damage to the cell membrane. AgNPs also cause neutralization of charge on the surface, changes in cell membrane permeability, and non-viability of cells. Fourier transmission infrared spectroscopy showed the cell membrane killing process and chemical decomposition of the cell membrane. Atomic force microscopy showed changes in the ultrastructure and nano-mechanical of the cell surface characteristics. Other studies described the activity of AgNPs against the biofilm production ability of MDR gram-negative bacteria [10]. So, this study aimed to assess the carbapenem resistance in *P.aeruginosa* isolated from burn units and to estimate the antibacterial activity of AgNPs in combating MDR *P. aeruginosa*.

METHODOLOGY

Study design and patients.

This cross-sectional research was carried out in the Departments of Medical Microbiology, Clinical Pharmacology, and General Surgery of Menoufia University Hospitals between January 2024 to June 2024. A total of 46 samples positive for *P. aeruginosa* were collected from patients in the burn unit having burn injuries that range from moderate to severe accompanied with clinical manifestations of burn infection. Every patient provided a written informed permission. The Ethical Committee, Faculty of Medicine, Menoufia University provided its approval to the study's protocol (1-2024 COM 10-1). Inclusion criteria included patients showing symptoms and signs of burn wound infection. Patients who refused participation in the study, and patients who were colonized not infected were excluded from the study.

Sample size calculation:

According to Elda Righi et al.[13] minimum calculated sample size was found to be 46 subjects using Gpower power program.

Identification of isolated *P. aeruginosa*

The specimens were cultivated on the following agar plates: MacConkey, and 5% Blood agar, cetrimide agar (Oxoid, Himedia) incubated in aerobic environment for 24- 48 hours at 37°C. *P. aeruginosa* species differs from other species by growing on cetrimide agar[14] where it produces a greenish-yellow color. Well-isolated colonies were confirmed as *P. aeruginosa* by growth at 42ºC, positive oxidase reaction, pigment production, and other biochemical characteristics. The diagnosis was confirmed by detecting the *OprL* gene using PCR technique. After identification, isolates were inoculated in tryptic soy broth containing 16% glycerol and kept at -80°C.

Antibiotic susceptibility testing:

Testing drug susceptibility was assessed by the Kirby-Bauer agar disk diffusion method for all isolates, and the inhibition zones were measured per the recommendations of CLSI, 2021 [15]. The susceptibility profiles were determined for antibiotic discs including (meropenem 10 µg, imipenem 10 µg, colistin 10 µg, ciprofloxacin 5 µg, amikacin 30 µg, ceftazidime 30 µg and norfloxacin 10 µg) (Oxoid disks, UK). MDR *P. aeruginosa* were identified as strains that are resistant to at least one drug in three or more antimicrobial groups**.**

Phenotypic characterization of the carbapenemase production:

Modified carbapenem inactivation method:

Ten ul of *Pseudomonas aeruginosa* colonies were inoculated in 2ml of tryptic soy broth (TSB). A disc containing 10 µg meropenem was inserted in the inoculated tube and the mixture was put on a vortex. Tubes were incubated for 4 hours. Preparing a mixture of the mCIM indicator bacteria (*Escherichia coli* ATCC 25922, a carbapenem-susceptible strain) and adjusting turbidity to approximately 0.5 McFarland standard before the carbapenem inactivation step, and the standard disc diffusion susceptibility testing protocol was used to inoculate the surface of an MHA plate. By utilizing 10 µl inoculating loop the meropenem (MEM) disc was extracted from the bacterial culture, which was pulled along the tube's edge to eliminate extra liquid, and the disc was put on the inoculated MHA plate, then the plate was inversly incubated for about 18 to 24 hours at 35°C in ambient atmosphere [16]. Results were observed.

Genotypic detection of Carbapenemase encoding genes (*blaIMP*, *blaNDM*, *blaVIM,* and *blaKPC)* by real-time PCR:

DNA extraction:

As directed by the manufacturer, the bacterial DNA was extracted and purified from 46 *P. aeruginosa* isolates using the geneJET™ genomic DNA purification kit (ThermoFisher Scientific, UK).

PCR amplification:

Real-time PCR system (Applied Biosystems 7500) for amplification and detecting amplified products. The used primers are shown in **Table 1**. Reaction mix was prepared for each primer in a separate well to a total volume of 25ul as follow: 12.5 µl Maxima SYBR green qPCR master mix, 1 µl Forward primer, 1µl Reverse primer, 5 µl Bacterial DNA, and 5.5 µl Water. Amplification was done by initial denaturation at 95˚C for 10 min for 1 cycle: then 40 cycles of denaturation at 95C° for 15 sec, annealing and extension at 60 ˚C for 1 min. To confirm the specificity and identification of the PCR products, a melting curve analysis was carried out.

Table-1: Primer sets used for molecular detection of *P. aeruginosa* genes.

Targeted gene	Forward primer	Reverse primer	Reference
OprL	5'-ATGGAAATGCTGAAATTCGGC-3'	5'-CTTCTTCAGCTCGACGCGACG-3'	50
blaSPM	5-CCTACAATCTAACGGCGACC-3	5-TCGCCGTGTCCAGGTATAAC-3	51
blaVIM	5'-GTT TGG TCG CAT ATC GCA AC-3'	5'-AAT GCG CAG CAC CAG GAT AG-3'	52
blaIMP	5'-GAA GGA GTT TAT GTT CAT AC-3'	5'-GTA CGT TTC AAG AGT GAT GC-3'	
blaKPC	5'-CGT CTA GTT CTG CTG TCT TG-3'	5'-CTT GTC ATC CTT GTT AGG CG-3'	
blaNDM	5'-GGT TTG GCG ATC TGG TTT TC-3'	5'-CGG AAT GGC TCA TCA CGA TC-3'	

Silver nanoparticles (AgNPs):

Nanotech, Egypt provided a stock solution of commercially accessible AgNPs measuring 19±5 nm. AgNPs were prepared by chemical reduction as reported by the manufacturer.

The activity of different dilutions of AgNPs was examined against *P. aeruginosa* MDR strains by agar well diffusion method [17]. Where 100 µl of bacterial culture were added on the surface of Muller Hinton agar and allowed to settle down for 5 minutes. A steel well borer was used to pierce five wells with a diameter of 5 mm at the proper locations. Then 100 µl of different concentrations (4 mg/ml, 3 mg/ml, 2 mg/ml, or 1mg/ml) of AgNPs were added to the wells except the control well. All the plates were left at 4 °C for 15-30 minutes to provide proper diffusion of AgNPs into media and then placed in an incubator for 24 hours at 37°C then the zone of inhibition was measured.

AgNPs antibacterial activity by calculating MICs:

In accordance with the CLSI standards 2018[18], the broth microdilution procedure was applied. A stock solution of of AgNPs in a concentration of 1mg/ml (prepared in DMSO) was used to evaluate MIC against *P. aeruginosa*. Briefly, isolated colonies broth suspension was generated, then the turbidity of the suspensions was adjusted to meet the 0.5 McFarland standard, and a 96-well microtiter plate was filled with 50 µl per well to achieve a final concentration of ~ 5×10^5 CFU/mL per well. Subsequently, 50 µl of the diluted AgNPs were added per well to the microtiter plate. The plates were incubated for 24 hours at 37◦C. Positive and negative growth control wells were included. The MIC was determined by visual inspection of turbidity/non-turbidity as well as by photometric measurement at wavelength 600nm. After incubation, aliquots from wells that showed no discernible growth were subcultured into MHA plates in order to define MBCs. The MBC was defined as the maximum dilution of AgNPs which prevented the bacteria from growing on agar plates after additional 24 h incubation [19]. Minimum inhibitory concentration and MBC tests were run in triplicate.

Checkerboard assay to estimate the synergistic effect of antibiotics and AgNPs:

The combined activity of different antibiotics and AgNPs was investigated by calculation of fractional inhibitory concentration (FIC) in the two-dimensional checkerboard method. In current research we checked

the activity of different antibiotics combined with Moringa oleifera synthesized silver nano particles against P. aeruginosa. The checkerboard method was used to perform FIC studies in a 96 well plate. A proper amount of Muller Hinton broth (MHB) was poured into all wells, then a two-fold serial dilution of antibiotics was poured in all the wells (in combination with AgNPs) from A to G (mention on 96 well plates). Similarly, the two-fold serial dilution of AgNPs (in combination with antibiotic) was also poured in all the wells from 1 to 11 (mentioned on 96 well plates). Column 1 contains a two-fold serial dilution of antibiotics alone to determine the MIC of antibiotics while row A contains a two-fold serial dilution of AgNPs alone to determine the MIC of AgNPs. Initial OD600 was determined just before completing the experiment and then incubate the plates at 37C for 18-24 hours.

Statistical Analysis:

SPSS, version 26 (IBM; Armonk, New York, USA) was used to analyze the data. The means ± standard deviations were used to present continuous data. Numbers and percentages were used to display the categorical data.

RESULTS

Forty-six (38.3%) of the 120 wound samples were identified as *P. aeruginosa*. Hot fluids (scalds) caused 53% of injuries and flames caused the other 47%. Indoors-related accidents resulted in 61% of injuries and 39% were due to outdoors-related accidents, with a median hospitalization period of 12.5 days. Only few patients (12%) had surgery in the form of skin grafts. The median total burn area (TBSA) was 13%, varying from 8% to 42%. More than half of cases had 1st-degree burns (55%), while 45% of cases had 2nd and 3rd-grade burns. *P. aeruginosa* isolates were identified microscopically and biochemically and were confirmed genotypically by using primers of the *OprL* gene **(Table 1).**

Out of 46 isolates of *P. aeruginosa*, about 25 (54.3%) were MDR strains. The majority of isolates were resistant to Gentamicin (82.6%), Cefepime (87.0%), Imipenem (76.1%), and Ciprofloxacin (65.3%) (**Table 2).**

Table 2: Antibiogram analysis of different antibiotics against *P. aeruginosa*

Antimicrobial drugs	Antibiotics	Sensitive (%)	Resistant (%)	Intermediate (%)
Polymyxin	Colistin	26 (56.5)	15 (32.6%)	5 (10.9%)
Aminoglycosides	Tobramycin	5 (10.9%)	39 (84.8%)	2 (4.3%)
	Amikacin	11 (23.9%)	28 (60.9%)	7 (15.2%)
	Gentamicin	5 (10.9%)	38 (82.6%)	3 (6.5%)
Carbapenems	Meropenem	11 (23.9%)	26(56.5%)	9 (19.6%)
	Imipenem	7 (15.2%)	35 (76.1%)	4 (8.7%)
Cephalosporins	Cefoxitin	10 (21.7%)	30 (65.2%)	6 (13.0%)
	Ceftriaxone	8 (17.4%)	32 (69.6%)	6 (13.0%)
	Cefepime	4 (8.7%)	40 (87.0%)	2 (4.3%)
	Ceftazidime	6 (13.0%)	36 (78.3%)	4 (8.7%)
Fluoroquinolones	Ciprofloxacin	10 (21.7%)	30 (65.3%)	6 (13.0%)
Penicillins	Ampicillin	9 (19.6%)	31 (67.4%)	6 (13.0%)
	Piperacillin	12 (26.1%)	27 (58.7%)	7 (15.2%)

Regarding Carbapenemase production, mCIM revealed that 39/46 (84.8%) of *P. aeruginosa* isolates produced carbapenemase (**Fiqure 1**).

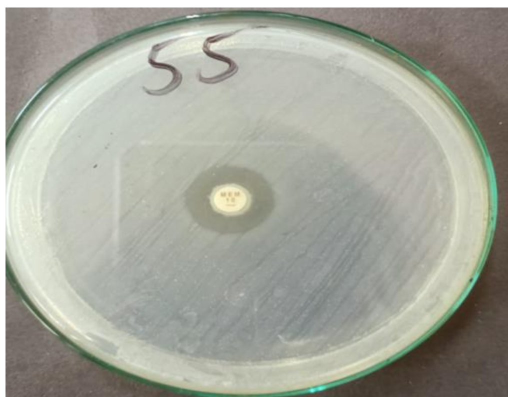

Fig. 1: Modified carbapenem inactivation method: representation of zone of inhibition (19mm) with pinpoints colonies around the disc, positive modified carbapenem inactivation

Thirty-five isolates of *P. aeruginosa* (76.1%) were positive for Carbapenemase-producing genes. Among these 35 cases, different carbapenemase genes were detected 60 times due to the coincidence of different carbapenemases. The prevalence of carbapenemase genes is shown in **(Table 3) and (Fiqure 2).** Regarding the 35 *P. aeruginosa* isolates positive for carbapenemase genes, 31/35 (91.4%) were phenotypically producing carbapenemases.

Table 3: Prevalence of carbapenemase genes in the studied *P. aeruginosa* bacteria

Name of gene	Gene prevalence	Gene frequency
blaSPM	9	15
blaVIM	15	25
blaNDM	23	38.3
blaKPC	2	3.3
blaIMP	11	18.3
Total	60	100

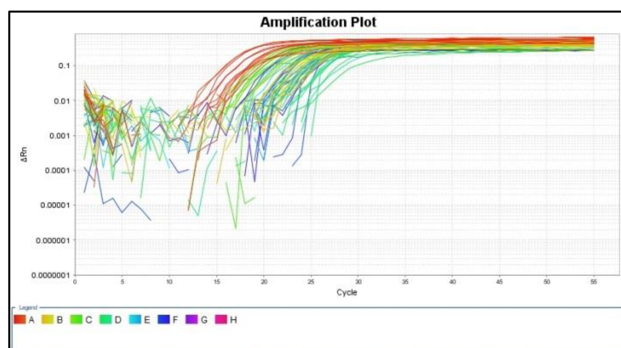

Fig. 2: Amplification blot of the studied genes in studied cases by real time PCR.

Regarding antibacterial activity of AgNPs, (**Figure 3**). The decline in bacterial growth increased with increasing the AgNP concentrations. The highest concentration (4 mg/ml) has a mean zone of inhibition of bacterial growth of (12 mm) which declined to a mean zone of inhibition of (2.5 mm) at the lowest concentration (1mg/ml) in all tested isolates of MDR *P. aeruginosa* MIC and MBC of the tested isolates were 250 µg/ml and 500 µg/ml respectively. Regarding the synergistic effect of combining AgNPs with colistin, ciprofloxacin, and gentamicin, all isolates exhibited additive and synergistic interactions with AgNPs and either colistin or gentamicin, however there was only partial synergism observed when AgNPs and ciprofloxacin were combined**.**

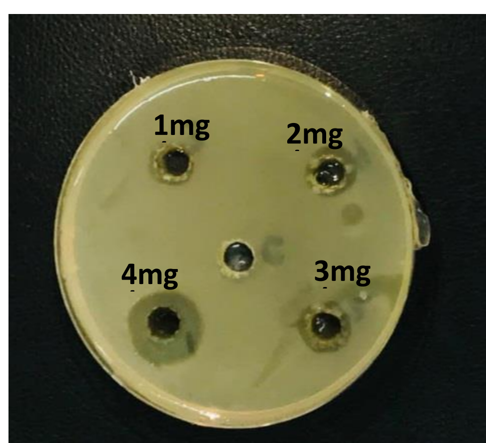

Fig. 3: Zone of inhibition of AgNPs by agar well diffusion

DISCUSSION

One hundred Twenty samples were collected from burn wounds of individuals diagnosed with burn and wound infections from Menoufia University Hospitals. *P. aeruginosa* was isolated from 38.3% of clinical samples, which is similar to Kabanangi et al., who found 39% of all specimens were *P. aeruginosa* [20]. Conversely, Ennab et al., [21]revealed a lower prevalence (11%), this difference may be due to the different characteristics of the patients studied. Also, may be due to differences in adherence to infection control practices, Makled et al [22]. *P. aeruginosa* is frequently identified as the causative agent of cutaneous infections, especially in hospitalized patients. Indwelling and trauma, surgical methods, prolonged hospitalization, poor sanitization, and patient neglect all contributed to the high prevalence.

The resistance to carbapenems is generally believed to be primarily due by the synthesis or acquisition of *MβL* genes such as *VIM* and *IMP* [23]. For the treatment of infections caused by *P. aeruginosa,* various antibiotic classes have been used. Resistance pattern exhibited

against antibacterial drugs included colistin (33%), tobramycin (85%), amikacin (61%), gentamycin (83%), meropenem (57%), imipenem (76%), cefoxitin (65%), ceftriaxone (70%), cefepime (87%), ceftazidime (78%), ciprofloxacin (65%), ampicillin (67%) and piperacillin (57%) which is similar to previous studies [23]. The main cause of the high level of resistance of *P. aeruginosa* is the irrational and inappropriate use of antibiotics in everyday clinical practice. The infection control practices and strategies followed in medical facilities affect the carbapenemase-producing *P. aeruginosa* in Egypt. In the current study, the prevalence of MDR *P. aeruginosa* bacteria was 25/46 (54.3%). Previous studies found varying percentages of MDR *P. aeruginosa* (38.46%, 31.42%, 28%, 65.2%, and 100%) [25&26]. Irrational antibiotic use, an unsanitary environment, and health-care personnel's activities were identified as contributing factors that may enhance the occurrence of MDR *P. aeruginosa* in hospitalized patients. The prevalence of *P. aeruginosa* positive for carbapenemase genes was 35/46 (76%) which is consistent with a previous study by Sheikh et al. who reported that carbapenemase genes were seen in 72.3% of *P. aerugino* isolates [27]. Another study by Aruhomukama et al. found a much lesser prevalence (7.4%) [28].

The prevalence of carbapenemase encoding genes were 9/60 (15%) for *blaSPM*, 15/60 (25%) for *blaVIM*, 23/60 (38.3%) for *blaNDM*, 2/60 (3.3%) for *blaKPC* and 11/60 (18.3%) for *blaIMP*. Similar results were obtained by previous studies [29-30]. Research from Iran reported that 9.75% of *P. aeruginosa* isolates that produced MBL were positive for *blaIMP* [31].

In the current study, 39/46 (84.8%) of the *P. aeruginosa* isolates produce carbapenemases whereas 35/46 (76%) showed positive carbapenemase producing genes. This difference is due to the presence of other carbapenemase encoding genes not included in the molecular design of our study. Out of the 35 *P. aeruginosa* isolates with positive PCR test for carbapenemase genes, the mCIM test was positive in 32/35 isolates (91.42%), negative in 2/35 (5.7%), and intermediate in 1/35 (2.85%). A previous study reported 27 cases were positive for carbapenemases, 16 were intermediate cases, and 57 were negative as detected phenotypically out of 100 cases with positive carbapenemase genes for carbapenemases [32]. Another study reported 29 (54.71%) *P. aeruginosa* isolates with carbapenemase positive phenotype out of 53 carbapenemase positive genotype while 21 (39.62%) isolates were negative, and 3(5.66%) isolates were intermediate [33].

Our results suggest that the detection of carbapenemase resistance in *P. aeruginosa* isolates by molecular detection and mCIM is highly concordant. PCR has the advantages of simplicity and speed; however, it is more expensive and requires special equipment.

In our study, 54.3% of isolates were multi-drug resistant. To overcome these complications and antibiotic resistance, scientists have focused on nanomaterials to solve this challenge for their use in the medical industry. Diagnostic and therapeutic procedures include the use of nanomedicines for different diseases. Nanocarriers have a significant effect on the development of medicine and the control of infection due to the secure distribution of drugs. Nanoparticles have a vital role in different fields of biology and medicine [34].

The results of the evaluation of AgNPs' antibacterial activity against *P. aeruginosa* showed that the bacterial growth in the wells decrease with increasing concentrations , which was similar to Ulagesan et al., who documented an increase in the zone of inhibition with the increase of the concentration of AgNPs [35]. The results of MIC and MBC of AgNPs were 250 µg and 500 µg for *P. aeruginosa,* respectively. Related results were recorded by Ulagesane et al. [35]. On the other hand, Abdolhosseini et al. reported that the MIC of AgNPs against all tested strains of *P. aerµginosa* was 1024 µg/ml [36]. Another study by Danjuma and Abdullahi reported that the MIC of AgNPs was 500 µg/ml [37]. Lower values for MBCs (25-100 µg/mL) and MICs (12.5-100 µg/mL) were reported in other research[38]. Habash et al. revealed AgNP activity at significantly lower concentrations with MICs were found to be between 0.312-2.50 µg/mL [39]. Rai et al. [40]state that many variables, including size, shape, stability, and concentration, are known to alter the antibacterial activity of AgNPs and may contribute to differences in the MIC levels.

By calculating the synergistic effect of AgNPs with Colistin, ciprofloxacin, and gentamicin, our results revealed the following: For colistin alone, the MIC was 16 µg/ml while the combination of colistin with AgNPs decreased the MIC of colistin to 4-8 µg/ml and MIC of AgNPs to 15.1-31.2 µg/ml and the mean FIC index of AgNPs with colistin was (0.49). Regarding gentamycin alone, the MIC ranged from 32-64 µg/ml while the combination of gentamycin with AgNPs decreased the MIC of gentamycin to 16 µg/ml and MIC of AgNPs to 15.1-31.2 µg/ml & and the mean FIC index of AgNPs with gentamycin was (0.47) these results are consistent with a synergistic effect. With ciprofloxacin, partial synergism was recorded with an FIC index of 0.7 which is concordant with *Fadwa et al.* who found equivalent results to our study [41]. Our results were different from results of the study performed by Danjuma and Abdullahi who showed complete synergism of ciprofloxacin with AgNPs [37]. Combining antibiotics with nanoparticles was a promising strategy to increase the effectiveness of antimicrobials against MDR *P. aeruginosa* at the lowest concentrations to avoid the

toxicity of synthetic chemicals. In our study, no single *P. aeruginosa* isolate showed any resistance against AgNPs either alone or in combinations with the tested antibiotics. However, to confirm the safe use of these combinations for the treatment of human bacterial infection, there is a need to perform cytotoxic assays on numerous human cell lines.

CONCLUSIONS

- Resistance to variable antimicrobial agents was reported in *P. aeruginosa* clinical isolates. Carbapenemase production is better evaluated by both standard phenotypic methods and PCR to identify the hidden genes. The majority of the isolates were MDR, a finding that indicates resistant isolates are alarmingly spreading. Infection management measures should be taken into account in order to stop the spread of resistant isolates.
- AgNPs have significant antibacterial effect and when used with other antimicrobials, it amplifies their efficacy. Treating diseases caused by MDR *P. aeruginosa* can be enhanced by this combination. Furthermore, the study can be expanded to the use of nanoparticles other than AgNPs, and cytotoxic activity would also be accomplished. Moreover, To evaluate the therapeutic safety of AgNPs, in-vivo investigations may also be carried out.

Declarations:
Consent for publication: Not applicable
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