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Evaluation of antibacterial activity of silver nanoparticles against multidrug-resistant Gram negative bacilli clinical isolates from Zagazig University Hospitals

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ABSTRACT

Background: The growing incidence of multidrug resistant (MDR) bacterial infections has become a public health crisis. This work aims to evaluate the *in-vitro* activity of silver nanoparticles (AgNPs), alone and in combination with the antimicrobials amikacin and ceftazidime, against MDR Gram-negative bacilli (GNB) isolated from clinical cases in Zagazig University Hospitals. **Methods:** In a cross sectional study, MDR GNB were isolated from different clinical specimens and were tested to determine the minimal inhibitory concentration (MIC), minimal bactericidal concentration (MBC) and bactericidal activity of AgNPs using broth microdilution method. The effect of combining subMIC levels of AgNPs (MIC/2 and MIC/4) with amikacin and ceftazidime, was also determined by broth microdilution. **Results:** A total of 63 MDR GNB was obtained during the study period (22 *E. coli*, 17 *Klebsiella*, 15 *Pseudomonas aeruginosa* and 9 *Acinetobacter* isolates). AgNPs demonstrated a bactericidal effect on all tested isolates with an MBC/MIC ratio of less than 4. When combined with amikacin, a synergistic effect was demonstrated on all tested *E.coli* and *Klebsiella* isolates at AgNPs MIC/2 and on 45.4%, 40% and 77.8% of *E.coli, P.aeruginosa* and *Acinetobacter* isolates, respectively at MIC/4. In combination with ceftazidime, AgNPs exhibited a synergistic effect on 100% of *E. coli* and 88.2% *Klebsiella* at both MIC/2 and MIC/4 and on 40% of *P. aeruginosa* isolates at AgNPs MIC/4. **Conclusions:** AgNPs exert a bactericidal activity on MDR GNB as well as a synergistic effect when combined with amikacin and ceftazidime suggesting them as a new weapon in the war against MDR GNB.

Introduction

Antimicrobial resistance in bacteria, especially in Gram negative bacilli (GNB), is a frightening problem that threatens the treatment and outcome of healthcare acquired infections increasing mortality rates and causing massive economic loss to both patient and nation [1].

According to the standardized terminology created by Centre for Disease Control and Prevention (CDC) and European Centre for Disease Control (ECDC), multi drug - resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories [2].

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This multidrug-resistance narrows antibiotic choices for definitive therapy. Additionally, resistance to last-line antibiotics and the limited availability of newly approved drugs affecting these bugs further aggravate the challenge. The use of abandoned antibiotics such as polymyxins and drug combinations have been introduced as a solution, however, it remains difficult to determine what combination would be most effective in any given clinical situation [3]. This makes alternative approaches to combat infections caused by MDR pathogens urgently needed and even mandatory [4].

In recent years, the use and research in nanomaterials have increased considerably. Nanoparticles (NPs), owing to their exceptionally small size and high surface to volume ratio, can penetrate the cell membrane of pathogenic microorganisms and interfere with important molecular pathways, formulating unique antimicrobial mechanisms [5].

Among these mechanisms, the disruption of membrane potential and integrity, inhibition of RNA and protein synthesis, as well as generation of reactive oxygen species have been suggested [6].

This antibacterial activity is exhibited by NPs, in particular metallic ones, against both Gam-positive and Gram-negative bacteria. Furthermore, when combined with optimal antibiotics, they demonstrate a synergistic effect which allows for using lower doses of both drugs decreasing their side effects and probably reducing the evolution of multidrug resistance mechanisms [7].

Among metallic NPs, silver NPs (AgNPs) gained much of interest owing to their powerful antimicrobial properties [8].

As data concerning this issue is limited in our hospital, this study aims to evaluate the *in-vitro* activity of AgNPs, alone and in combination with other antimicrobials, against MDR GNB isolated from clinical cases in Zagazig University Hospitals.

Material and Methods

A cross-sectional study was carried out at the Microbiology and Immunology Department, Faculty of Medicine, Zagazig University and Zagazig University Hospitals in the period between August 2018 and January 2019. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Reviewer Board (IRB), Faculty of Medicine, Zagazig University.

Patient selection and collection of samples

This study was conducted on 147 hospitalized patients who were admitted for more than 48 h to Zagazig University Hospitals and developed different infections. They included 64 males and 83 females with their ages ranging from 8 years to 82 year. A written informed consent was obtained from each patient or from patients' guardians before obtaining the samples. Clinical and laboratory data of each patient were obtained through a worksheet filled for each case.

Using standard microbiologic methods, different samples e.g. endotracheal aspirates, urine, pus and blood, were collected from patients, according to the type of their infections, and transported to the microbiology laboratory as soon as possible.

Isolation and identification

Following their isolation, identification of Gram negative colonies was primarily done by conventional microbiologic methods. *Acinetobacter* spp. identification was confirmed by the bioMerieux VITEK 2 compact 15 system.

Antibiotic susceptibility tests

Isolated GNB were tested for their susceptibility to different antimicrobials by disc diffusion method. Antibiotic discs included amikacin 30 µg, aztreonam 10 µg, cefotaxime 30 µg, amoxicillin/clavulanic acid (20/10µg), tetracycline 15 µg, piperacillin/tazobactam (100/10) µg, ceftazidime 30 µg, trimethoprim/sulphamethoxazole (1.25/23.75) µg, imipenem 10 µg, cefoxitin 30 µg, ciprofloxacin 5 µg, chloramphenicol 30 µg and nitrofurantoin 300 µg (for urine samples only). All discs were purchased from HiMedia Chemicals Pvt. Ltd., Mumbai, India.

Interpretation of inhibition zones diameters was done according to Kirby-Bauer zone diameter interpretative standards as documented by Clinical Laboratory Standard Institute guidelines[9]. In all antibiotic susceptibility tests, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC® 27853 (Global Bioresource, Center of American Type Culture Collection KWIK-STIK TM) served as a quality control strain. Multi drug -resistance (MDR) was defined as according to **Magiorakos *et al*.** [2].

Testing the antibacterial activity of AgNPs by broth microdilution

In a 96 well flat-bottom microtitre plate, 50 µl of the test bacterial suspension in Muller Hinton broth (MHB) (1×10^6 CFU/ml) were mixed with 50 µl of two fold serially diluted spherical AgNPs with an average size of 20±5 nm (NanoTech, Egypt) (range from 64 µg/ml to 2 µg/ml) to attain a final bacterial concentration of 5×10^5 CFU/ml [9]. The wells were mixed thoroughly, covered with a tight-fitting plastic cover and kept at 37ºC for 24 h. Wells with MHB, bacterial suspension and AgNPs solution alone served as sterility, positive and negative controls, respectively. The wells were visually inspected for growth

turbidity. A spectrophotometer reader device (BioTek, USA) was used to measure the optical density (OD) for each well of tested bacterial strain to be compared with that of negative and positive control wells. The absorbance was recorded at 600 nm wave length [10].

The MIC was measured as the lowest concentration with an OD below or equal to that of negative control. MBC was determined by subculturing the MIC dilutions as well as higher concentrations onto sterile Mueller Hinton agar plates incubated at 37ºC for 24 h. The lowest concentration of AgNPs which completely killed the tested bacteria was documented as MBC level. All steps were implemented in duplicate and the mean values were recorded. The MBC/ MIC ratio was calculated and if found lower than or equal to 4, AgNPs were considered to have a bactericidal effect [11].

Evaluation of the combined activity of AgNPs and antibiotics by broth microdilution

Two antibiotics (amikacin and ceftazidime) were tested in combination with sub MIC doses [MIC/2 (S1) and MIC/4 (S2)] of AgNPs to evaluate their efficacy using broth microdilution method. Two fold serial dilutions from both antibiotics were prepared and kept refrigerated at 2–4 °C. Two concentrations below the MIC values of AgNPs, detected in the previous step, were prepared (MIC/2 and MIC/4). Then, in a 96 well flat-bottom microtitre plate, 100 µl of two-fold serial dilution of each antimicrobial were mixed with 100 µl of bacterial suspension in MHB (final concentration 5×10^6 CFU/ml). The final concentrations of the tested antibiotics ranged from128 µg/ml to 0.0625 µg/ml. For each antibiotic, three rows were prepared; one tests the effect of the antibiotic alone and to the other two rows, AgNPs MIC/2 and MIC/4 were added, respectively.

Sterility, negative and positive controls were included in each plate. The inoculated plates were incubated at 37 °C for 16–20 h, then MIC values of the chosen antibiotics alone and with combination with AgNPs were detected by measuring their OD using spectrophotometer reader (BioTek, USA) at 600nm [12]. All steps were implemented in duplicate and the mean values were recorded.

Collected data from susceptibility tests were interpreted for each bacterial strain according to CLSI guidelines [9]. Fold change was calculated using the formula (MIC of A alone /MIC of A and B in combination). The fractional inhibitory concentration index (FICI) for antibiotic A was calculated using the formula (FIC of antibacterial A = MIC of antibacterial A in combination/MIC of antibacterial A alone).The FIC of antibacterial agent B was calculated in the same manner and the sum of the two agents FIC was combined to give the ΣFIC index (ΣFIC index = FIC of antibacterial A + FIC of antibacterial B), where A and B are the antibiotic and AgNPs, respectively. The values published by the American Society of Microbiology were used to decide the nature of the interaction; FICI < 0.5 synergy, 0.5 ≤ FICI < 1 partial synergy, FICI = 1 additive, 2 ≤ FICI < 4 indifferent, and 4 < FICI antagonism [13].

Statistical analysis

Data was analyzed using Statistical Package for the Social Sciences (SPSS version 19.0) software for analysis. Continuous data were checked for normality by using Shapiro Walk test. Mann-whitney test was used to compare two groups of not-normally distributed data. Kruskall-wallis test was used to compare more than two groups of not-normally distributed data. Categorical data were compared using Chi-square test (χ 2 test). P value of < 0.05 was considered significant.

Results

Among a total of 106 obtained GNB, 63 strains (59.4%) were found to be MDR. They consisted of 22 *E. coli*, 17 *Klebsiella*, 15 *P. aeruginosa* and 9 *Acinetobacter* isolates.

The MIC and MBC values of AgNPs with different MDR GNB species as well as MBC/MIC ratios are demonstrated in **Table 1 and Figure 1**. AgNPs demonstrated a bactericidal effect on all tested isolates with an MBC/MIC ratio of almost less than 4.

Regarding the combination with amikacin**, Table 2** demonstrates the MIC fold change as well as FICI values of different tested species when amikacin was combined with MIC/2 and MIC/4 concentrations of AgNPs. The table shows that there was a synergistic effect between amikacin and AgNPs (MIC/2) against all examined *E.coli* and *Klebsiella* isolates and against 88.9% and 86.7% of *P.aeruginosa* and *Acinetobacter* isolates respectively, while the combination with AgNPs (MIC/4) recorded a synergistic effect against only 45.4% of *E.coli,* 40% of *P.aeruginosa* and 77.8% of *Acinetobacter* isolates and a partial synergistic effect against 41.2 % *of Klebsiella* isolates.

Table 3 and Figure 2 demonstrate the MIC fold change as well as FICI values of different tested species when ceftazidime was combined with MIC/2 (S1) and MIC/4 (S2) concentrations of AgNPs. Regarding, the combination effect, there was a synergistic effect between ceftazidime and AgNPs (MIC/2) against all *E. coli* isolates (100%), 88.2% of *Klebsiella,* 46.7% of *P.aeruginosa* isolates, while 66.7% of *Acinetobacter* isolates showed indifferent effect. In combination with AgNPs (MIC/4), the same previous ratio of *E.coli* and *Klebsiella* and 40% of *P.aeruginosa* isolates showed synergistic effect, while

77.8% of *Acinetobacter* isolates showed indifferent effect.

Table 1. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of silver nanoparticles (AgNPs) and their ratio among MDR GNB

Variable (µg/ml)	*E. coli* (n=22)	*Klebsiella* (n=17)	*P. aeruginosa* (n=15)	*Acinetobacter* spp. (n=9)	P
MIC:					
Mean ± SD	8.55 ± 3.33	7.29 ± 2.91	7.33 ± 5.05	12.4 ± 4.21	
Median	8	8	8	16	**0.01**
Range	4 – 16	4 – 16	2 – 16	8 – 16	**(S)**
MBC:					
Mean ± SD	15.6 ± 7.6	14.3 ± 6.17	12.4 ± 9.20	23.1 ± 8.43	**0.01**
Median	16	16	8	16	**(S)**
Range	8 – 32	4 – 32	2 – 32	16 – 32	
MBC/MIC ratio:					0.89
Mean ± SD	1.86 ± 0.63	2.11 ± 0.99	1.90 ± 1	2.11 ± 1.16	(NS)
Median	2	2	2	2	
Range	1 – 4	1 – 4	0.5 – 4	1 – 4	

*S; significant, NS; non-significant

Figure 1. A microtiter plate for testing antibacterial activity of AgNPs on different bacterial clinical isolates (each isolate in a vertical row), the black arrow is pointing to MIC value equalling 8 µg/ml of a *P. aeruginosa* isolate obtained from a case of ventilator-associated pneumonia (VAP).

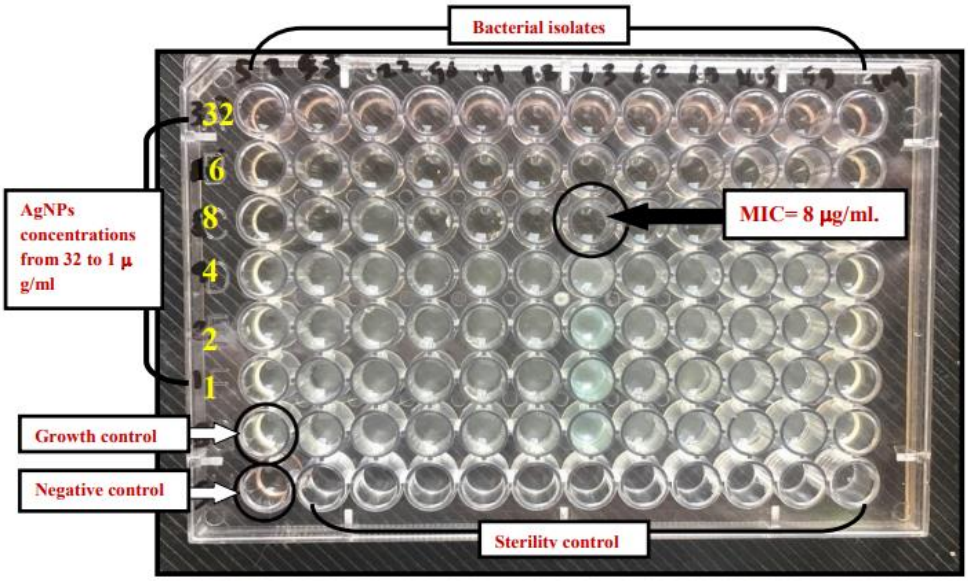

Table 2.: Results of combination of sub-MIC concentrations of AgNPs (MIC/2, S1) and (MIC/4, S2) with amikacin among MDR GNB by broth microdilution

Variable	*E. coli* (n=22)	*Klebsiella* (n=17)	*P. aeruginosa* (n=15)	*Acinetobacter* spp. (n=9)	P
Fold change(S1):					
Mean ± SD	35.6 ±36.5	71.7 ±106.3	57.07 ±59	21.3 ±8	0.148
Median	16	16	64	16	(NS)
Range	4 – 128	4 – 256	8 – 256	16 – 32	
Fold change (S2):					
Mean ± SD	6.67 ±4.91	6.12 ±5.85	8.93 ±7.12	16 ±6.92	
Median	4	4	8	16	**0.001**
Range	2 – 16	2 – 16	2 – 32	8 – 32	**(S)**
FICI (S1):					
Mean ± SD	0.15 ±0.08	0.22 ±0.17	0.21 ±0.27	0.21 ±0.15	
Median	0.12	0.14	0.14	0.15	0.642
Range	0.03 – 0.37	0.03 – 0.53	0.03 – 1.01	0.04 – 0.56	(NS)
FICI (S2):					
Mean ± SD	0.69 ±0.39	0.87 ±0.41	0.83 ±0.66	0.28 ±0.18	
Median	0.56	0.75	0.75	0.25	**0.001**
Range	0.3 – 2.06	0.5 – 2.25	0.25 – 2.5	0.04 – 0.56	**(S)**
Combination effect (S1):					
IN:	0 (0%)	0 (0%)	1 (6.7%)	0 (0%)	
PS:	0 (0%)	0 (0%)	1 (6.7%)	1 (11.1%)	0.316
SN:	22 (100%)	17 (100%)	13 (86.7%)	8 (88.9%)	(NS)
Combination effect (S2):					
AD:	0 (0%)	3 (17.6%)	0 (0%)	0 (0%)	
IN:	4 (18.2%)	4 (23.5%)	5 (33.3%)	0 (0%)	
PS:	8 (36.4%)	7 (41.2%)	4 (26.7%)	2 (22.2%)	**0.04**
SN:	10 (45.4%)	3 (17.6%)	6 (40%)	7 (77.8%)	**(S)**

AgNPs MIC/2 (S1) ranged between 1 and 8 µg/ml, while MIC/4 (S2) ranged between 0.5 and 4 µg/ml. *FICI; Fractional inhibitory concentration index, IN; indifference, PS; partially synergistic, SN; synergistic, AD; additive, S; significant, NS; non-significant

Figure 2. A microtiter plate showing combined antibacterial activity of sub MIC values of AgNPs (S1 = 4µg/ml and S2 = 2 µg/ml) and ceftazidime (A) against 2 MDR isolates (E. coli and Klebsiella) obtained from patients with VAP and urinary tract infection (UTI), respectively. The black circles represent MIC values before and after combination.

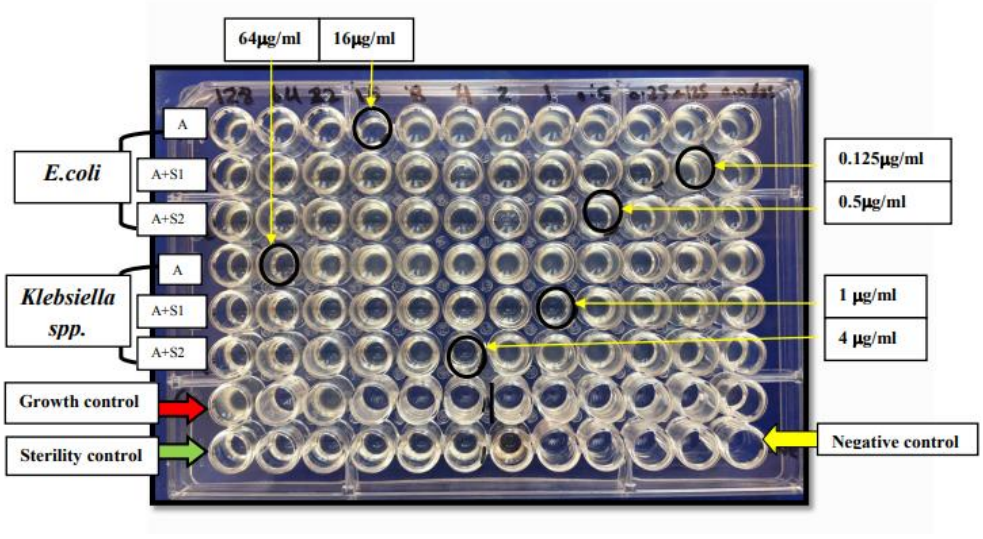

Table 3. Results of combination of sub-MIC concentrations (MIC/2, S1) and (MIC/4, S2) of AgNPs with ceftazidime among MDR GNB by broth microdilution

Variable	*E. coli* (n=22)	*Klebsiella* (n=17)	*P. aeruginosa* (n=15)	*Acinetobacter* spp. (n=9)	P
Fold change(S1):					
Mean ± SD	152 ±150	58.5 ±43.4	49.3 ±40.4	7.11 ±4.25	**<0.001**
Median	128	32	32	8	**(HS)**
Range	32 – 512	4 – 128	8 – 128	2 – 16	
Fold change(S2):					
Mean ± SD	75.2 ± 37.8	67.3 ± 135	65.1 ± 71.6	4.78 ± 2.63	**<0.001**
Median	64	32	32	4	**(HS)**
Range	32 – 128	4 –128	8 – 128	1 - 8	
FICI(S1):					
Mean ± SD	0.06 ±0.06	0.34 ±0.50	0.52 ±0.59	1.32 ±0.82	**<0.001**
Median	0.05	0.23	0.51	1.12	**(HS)**
Range	0.01 – 0.26	0.03 – 2.25	0.01 – 2.12	0.31 – 2.5	
FICI(S2):					
Mean ± SD	0.09 ± 0.07	0.38 ± 0.56	0.76 ± 0.78	1.76 ± 0.84	**<0.001**
Median	0.09	0.28	0.53	2.12	**(HS)**
Range	0.01 – 0.26	0.01 – 2.25	0.03 – 2.12	0.62 - 3	
Combination effect(S1):					
IN:	0 (0%)	1 (5.9%)	4 (26.7%)	6 (66.7%)	**<0.001**
PS:	0 (0%)	1 (5.9%)	4 (26.7%)	1 (11.1%)	**(HS)**
S:	22 (100%)	15 (88.2%)	7 (46.7%)	2 (22.2%)	
Combination effect(S2):					
IN:	0 (0%)	1 (5.9%)	6 (40%)	7 (77.8%)	**<0.001**
PS:	0 (0%)	1 (5.9%)	3 (20%)	2 (22.2%)	**(HS)**
S:	22 (100%)	15 (88.2%)	6 (40%)	0 (0%)	

AgNPs MIC/2 (S1) ranged between 1 and 8 µg/ml, while MIC/4 (S2) ranged between 0.5 and 4 µg/ml.
*FICI; Fractional inhibitory concentration index, IN; indifference, PS; partially synergistic, S; synergistic, AD; additive.

Discussion

This study aimed to detect the antibacterial activity of spherical AgNPs with an average size of 20±5 nm against MDR GNB isolates from nosocomial infections in Zagazig University Hospitals and also to investigate the effect of combination between AgNPs and two antibiotics (amikacin and ceftazidime) against the isolated MDR GNB.

The frequency of MDR GNB (59.4%) detected in the current study comes in line with the findings recorded previously [14] where among 143 rectal swabs obtained from refugee patients at Germany University Hospital, 60.8% were positive for MDR GNB. Higher frequency (≥ 70%) was reported [15] among fermentative GNB recovered from intensive care unit (ICU) patients in Ethiopia. In contrast, lower prevalence of MDR infections was recorded at ICUs of Germany University Hospital [16] and Mexico cancer center [17] recording 33.8%, and 39.5% among 325 and 266 isolated bacteria, respectively. The higher isolation frequency of MDR GNB in the current study could be attributed to differences in antibiotic policy or the injudicious usage of antibiotics in some cases in our hospital.

The recorded MICs values of AgNPs in the current study come very close to the values reported previously [18] when AgNPs of an average size of 8.23 nm ± 0.91 nm were tested against MDR uropathogens including *E.coli*, *Klebsiella*, *P.aeruginosa* and *Acinetobacter* isolates with MIC values of (8, 4, 8 and 16 µg/ml). Higher MIC values of 31.25µg/ml and 62.5µg/ml were reported [19] on using 11-21 nm AgNPs against *E.coli* and *Klebsiella pneumoniae*, respectively. An obviously lower MIC value was reported [20] with AgNPs MIC of 1 µg/ml against *P.aeruginosa*. This apparent disparity in MIC values between the current study and other studies could be due to different size, shape and manufacturing method of the used AgNPs [21].

In the current study, AgNPs exhibited a bactericidal effect against all tested MDR GNB with an

MBC/MIC ratio of 4 or less. A previous report [11] recorded a bactericidal activity of AgNPs with an MBC/MIC ratio of 2 which supports this result.

Furthermore, we have investigated the combined antibacterial activity of AgNPs at sub MIC concentrations (MIC/2 and MIC/4) with amikacin and ceftazidime in an attempt to use lower doses of both drugs yet the same or even augmented antibacterial effects. Regarding amikacin, the MIC fold changes and FICI values demonstrated a synergistic or partially synergistic effect between AgNPs and amikacin against most of tested GNB regardless the used AgNPs concentration (either MIC/2 or MIC/4). Additionally, *P.aeruginosa* isolates showed a considerable decrease in the MIC fold change of amikacin (median of 64µg/ml) on using MIC/2 concentration of AgNPs. Similarly, the MIC values of *Acinetobacter* isolates decreased (median fold change of 16 µg/ml) on using MIC/4 concentration of AgNPs.

A previous study [18] strongly supports the obtained results recording a synergistic effect of AgNPs of 8.23 nm size and at concentration of 4-16 µg/ml where a decrease in the MIC of amikacin by 16, 2, 8, 32 folds against *E.coli*, *Klebsiella*, *P.aeruginosa* and *Acinetobacter* isolates were recorded. These findings locate within our recorded ranges against the same isolates respectively (2-16, 2-16, 2-32 and 8-32). Moreover, They also recorded FICI of (0.56, 0.75, 0.63 and 0.28) against *E.coli*, *Klebsiella*, *P.aeruginosa* and *Acinetobacter* isolates and these are almost the same recorded results of the current study on using MIC/4 AgNPs(median FICI of 0.56, 0.75, 0.75 and 0.25). A significant synergistic effect of 8-12 nm AgNPs at a concentration of 15µg/ml was reported previously [22] when combined with amikacin against *Acinetobacter* isolates, where amikacin MICs values markedly decreased by 64 folds (from 128 to 2 µg/ml). This comes in partial agreement to our finding where the fold change of amikacin MIC was at range of 16 – 32 when combined with MIC/4 AgNPs against *Acinetobacter* isolates.

Regarding the combination with ceftazidime**,** we observed a significant synergistic effect against 100% of *E. coli* either with MIC/2 or MIC/4 AgNPs. Meanwhile, this was not the case with *P.aeruginosa* and *Acinetobacter* isolates where at MIC/4, only 40% of *P.aeruginosa* isolates showed a synergistic effect and only 22.2% of *Acinetobacter* isolates showed partial synergy.

Very interestingly, the current study also revealed that some of the tested bacteria which were completely resistant to ceftazidime became susceptible after combining it with AgNPs. This result may provide a novel helpful approach in the development of new antimicrobial agents.

In agreement with the current study, a synergistic effect was confirmed previously on combining AgNPs with 26 nm size with different antibiotics and was exhibited against almost all resistant bacterial strains [23]. The greatest enhancement was observed at AgNPs concentrations of MIC/2 and MIC/4, with the MICs of the antibiotics being as much as 100–fold lower. In addition, FICI mean values of ceftazidime combined to different concentrations of AgNPs below MIC levels were found to be 0.36 and 0.2 in case of *E.coli* and *Klebsiella* strains respectively [23]. This comes nearly similar to the current study findings that recorded a synergistic effect with mean FICI values of 0.05 and 0.23 for ceftazidime with MIC/2 AgNPs against *E.coli* and *Klebsiella* isolates, respectively.

On the other hand, the antibacterial effect of ceftazidime was enhanced after adding of 10-60 nm AgNPs at concentration of 15µg/ml against *E.coli* and *P.aeruginosa* strains with MIC values reduced from 0.125 to 0.03 µg/ml (4 fold change) and from 0.5 to < 0.015µg/ml(> 34 fold change), respectively, while no enhancement was recorded against *Acinetobacter* strains [22]. These findings are in partial agreement with the findings of the current study which revealed a higher fold change of ceftazidime MIC (median 128, 32, 8) against *E.coli*, *P.aeruginosa* and *Acinetobacter*, respectively on adding AgNPs MIC/2 to ceftazidime.

Whether different mechanisms of antimicrobial resistance, as production of extended-spectrum beta lactamases, could have an influence on the antibacterial activity of AgNPs, this was not addressed in the current study and needs o be more clarified.

Still there are some limitations in the current study as we did not compare AgNPs of different sizes in order to determine those with the best antibacterial effect, also the limited number of antibiotics used is another limitation.

Conclusion

In spite of their variable behavior with different species of MDR GNB, AgNPs were shown to exert not only a bactericidal activity on MDR GNB, but furthermore, a synergistic effect when combined with other antibiotics (amikacin and ceftazidime). This could probably allow using lower doses of antimicrobials and hence better management of infections caused by MDR GNB.

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Competing interests

Non declared

Authorship:

Each author listed in the manuscript had approved the submission of this version of the manuscript and takes full responsibility for it.

Ethical approval

The study was approved by the Institutional Reviewer Board (IRB), Faculty of Medicine, Zagazig University, Egypt.

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