Research Article

Estimation of antibacterial activity of zinc oxide, titanium dioxide, and silver nanoparticles against multidrug-resistant bacteria isolated from clinical cases in Amara City, Iraq

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ABSTRACT

Background: Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are responsible for health-care-associated infections and hospital outbreaks, with multidrug resistance to antimicrobial agents such as aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, and polymyxins. It is found that the frequent use of antibiotics has led to the emergence of nearly all antibiotic super bacteria which carry the super-resistance gene called NDM. **Materials and Methods:** Three species *P. aeruginosa*, *A. baumannii,* and *Serratia marcescens* isolated from clinical cases, their diagnosis and antibiotic susceptibility testing were achieved using Vitek2 systems depending on Gram-negative diagnosis and sensitivity cards. **Results:** The results revealed that *P. aeruginosa* and *A. baumannii* were resistant to antibiotics (ticarcillin, piperacillin, piperacillin/tazobactam, ceftazidime, aztreonam, imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin, minocycline, and trimethoprim/sulfamethoxazole) used in this study, oppositely, *S. marcescens* was sensitive. The results also showed that minimum inhibitory concentration (MIC) of nanoparticles (NPs) is varying according to the type of NPs, the dimension, and bacterial species; the MIC for zinc oxide (Z_nO) NP sizes (20, 30, 50, and ~ 150 nm) was determined between 625 and 2500 µg/ml, titanium dioxide (TiO_2) NP sizes (10, 50, and 100 nm) were 3125–50,000 µg/ml, and silver NPs (AgNPs) (90 nm) were 2500 µg/ml for all isolates. In addition, our results reported that *S. marcescens* isolate was resistant to TiO_2 (10 nm) NPs. **Conclusion:** Z_nO NPs found to have best activity against bacterial growth compared to AgNPs and TiO_2 NPs, suggesting the use of these NPs in special preparation to treat such multidrug-resistant bacteria.

KEY WORDS: Antimicrobials agents, Minimum inhibitory concentration, Multidrug-resistant bacteria, Nanoparticles

INTRODUCTION

Millions of people around the world are infected with bacterial infections, morbidity, and mortality and have a major impact on the health-care economy.[1] Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are responsible for health-care-associated infections and hospital outbreaks, with multidrug resistance to antimicrobial agents such as aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, and polymyxins.[2] It is found that the frequent use of antibiotics has led to the emergence of nearly all antibiotic super bacteria which carry the super-resistance gene called NDM-1.[3] Most of the enzymes of Gram-negative bacteria are cell bounded so that they exert their effects only if the antibiotic enters the bacterial cell.[4] Nanoparticles (NPs) are particles with a dimension range between 10 and 100 nm. The most important property of these particles is their greater surface area which is reflected by their antimicrobial activity against both Gram-negative and Gram-positive bacteria; zinc oxide nanoparticles (Z_nO Nps) were found to inhibit *Staphylococcus aureus*, its antibacterial activity against a broad range of Gram-positive and Gram-negative bacteria.[5] Silver nanoparticles (AgNPs) exhibit a bactericidal activity against *Escherichia coli* and also titanium dioxide (TiO_2) allows bacteria to compress DNA, degeneration, and fragmentation, especially sequences rich in GC

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base pair.[6,7] The effective antimicrobial properties of these substances are mainly due to their size, which provide them with unique physical and chemical properties and from surface increase to volume ratio and high reactivity.[8] The emergence of multidrug-resistant super bacteria in Iraqi hospitals and its consequences on public health has been increased recently; hence, the present study aimed to estimate the antibacterial activity of different NPs against three nosocomial Gram-negative pathogens isolated from clinical cases in Amara City, Iraq.

MATERIALS AND METHODS

Bacterial Strains

The present study involved three Gram-negative bacterial species (*P. aeruginosa*, *A. baumannii*, and *Serratia marcescens*) isolated from clinical cases of burns, injuries, and bronchial infection and identified primarily by routine laboratory procedures which included the cultural and microscopically morphology and biochemical tests[9] and diagnosed using Vitek2 (performed by BioMerieux Comp., France, 2017) tools in the bacteriology unit at Al-Sader Teaching Hospital in Amara City, Iraq.

Antibiotics Sensitivity Testing and Minimum Inhibitory Concentration (MIC)

The antibiotics used in the current study are (ticarcillin, piperacillin, piperacillin/tazobactam, ceftazidime, aztreonam, imipenem, meropenem, gentamicin, tobramycin, ciprofloxacin, minocycline, and trimethoprim/sulfamethoxazole) supplied in Gram-negative sensitivity testing cards that used and analyzed specifically by Vitek2 system, performed by BioMerieux Comp., France, 2017. The sensitivity test was achieved as described by the supplier company leaflet (BioMerieux Comp. France, 2017), and thus, the Vitek2 system will determine the MIC for each antibiotic against each bacteria after 24 h of incubation.

TESTED NANOPARTICLES

The present study used, AgNPs (dimension: 90 nm, purity 99.9%), Z_nO NPs (dimension: 20, 30, and 50 nm, purity 99.9%), and TiO_2 NPs (dimension: 10, 50, and 100 nm, purity 99.9%). These NPs were supplied by MK Impex. Corp., Canada. The NPs stock solution was prepared by adding 100 mg of NPs powder into 10 ml of deionized water with high shaking to separate the NPs accumulation for 5 min to obtain a homogenous solution which was sterilized by autoclaving and left at room temperature. The final concentration was 10 mg/ml.[10]

MIC for NPs

The MICs of the studied nanoparticles against the three bacterial species were identified using modified broth dilution assay.[11] An aliquot (25 µl) of a 24 h culture of bacterial species was added to (10) test tubes containing 0.5 ml of sterile nutrient broth. Thereafter, 0.5 ml of NPs stock solution (5000 µg/ml) of NPs was added to the first tube to obtain a concentration of 2500 µg/ml and mixed gently, then, 0.5 ml of the suspension was transferred from the first tube to the second to obtain 1250 µg/ml concentration of NPs. Dilutions were continued to the 10th tube (0.5 ml was transferred from the 10th tube and neglected) to obtain the concentrations (625, 312.5, 156.25, 78.125, 39.06, 19.53, and 9.76 µg/ml) for each of NPs in use. In the control, only the organism was grown without NPs nanoparticles. These steps were repeated for each NP in use. All the tubes were incubated at 37°C for 24 h.

RESULTS AND DISCUSSION

Results of antibiotic testing showed that both *P. aeruginosa* and *A. baumannii* were resistant to all used antibiotics, while *S. marcescens* was sensitive to all except one of the antibiotics listed in Table 1.

The result of the present study found that the MICs for studied NPs differ between the three bacterial species

Table 1: Antibiotic sensitivity testing of *P. aeruginosa*, *A. baumannii*, and *S. marcescens*

Antibiotics	*P. aeruginosa*		*A. baumannii*		*S. marcescens*	
	MIC (IU/ml)	Suscep.	MIC (IU/ml)	Suscep.	MIC (IU/ml)	Suscep.
Ticarcillin	≥128	R*	≥128	R*	≤8	S*
Piperacillin	≥128	R	≥128	R	≤4	S
Piperacillin/tazobactam	≥128	R	≥128	R	≤1	S
Ceftazidime	≥64	R	≥64	R	≤1	S
Aztreonam	≥64	R	≥64	R	≤1	S
Imipenem	≥16	R	≥16	R	≤0.25	S
Meropenem	≥16	R	≥16	R	≤2	S
Gentamicin	≥16	R	≥16	R	≤1	S
Tobramycin	≥16	R	≥16	R	≤1	S
Ciprofloxacin	≥4	R	≥4	R	≤0.25	I*
Minocycline	≥16	R	≥16	R	≤8	S
SXT	≥320	R	≥320	R	≤20	S

*R: Resistant, *S: Sensitive, *I: Intermediate sensitive. *P. aeruginosa*: *Pseudomonas, aeruginosa*, *A. baumannii*: *Acinetobacter baumannii*, *S. marcescens*: *Serratia marcescens*

Table 2: MIC of zinc oxide NPs against studied bacteria

Bacterial species	NPs MIC (µg/ml)		
	Z_nO (20 nm)	Z_nO (30 nm)	Z_nO (50 nm)
Pseudomonas aeruginosa	625	1250	2500
Acinetobacter baumannii	1250	625	2500
Serratia marcescens	1250	1250	1250

MIC: Minimum inhibitory concentration, NPs: Nanoparticles, Z_nO: Zinc oxide

Table 3: MIC of TiO_2 NPs against studied bacteria

Bacterial species	NPs MIC (µg/ml)		
	TiO (10 nm)	TiO (50 nm)	TiO (100 nm)
Pseudomonas aeruginosa	25,000	3125	50,000
Acinetobacter baumannii	50,000	25,000	50,000
Serratia marcescens	*R	12,500	50,000

*R: Resistant. MIC: Minimum inhibitory concentration, NPs: Nanoparticles, TiO: Titanium (II) oxide

Table 4: The minimum inhibitory concentration of silver nanoparticles against studied bacteria

NPs	Bacterial species		
Silver (90 nm)	*Acinetobacter baumannii* 2500	*Pseudomonas aeruginosa* 2500	*Serratia marcescens* 2500

understudying; in this regard, the results showed that the MIC of Z_nO NPs (20 nm) against *P. aeruginosa* was 1250 µg/ml, also it reached 2500 µg/ml against both *A. baumannii* and *S. marcescens*; in addition, MIC of Z_nO NPs (30 nm) was 2500, 625, and 2500 µg/ml against *P. aeruginosa*, *A. baumannii,* and *S. marcescens*, respectively; however, MIC of Z_nO NPs (50 nm) was 625, 2500, and 2500 µg/ml against the three bacterial species, as shown in Table 2, respectively. The antibacterial activity of Z_nO nanoparticle against multidrug-resistant bacteria increases when the particle size decreases.[12] Z_nO NPs have the ability to generate reactive oxygen species and damage caused to the bacterial cell membrane.[13]

In the same vein, Table 3 elucidates that titanium dioxide NPs are the weakest NPs affecting growth of the studied bacteria; hence, *S. marcescens* was resistant to TiO_2 (10 nm) NPs, while the MIC of same NPs against *P. aeruginosa* and *A. baumannii* was 25,000 and 50,000 µg/ml, respectively. The MIC of TiO_2 (50 nm–100 nm) NPs against the same bacterial species was 3125, 25,000, 12,500, and 50,000 µg/ml, respectively, and these results are comparable with Prasad *et al*.[14] Generation of active free hydroxyl radical (-OH) by TiO_2 particles is probably responsible for the antibacterial activity.[15]

Likewise, MIC of silver NPs (90 nm) was (2500 µg/ml) against all bacterial species as shown in [Table 4]. The results demonstrating that the isolates of bacteria have more resistant and needs a higher concentration of nanoparticles to affect the growth of bacteria . This concords with the results.[16] Also was in agreement with.[17] The silver nanoparticles sticks to the bacterial cell membrane causing degeneration and disruption of the penetrability of membrane.[18,19]

CONCLUSION

This study showed that the efficiency of Z_nO NPs against multidrug-resistant bacteria is higher compared with AgNPs and TiO_2 NPs used in the study. Further studies should be conducted on synergistic effect between antibiotics and nanoparticles to be used as an antibacterial agent in hospitals and health-care system.

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