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ORIGINAL ARTICLE

Antibacterial potentials of silver nanoparticles on multi-drug resistant *Pseudomonas aeruginosa* isolated from patients with catheter associated urinary tract infections

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ABSTRACT

Background*: A great alertness should be paid for the alarming rate of multi-drug resistance (MDR) in Pseudomonas aeruginosa (P.aeruginosa); the third most prevalent pathogen correlated to nosocomial catheter-associated urinary tract infections (CAUTIs), making traditional treatment much more difficult. Nanoparticles represent a promising alternative.* ***Objective****: To assess the antibacterial effect of silver nanoparticles (AgNPs) against MDR P. aeruginosa.* ***Methodology****: Minimum inhibitory concentrations (MICs) of AgNPs evaluated using broth microdilution assay and minimum bactericidal concentrations (MBC) determined by the lowest concentrations required to kill 99.9% of the initial inoculum.* ***Results:*** *The MIC and MBC values ranged from 1 to 8 µg/ml and 2 to 32 µg/ml, respectively.* ***Conclusion:*** *Obtained results were very promising suggesting that AgNPs exhibited considerable bacteriostatic and bactericidal effect, thus may be considered as an effective solution in fighting against MDR P.aeruginosa.*

INTRODUCTION

Health care associated infections (HAIs); are infections acquired when receiving health care, in any health care facility. Health care-associated infections appear 48 hours or increasingly after hospitalization, or within 30 days after discharge [1]. Urinary tract infections (UTIs) are accounted for to be the most prevalent type of HAIs and among healthcare associated UTIs, nearly 75% are associated with a urinary catheter [2]. Catheter associated urinary tract infections account for 12–16% of all hospitalized patients and up to 81.8% of all intensive care patients having an indwelling urinary catheter during their hospitalization [3].

Catheter associated urinary tract infection is defined as a UTI where an indwelling urinary catheter was in place for more than 2 calendar days on the date of event, starting from the day of device placement. At least one of the following criteria should be present in absence of other recognized cause: fever (>38°C), dysuria, urgency, frequency or suprapubic tenderness and a urine culture of $\geq 10^5$ CFU/ml with maximally two identified species of organisms [4].

Pseudomonas aeruginosa is a non-fermenter Gram-negative bacillus with a large intrinsic resistance to multiple antibiotics. This characteristic, paired with its quick ability to acquire new antimicrobial resistance determinants, makes this pathogen a seriously growing problem in the field of communicable diseases, particularly when it is nosocomial in origin[5]. *Pseudomonas aeruginosa* comes third among pathogens associated with nosocomial CAUTIs [6]. Despite advances in the anti-microbial therapy, the morbidity and mortality associated with *P. aeruginosa* induced UTIs remain significantly high [7].

Nowadays, the most troublesome part is the rapid increase in the number of HAIs due to MDR *P. aeruginosa*. Multi-drug resistant strains, have spread worldwide and have a worse prognosis being associated with significant morbidity and mortality, particularly in critically ill patients [8]. The main problem with MDR strains is that they are severely limiting the remaining treatment options, posing a major challenge for health care providers, since few secondary treatment options remain active, and those available have side effects [9].

The increasing utilization and immense studies of nanomaterial have brought new perspectives towards new antimicrobial material that could hinder the MDR bacteria pandemic currently faced [10]. Particularly, AgNPs are reported to exhibit strong biocidal properties on different bacterial species, including MDR bacteria [11-12]. In recent years, AgNPs have proven to be a promising nominee for the development of various antimicrobial products that can be used effectively when antibiotics fail to act [13].

Hence, the aim of this study was to evaluate the antibacterial effect of AgNPs against MDR *P. aeruginosa* isolated from patients with CAUTIs.

METHODOLOGY

A cross-sectional study was carried out in Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University and Zagazig University Hospitals. The study was approved by the Institutional Reviewer Board (IRB), Faculty of Medicine, Zagazig University.

Patients:

Inclusion criterion:

1. Hospitalized patients with CAUTIs [4].

Exclusion criteria:

1. Patient's refusal.
2. Patients not fulfilling the criteria of CAUTIs.

Informed written consents were gathered from patients or their relatives.

Bacterial strains:

One hundred and forty-eight urine samples were collected from hospitalized patients in Zagazig University Hospitals. Samples were collected from catheterized patients, according to the method described by Collee and Marr [14]; the catheter was clamped off to allow collection of freshly voided urine, then the wall of the catheter was disinfected with 70% alcohol above the level of clamping and urine was aspirated using needle and syringe and carried in a sterile container to the laboratory for processing.

Silver nanoparticles:

A stock solution of commercially available AgNPs (19±5 nm) were obtained from (Nano-Tech, Egypt).

Bacterial identification:

Bacterial cultures were identified morphologically and biochemically by standard laboratory procedures; specimens were inoculated on blood agar and MacConkey agar, incubated at 37°C for 24 to 48 h. Grown colonies were isolated, purified, identified by colonial morphology, Gram's staining, cytochrome oxidase, motility, and other biochemical tests) [15].

Antibiotics Susceptibility Test:

Antimicrobial sensitivity tests were performed by modified Kirby-Bauer's disk diffusion method [15]. Direct broth suspensions of isolated colonies and control strains were made and adjusted to a 0.5 McFarland standard turbidity "approximately cell density 1.5 x10^8 CFU/ml". From these suspensions, inocula were applied by sterile swabs on Muller Hinton Agar (MHA) plates. Antimicrobial disks (Oxoid and Liofilchem), were placed on the agar plates using sterile forceps and plates were incubated for 18-20 hours at 37°C. The antibiotic discs included ceftazidime-avibactam (CZA), ceftolozane-tazobactam (C/T), piperacillin/tazobactam (TZP), ticarcillin-clavulanic acid (T/C), ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), imipenem (IPM), meropenem (MEM), gentamicin (CN), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR). *Pseudomonas aeruginosa* ATCC 27853 was used for quality control of the susceptibility testing. Diameters of the inhibition zones were interpreted following the CLSI guidelines [16].

Antibacterial activity of AgNPs:

For determining MICs of AgNPs, broth microdilution method was used following the CLSI standard guidelines [17]. Briefly, direct broth suspensions of isolated colonies were made, then suspensions were adjusted to a 0.5 McFarland standard turbidity, diluted and 50µl was dispensed per well into a 96-well microtiter plate to reach a final concentration of ~ 5 × 10^5 CFU/mL per well. Silver nanoparticles concentrations ranged from 64 to 0.125 µg/ml in series of two-fold dilutions. The diluted AgNPs were then added to the microtiter plate (50 µl) per well. Each plate included growth and negative growth control wells, incubated at 37 °C for 20 h. The MIC was determined by visual inspection of turbidity/noturbidity. For defining MBCs, aliquots from wells that did not exhibit any visible growth after incubation were sub-cultured onto MHA plates. The MBC was defined as the highest dilution of AgNPs which prevented the growth of bacteria on agar plates after further 24 h incubation [18]. Minimum inhibitory concentration and MBC assays were performed in triplicate.

Statistical Analysis:

Data were analyzed using SPSS, version 24 (IBM; Armonk, New York, USA). Continuous data were presented as mean ± standard deviation. Categorical data were presented by the frequency and percentage.

RESULTS

One hundred and forty-eight hospitalized patients were included in this study; 68 males and 80 females with their ages ranging from 27- 56 years.

Thirty *P. aeruginosa* strains were identified and recovered from the 148 urine samples (20.3%).

Antibiotic susceptibility test:

Out of 30 isolates of *P. aeruginosa*, 28 (93 %) isolates were MDR, the highest resistance levels were recorded for ceftazidime (87%), piperacillin-tazobactam (87%) and gentamycin (83%). On the other hand, the lowest resistance levels were recorded for ceftazidime/avibactam (7%) and ceftolozane/ tazobactam (13%). Pattern of resistance of *P. aeruginosa* isolates to the tested antibiotics were presented in figure1.

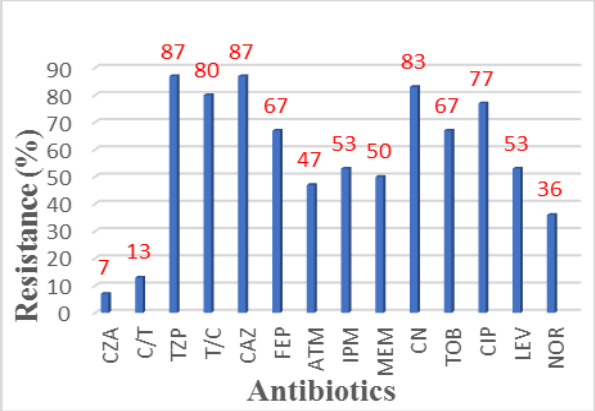

Fig. 1: Antibiotic resistance pattern of *P.aeruginosa* isolates

Antibacterial activity of AgNPs:

The result of AgNPs antibacterial assay showed that, the MIC and MBC values of the different concentrations of AgNPs against MDR *P.aeruginosa* ranged from 1-8 µg/mL and 2-32 µg/mL, respectively (Table 1).

Table 1 MIC and MBC values of AgNPs tested against MDR *P. aeruginosa* *(n=30)*

No. of isolates	MIC (µg/ml)	No. of isolates	MBC (µg/ml)
1	1	1	2
5	2	3	4
14	4	11	8
10	8	8	16
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MIC: minimum inhibitory concentration, M**BC**: minimum bactericidal concentration, **MDR**: multidrug resistant

DISCUSSION

In the current study, 30 *P. aeruginosa* were isolated from the 148 urine samples (20.3%). This result was in agreement with Helmy and Kashef [19], who reported that (22%) of the isolates *were P.aeruginosa*. Higher result was reported by Fattouh et al., where (35.3%) of the isolates were *P.aeruginosa* [20]. Variations in the prevalence rate of *P.aeruginosa* between studies might be due to differences in the study design, and period.

Out of 30 isolates of *P. aeruginosa*, 28 (93 %) isolates were MDR, this rate was higher than other Egyptian studies where the MDR isolates ranged from 34%- 56% [21-23]; which may reflect higher resistance rate in case of urinary tract infections also might be an indication of the increasing rate of MDR over times. Other global studies showed even lower MDR rates; 15.4 % in USA [24], 21 % in Spain [5], 24.8% in Thailand [25] and 50% in India [26]. Variations in the rate of MDR usually reflect geographical differences. MDR rate in the Egyptian studies is alarming and necessitates application of stringent antibiotic prescription policies in our country.

The highest percentages of resistance among *P.aeruginosa* isolates were for ceftazidime (87%) and piperacillin-tazobactam (87%). Low rates of resistance were recorded against ceftazidime-avibactam (7%) and ceftolozane tazobactam (13%). High rates of resistance to ceftazidime and piperacillin-tazobactam were also reported by Mahmoud et al. [21] and Ahmed and Asghar [27]**.** This result might be due to the wide use of third generation cephalosporins and piperacillin-tazobactam for treatment of UTIs.

Many researchers have proven the effectiveness of AgNPs against MDR bacteria such as MDR *Escherichia coli*, MDR *P.aeruginosa* and methicillin-resistant *Staphylococcus aureus* [28-30]. The MIC values of AgNPs against *P.aeruginosa* ranged from 1-8 µg/mL and the MBC values 2-32 µg/mL. This was comparable to, Liao et al. who reported MIC values ranging from 1.406–5.625 µg/mL [31]. These results were also consistent with Ebrahimi et al who reported a mean MICs of 3 µg/mL against MDR strains of *P.aeruginosa* [32].

On the other hand, Nasiri et al., reported higher values for MICs (12.5-100 µg/mL) and for MBCs (25-100 µg/mL) [18]. Similarly, Singh et al reported high MICs and MBCs range 50-100 µg/mL [33]. This was not the case with Habash et al. who reported AgNPs activity at much lower concentrations in which the MICs were found to be in the range of 0.312-2.50 µg/mL [34]. According to Rai et al., variations in the MIC levels might be caused by many factors that are known to modify the antibacterial effect of AgNPs, such as size, shape, stability, and concentration [35].

Results of the present study revealed that the antibacterial effect of AgNPs is concentration dependent in which the increase in the AgNPs concentration was associated with enhanced bacterial inhibition. In agreement with this finding Pazos-Ortiz et al. also stated that the antibacterial effect of AgNPs was concentration dependent [36]. This concorded with the results from Karimipour and Tanomand also studying *P. aeruginosa* [37]. This finding disagreed with Gheidar et al. who mentioned a non-dose dependent effect [38]. This variation could be attributed to differences in AgNPs concentration and size among different studies.

CONCLUSION

Pseudomonas aeruginosa can be considered as one of the most important and intractable nosocomial pathogens. Multi-drug resistant strains are rapidly disseminating limiting the remaining

therapeutic options. This study demonstrated a significant antibacterial effect of AgNPs against MDR *P.aeruginosa* making it a promising alternative for treatment of infections caused by MDR bacteria. Further investigations, particularly in vivo experiments, should be carried out to ensure effectiveness of nanoparticles, investigations to assay the probable cytotoxicity of this material are also important.

Conflicts of interest: The authors declare that they have no financial or non-financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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