RESEARCH PAPER

Antibacterial Activity of Bio-Silver Nanoparticles in Combination with Antibiotics

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Abstract: Drug resistance among bacteria is a concerning issue in the medical field. Silver nanoparticles (AgNPs) are one of the promising novel nano-antibiotics. In the present study, AgNPs were synthesized using a cell-free extract of Acinetobacter sp. challenged with silver nitrate. Preliminary observation was done using UV-Vis spectrophotometry at 420 nm. The complete reduction of silver ions to AgNPs was confirmed through cyclic voltammetry. Electron microscopy revealed the formation of spherical-shaped nanoparticles of size up to 20 nm. These AgNPs were further used to determine their effect on the activity of various antibiotics against pathogenic bacteria such as Neisseria and Xanthomans. Higher antibacterial activity of AgNPs was observed against Gram-negative bacteria. Enhanced antibacterial action of AgNPs was observed with selective β-lactam antibiotics producing up to a 3-fold increase in area of zone of inhibition. On exposure to AgNPs, the minimum inhibitory concentration and minimum bactericidal concentration of antibiotics were lowered by up to 2000 times indicating potential synergistic action of AgNPs. This study signifies that the drug, which proved to be inefficient due to bacterial resistance, could be made functional again in the presence of AgNPs. This will help in the development of novel antibacterial formulations containing antibiotics and nanoparticles to combat multiple drug resistance in microorganisms.

Keywords: Silver nanoparticles (AgNPs), Antibacterial, Synergy, Biomedical application.

1. INTRODUCTION

Drug resistance among pathogenic microorganisms is one of the concerns in health. The development of drugs and their analogues kept pace with the advent of resistance to issues concerning human microorganisms until 30 years ago [1]. Since then, multi-drug resistant strains have emerged in many species causing infections and diseases. It is estimated that at least 700,000 people die worldwide every year due to drug-resistant infections, and this number could rise to 10 million in three decades if the problem of antimicrobial resistance is not addressed [2]. Horizontal gene transfer by conjugation, transduction, transformation and outer membrane vesicles can be the possible ways to acquire antibiotic resistance [3]. However, antibiotic therapy may also lead to inheritable resistance among microorganisms due to misuse, overuse and anomalous combinations [4]. Resistance to conventional antibiotics either prohibits their usage due to ineffectiveness or enforces their high-dose administration leading to intolerable toxicity [5]. Moreover, the successful availability of a single new drug in the market takes years of

research and billions of currencies. This is evident from the fact that only three new classes of drugs have been launched since 2010 for human use. Furthermore, enough analogues are not reaching the market to curb the high tide of antibiotic resistance [6, 7]. Therefore, there is an indispensable need for alternative strategies to combat microbial resistance, one of which could be nanomaterials.

Recently, nanotechnology has introduced new paradigms in diagnosis, therapeutics and medicine, where nanoparticles constitute a novel alternative owing to ease of synthesis, reduced cost and upscale production with controlled morphology [8, 9]. Metal nanoparticles exhibit high specificity and sensitivity due high surface area to volume ratio [10]. These have unique size-dependent physical, chemical, optical, electrical, thermal, magnetic, mechanical and biological properties as compared to their bulk counterparts [11]. Silver nanoparticles (AgNPs) are well known for excellent antimicrobial activity against bacterial and fungal pathogens [12-14]. Few reports have also suggested the enhanced activity of antibiotics in the presence of AgNPs [15, 16]. Biological AgNPs synthesized using plants [15,

17] or microorganisms [12, 13, 18] are safe, less toxic and biocompatible as compared to those synthesized through physical or chemical approaches [11]. In recent years, bacteria-mediated synthesis of metal nanoparticles has gained importance due to its ease of handling and eco-friendliness [19]. Both Gram-negative and Gram-positive bacteria have been employed to synthesize AgNPs through bacterial components, cellular biomass, supernatant and aqueous cell-free extract [5]. Cell-free extract (CFE) provides the benefits of extracellular synthesis in an aqueous environment eliminating the need for downstream processing to recover nanoparticles. In this view, the present study has been carried out to elucidate the effect of AgNPs, synthesized through CFE, on the bactericidal activities of various antibiotics against drug-resistant bacteria.

2. EXPERIMENTAL PROCEDURES

2.1. Cultures

An environmental isolate, *Acinetobacter calcoaceticus*, was employed for AgNP synthesis. The cultures used for antibacterial assays were procured from the Microbial Type Culture Collection (MTCC), Chandigarh, India. These include Gram-negative (*Neisseria mucosa* MTCC 1772, *Serratia odorifera* MTCC 495, *Xanthomonas campestris* MTCC 2286) and Gram-positive (*Bacillus subtilis* MTCC 441, *Micrococcus luteus* MTCC 2470) bacterial pathogens.

2.2. Antibiotics

Eighteen antibiotics, namely amikacin, amoxicillin, ampicillin, ceftriaxone, ciprofloxacin, chloramphenicol, doxycycline, gentamicin, kanamycin, piperacillin, streptomycin, tetracycline, vancomycin (HiMedia, Mumbai, India), ceftazidime, faropenem, carbenicillin (GlaxoSmithKline Pharmaceutical Limited, Nashik, India), trimethoprim (Sigma-Aldrich, St. Louis, MO, USA), and penicillin (Alembic Pharmaceutical Limited, Vadodara, India) were used for antibacterial assay.

2.3. Synthesis and Characterization of Silver Nanoparticles (AgNPs)

AgNPs were synthesized through the CFE of *Acinetobacter calcoaceticus* as described in our earlier study [16]. Briefly, cells of 24 h grown culture were suspended in sterile distilled water after thorough washing at 30°C/150 rpm for 72 h. Aqueous CFE was collected by centrifugation at 10,000 rpm and then passing the supernatant through a 0.2-micron filter. The CFE was then challenged with 0.7 mM silver nitrate (HiMedia) and incubated at 70°C for 7 days at static conditions. AgNP biosynthesis was monitored by visual observation for color change and measuring UV-Vis spectrum between 300 to 800 nm on SpectraMax M5 Multi-mode Microplate Reader (Molecular Devices LLC, Sunnyvale, CA, USA). To ensure a complete reduction of silver ions, cyclic voltammetry (PGSTAT302N, Metrohm Autolab BV, The Netherlands) was performed. The size and shape of AgNPs were analyzed under the transmission electron microscope (TEM) (FEI Company, Eindhoven, The Netherlands).

2.4. Antibacterial Activity

The disc-diffusion assay was performed to determine the antibacterial effect of AgNPs on antibiotics against pathogenic bacteria [16]. In brief, 100 μl of OD adjusted ($OD_{620} \approx 1.0$) overnight grown test strains were applied to the Muller Hinton (MH) agar (HiMedia) plates containing discs impregnated with AgNPs (1-1024 μg/disc) and antibiotics (30 μg/disc). A synergistic effect was determined with a combination of AgNPs (15 μg/disc) and antibiotics (30 μg/disc). The plates were incubated at 37°C. After 20 h incubation, the zone of inhibition was recorded and the fold increase in the area of the zone of inhibition was calculated using the formula-

Fold increase in area of zone of inhibition (C)

$$= \frac{B^2 - A^2}{A^2}$$

where A and B are the zones of inhibition (mm) obtained for antibiotics in the absence and presence of AgNPs, respectively. The experiment was performed in duplicate.

The minimum inhibitory concentration (MIC) of AgNPs, antibiotics and a combination of both was determined by broth micro-dilution assay as described by the Clinical Laboratory Standards Institute (CLSI). Two-fold serial dilutions of AgNPs and antibiotics ranging from 4096 to 0.0015 μg/ml were made in microtitre plates using MH broth. To each well, 5 μl inoculum containing 5×10^5 CFU/ml was added. To evaluate

the synergistic effect, a sub-inhibitory concentration of AgNPs (final concentration 15 µg/well) was added to each well having different concentrations of antibiotics. The plates were then incubated at 37°C for 20 h and results were recorded. The lowest concentration inhibiting the bacterial growth as observed with the naked eye was taken as the MIC. The experiment was further extended to determine the minimum bactericidal concentration (MBC) by spotting 5 µl aliquot from the wells showing no visible growth onto MH agar plates. The lowest concentration showing no colony after 20 h incubation at 37°C was taken as MBC. The assay was performed in duplicate.

3. RESULTS AND DISCUSSION

Acinetobacter is a Gram-negative bacterium having nutrition, metabolic and genetic versatility and excellent biofilm-forming capability [16, 20]. It is commonly found in environments including soil, human skin, sludge, etc [21-24]. Species of *Acinetobacter* have been reported to exhibit silver ion resistance indicating their ability to survive in silver-rich environments [25]. In the present study, biosynthesis of AgNPs employing 72 h CFE of *A. calcoaceticus* was carried out. A prominent color change from colorless to reddish brown indicated the synthesis of AgNPs (Fig. 1).
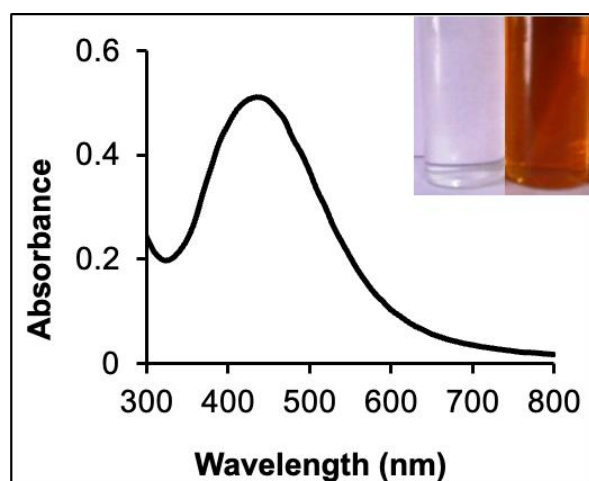

Fig. 1. UV-Vis spectra of AgNPs depicting peak at 420 nm. Inset: Color change observed in CFE on the addition of silver nitrate.

A single surface plasmon resonance peak at 420 nm in the UV-Vis spectrum also confirmed the formation of AgNPs through bacterial CFE. This peak has been observed between 400-450 nm for AgNPs having sizes ranging from 2 to 100 nm [26]. DNA, amino acids, peptides and proteins have been reported to act as reducing agents to form nanoparticles [27-29]. Aqueous CFE contains the biomolecules released by bacterial cells owing to autolysis or starvation, which may act as reducing agents to convert silver ions to nanosilver [5, 16].

In cyclic voltammetry, silver nitrate showed one reduction peak for reduction of Ag^+ to Ag^0 and one oxidation peak indicating oxidation of Ag^0 to Ag^+ (Fig. 2). However, no such peaks were observed in AgNP solution confirming the complete reduction of silver ions of silver nitrate to AgNPs. In another study, gold chloride salt was shown to have two peaks for conversion of Au^{+3} to Au^{+1} and Au^{+1} to Au^0 while no such peaks were found in gold nanoparticles [24].
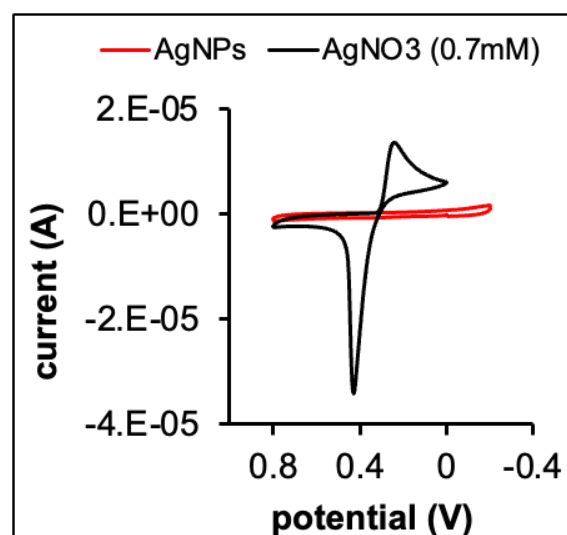

Fig. 2. Cyclic voltammetry of silver nitrate and AgNPs.

TEM analyses revealed the formation of spherical and oval-shaped AgNPs with size up to 20 nm at 70°C with 0.7 mM silver nitrate (Fig. 3). Synthesis of 45-60 nm AgNPs has been reported in *Lactobacillus acidophilus* employing CFE of dried bacterial biomass [30].

Silver compounds are well known for their Antimicrobial nature [31]. AgNPs, however, have enhanced properties due to a high surface area-to-volume ratio [10]. In the current study, the effect of AgNPs on the activities of antibiotics was investigated against pathogenic bacteria. AgNPs were found to have higher antibacterial activity

against *N. mucosa* and *S. odorifera* as compared to *M. luteus* and *B. subtilis* (Table 1). These results are following the published literature [15, 16, 32]. The difference in the action of AgNPs against Gram-negative and Gram-positive bacteria is due to the structural difference in their cell wall composition. The thick peptidoglycan layer in the cell wall of Gram-positive bacteria acts as a barrier to AgNP penetration making these bacteria more resistant to nanoparticle treatment [32]. In the disc-diffusion assay, a zone of inhibition on exposure to AgNPs was observed in the concentration range of 1024 to 32 µg/disc (Table 1). Therefore, sub-inhibitory concentration (15 µg/disc) of AgNPs was used to determine the effect on antibiotics (30 µg/disc). Wide inter- and intra-group variations in antibiotic activity have been observed in the presence of AgNPs, which is interpreted in terms of fold increase in the area of the zone of inhibition (Table 2).

Aminoglycosides showed a very minute increase of up to 0.8-fold. Considerable results were obtained with the combination of β-lactam and AgNPs, where a 3.0-fold increase was observed with piperacillin and 1.8-fold with ampicillin and penicillin against *X. campestris*. For *B. subtilis*, a 1.8-fold increase in the inhibition zone was seen with piperacillin. A similar result was obtained with vancomycin. The disc-diffusion assay revealed higher activity of AgNPs with β-lactam antibiotics. This might be due to the inhibitory action of β-lactams in the synthesis of outer cell walls making bacteria more susceptible to AgNP penetration. In another study, synergy of 30 µg/disc AgNPs, produced from *Dioscorea bulbifera* tuber extract, has been reported with 500 µg/disc of antibiotics [15]. Here, we have obtained comparable results despite using lower concentrations of both antibiotics and AgNPs (Fig. 4).
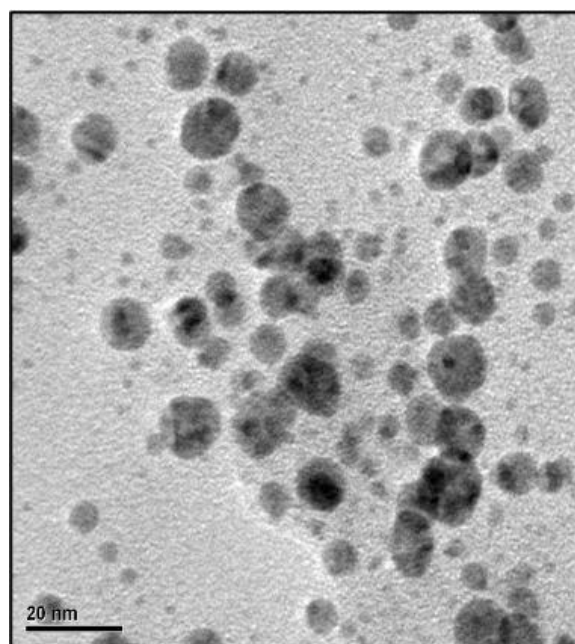

Fig. 3. TEM image of AgNPs.

To have a greater understanding of the effect of AgNPs on antibiotics, MIC was determined using broth micro-dilution assay. MICs for AgNPs alone were found to be in the range of 128-500 µg/ml against the tested bacteria. Up to a 2000-fold decrease in MICs of antibiotics was obtained with the addition of AgNPs (Table 3). Among all the bacteria, *B. subtilis* showed the highest degree of resistance against antibiotics. However, treatment with a combination of antibiotics and AgNPs made them very susceptible indicated by the lowered MIC values. Only a few β-lactam antibiotics, such as amoxicillin, ampicillin, carbenicillin, and cephalosporins were unable to show any reduction in MIC in the presence of AgNPs. This kind of specificity could be due to the interaction of AgNPs with the active groups in antibiotics resulting in enhanced activity [33], although further investigation is required.

Table 1. Zone of inhibition obtained with different concentrations of AgNPs against bacterial pathogens

AgNPs (µg/disc)	Zone of inhibition (mm) of pathogenic bacteria				
	Neisseria mucosa	Serratia odorifera	Xanthomonas campestris	Bacillus subtilis	Micrococcus luteus
1024	17	12	12	11	11
512	14	11	10	10	10
256	12	9	9	8	9
128	11	8	8	NI	7
64	10	7	NI	NI	NI
32	8	NI	NI	NI	NI

Note: NI – no inhibition.
All experiments were performed in duplicate and standard deviations were negligible.

Table 2. Zone of inhibition obtained for antibiotics against bacterial pathogens in the presence and absence of AgNPs.

Antibiotics	N. mucosa			S. odorifera			X. campestris			B. subtilis			M. luteus		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Aminoglycosides															
Amikacin	30.0	32.0	0.1	24.0	26.0	0.2	24.0	24.0	0.0	6.0	6.0	0.0	22.0	24.0	0.2
Gentamicin	30.0	36.0	0.4	23.0	23.0	0.0	26.0	28.0	0.2	6.0	6.0	0.0	22.0	25.0	0.3
Kanamycin	22.0	24.0	0.2	22.0	22.0	0.0	26.0	28.0	0.2	6.0	6.0	0.0	23.0	23.0	0.0
Streptomycin	24.0	24.0	0.0	19.0	21.0	0.2	6.0	8.0	0.8	6.0	7.0	0.4	6.0	6.0	0.0
β-lactams															
Amoxicillin	34.0	35.0	0.1	14.0	14.0	0.0	6.0	8.0	0.8	6.0	6.0	0.0	10.0	10.0	0.0
Ampicillin	32.0	36.0	0.3	15.0	20.0	0.8	6.0	10.0	1.8	6.0	6.0	0.0	11.0	14.0	0.6
Carbenicillin	6.0	6.0	0.0	6.0	6.0	0.0	8.0	10.0	0.6	6.0	6.0	0.0	6.0	8.0	0.8
Faropenem	42.0	46.0	0.2	26.0	28.0	0.2	35.0	38.0	0.2	15.0	16.0	0.1	31.0	31.0	0.0
Piperacillin	34.0	38.0	0.2	25.0	25.0	0.0	6.0	12.0	3.0	6.0	10.0	1.8	15.0	19.0	0.6
Penicillin	38.0	44.0	0.3	14.0	14.0	0.0	6.0	10.0	1.8	6.0	6.0	0.0	14.0	15.0	0.1
Cephalosporins															
Ceftazidime	25.0	30.0	0.4	29.0	35.0	0.5	22.0	23.0	0.1	6.0	8.0	0.8	9.0	9.0	0.0
Ceftriaxone	31.0	40.0	0.7	23.0	23.0	0.0	25.0	26.0	0.1	6.0	6.0	0.0	13.0	13.0	0.0
Glycopeptides															
Vancomycin	21.0	25.0	0.4	6.0	6.0	0.0	20.0	20.0	0.0	6.0	10.0	1.8	19.0	20.0	0.1
Quinolones															
Ciprofloxacin	30.0	33.0	0.2	28.0	32.0	0.3	30.0	32.0	0.1	6.0	7.0	0.4	34.0	35.0	0.1
Tetracyclines															
Doxycycline	30.0	40.0	0.8	20.0	24.0	0.4	30.0	32.0	0.1	12.0	12.0	0.0	26.0	32.0	0.5
Tetracycline	32.0	35.0	0.2	16.0	19.0	0.4	29.0	31.0	0.1	14.0	16.0	0.3	26.0	26.0	0.0
Others															
Chloramphenicol	30.0	32.0	0.1	22.0	22.0	0.0	26.0	29.0	0.2	8.0	10.0	0.6	21.0	21.0	0.0
Trimethoprim	26.0	33.0	0.6	24.0	25.0	0.1	7.0	9.0	0.6	6.0	6.0	0.0	6.0	6.0	0.0

Note: Fold increase (C) was calculated using the formula $(B^2-A^2)/A^2$, where A and B are the zone of inhibition (mm) obtained for antibiotic alone and antibiotic in combination with AgNPs, respectively. In case of no inhibition, disc diameter (6 mm) was taken for calculation. The experiment was performed in duplicate and standard deviations were negligible.
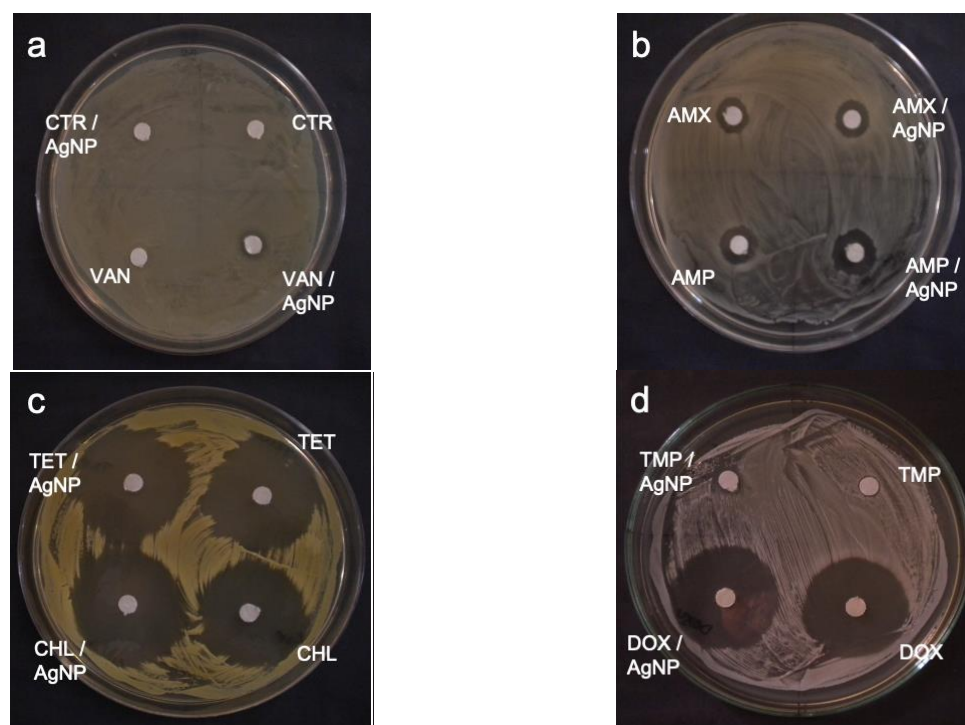

Fig. 4. Disc-diffusion assay representing the effect of AgNPs on antibiotics against (a) *B. subtilis*; (b) *M. luteus*; (c) *N. mucosa*; (d) *X. campestris*. (Abbreviations- AMX- amoxicillin; AMP- ampicillin; CHL- chloramphenicol; CTR- ceftriaxone; DOX- doxycycline; TET- tetracycline; TMP- trimethoprim; VAN- vancomycin).

Table 3. MIC and MBC of antibiotics against bacterial pathogens in the presence and absence of AgNPs.

Antibiotics		*N. mucosa*		*S. odorifera*		*X. campestris*		*B. subtilis*		*M. luteus*	
		A	B	A	B	A	B	A	B	A	B
Aminoglycosides											
Amikacin	MIC	0.5	<0.015	1	<0.015	0.5	<0.015	4096	2	0.25	<0.015
	MBC	0.5	0.12	1	<0.015	0.5	<0.015	>4096	16	0.25	<0.015
Gentamicin	MIC	0.12	<0.015	0.5	<0.015	0.25	<0.015	2048	2	0.25	<0.015
	MBC	0.12	<0.015	0.5	<0.015	0.25	0.06	2048	2	0.25	<0.015
Kanamycin	MIC	2	<0.015	1	<0.015	2	<0.015	4096	2	0.5	<0.015
	MBC	2	0.12	1	<0.015	2	0.06	>4096	8	0.5	<0.015
Streptomycin	MIC	1	<0.015	16	<0.015	4096	2	512	2	2048	2
	MBC	1	0.12	16	<0.015	4096	2	512	4	4096	2
β-lactams											
Amoxicillin	MIC	0.5	<0.015	32	<0.015	>4096	4096	4096	4096	16	<0.015
	MBC	0.5	0.12	32	<0.015	>4096	4096	4096	>4096	16	0.03
Ampicillin	MIC	0.25	<0.015	32	<0.015	>4096	4096	4096	4096	32	<0.015
	MBC	2	0.12	32	<0.015	>4096	4096	4096	4096	32	0.12
Carbenicillin	MIC	128	2	1024	<0.015	>4096	2	>4096	4096	2048	2
	MBC	256	16	1024	<0.015	>4096	2	>4096	>4096	2048	16
Faropenem	MIC	<0.015	<0.015	0.5	<0.015	0.25	<0.015	8	0.12	<0.015	<0.015
	MBC	<0.015	<0.015	0.5	<0.015	0.25	0.03	8	0.12	0.06	<0.015
Piperacillin	MIC	0.5	<0.015	0.5	<0.015	128	2	128	2	2	<0.015
	MBC	1	0.12	1	<0.015	256	2	256	2	16	<0.015
Penicillin	MIC	<0.015	<0.015	32	<0.015	4096	2	512	2	1	<0.015
	MBC	0.06	0.06	64	<0.015	4096	2	512	2	1	<0.015
Cephalosporins											
Ceftazidime	MIC	4	<0.015	0.03	<0.015	4	<0.015	2048	2048	128	0.12
	MBC	32	0.12	0.06	<0.015	4	<0.015	4096	4096	128	1
Ceftriaxone	MIC	0.5	<0.015	0.25	<0.015	4	<0.015	2048	1024	32	0.12
	MBC	0.5	0.12	0.25	<0.015	4	0.06	2048	2048	32	0.12
Glycopeptides											
Vancomycin	MIC	0.12	<0.015	256	<0.015	128	0.12	32	0.12	2	<0.015
	MBC	0.25	0.12	256	<0.015	128	1	32	0.12	2	<0.015
Quinolones											
Ciprofloxacin	MIC	1	<0.015	<0.015	<0.015	4	0.06	64	2	0.06	<0.015
	MBC	1	0.12	0.03	<0.015	4	0.06	128	2	0.06	<0.015
Tetracyclines											
Doxycycline	MIC	0.12	<0.015	2	<0.015	0.25	0.06	16	0.12	0.06	<0.015
	MBC	1	0.12	4	<0.015	16	1	256	0.12	0.06	<0.015
Tetracycline	MIC	0.25	<0.015	2	<0.015	0.5	0.06	4	0.12	0.25	<0.015
	MBC	1	0.12	8	<0.015	32	0.06	64	0.12	2	0.06
Others											
Chloramphenicol	MIC	0.5	<0.015	1	<0.015	256	2	256	2	2	<0.015
	MBC	4	0.12	2	<0.015	256	2	4096	2	2	0.06
Trimethoprim	MIC	1	<0.015	0.5	<0.015	16	0.06	512	2	1024	2
	MBC	1	0.12	2	<0.015	64	0.25	4096	2	4096	16

Note: All the values are expressed as μg/ml.
Columns A and B represent the MIC and MBC values of antibiotics obtained in the absence and presence of AgNPs, respectively.

In our previous report with *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, MIC values were interpreted as per the MIC breakpoints given in the CLSI guidelines [16]. We showed that the bacteria exhibiting resistance to an antibiotic could be made susceptible to that antibiotic in the presence of AgNPs. However, MIC breakpoints are not provided in the CLSI guidelines against the bacteria used in the current study. Hence, it is

difficult to define whether the bacteria fall into the resistant, intermediate or sensitive category for a particular antibiotic. Also, the efficiency of the combinatorial therapy cannot be determined. Nevertheless, the experiment surely indicates the reduction in MIC values of these bacteria on exposure to AgNPs along with antibiotics. Similar results were obtained with MBC assays (Table 3). In contradiction to our study, garlic-synthesized AgNPs showed an enhanced effect on Gram-positive bacteria like *B. subtilis* and *M. luteus* when combined with ampicillin [33]. This can be attributed to the difference in the cell wall composition of bacteria and its interaction with nanoparticles [32].

Although multi-drug resistance is extensively studied in well-known pathogens [14-19, 34], a high prevalence of antibiotic resistance among opportunistic pathogens has also been reported [35, 36]. *N. mucosa*, one such pathogen, has received very little attention compared to pathogenic *N. meningitidis* and *N. gonorrhoeae*. Despite *N. mucosa* has low pathogenicity and does not rank among major pathogens, it has been reported to cause serious infections such as endocarditis and complicated urinary tract infections [37]. Moreover, a recent report has shown antibiotic resistance among *Neisseria* species including *N. mucosa* [38]. Similar reports of pathogenicity and antibiotic resistance are available on *Bacillus subtilis*, *Serratia odorifera* and *Micrococcus luteus* [39-42]. *X. campestris* is a plant pathogen causing citrus canker among citrus fruits, such as orange, lemon and grapefruit [43]. Such novel treatment approaches may help in the prevention of fruit spoilage.

Unlike traditional antibiotics, multiple mechanisms have been suggested to explain the antibacterial action of AgNPs, such as disruption of cellular morphology, enzyme inactivation, inhibition of DNA replication and generation of oxidative stress [44, 45]. This implies that bacteria would have to acquire resistance through multiple mutations simultaneously to survive against nanoparticles, which makes these AgNPs very promising nano-antibiotics.

4. CONCLUSIONS

This is the first study to evaluate the combined effect of AgNPs and antibiotics against these lesser-studied clinically harmful bacteria using both disc susceptibility and broth micro-dilution assay. Although no standards are provided in CLSI for the tested pathogens, it has been proved from the study that a small amount of AgNPs in antibiotics can enhance their activities rendering resistant pathogens more susceptible. It implies that the drug, against which bacteria has developed resistance, can be made functional again using such combinations. Moreover, this will certainly decrease the required treatment dose of the drug by many folds, as indicated by lowered MICs, which will further eliminate the toxicity and side effects. This study will help in the development of a newer generation of antimicrobials comprising drugs and AgNPs.

ACKNOWLEDGEMENT

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