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Antimicrobial Activity of Metallic Nanoparticles: Their Implications for Multidrug Resistance Acinetobacter baumannii

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Abstract: In recent years, the demand for biocides from alternative compounds has increased due to reasons such as multi-drug resistant bacteria and limited antibiotic approval to prevent their resistance, and the inability of existing antibiotics to fully combat bacterial infections. With this in mind, we examined the effect of silver and zinc oxide nanoparticles on Acinetobacter baumannni, which has multi-drug resistance. The blood sample was taken and placed in the Vitek II device. A. baumannii was examined by molecular method and the presence of multi-drug resistance gene was investigated. Anitibiogram sensitivities were examined by disk diffusion method (bioMerieux; Durham, NC). It was interpreted according to the Current Clinical and Laboratory Standards Institute (CLSI) criteria. It was determined that the gene region of A. baumnannii with multi-drug resistance was blaOXA-51. A. baumannii with multi-drug resistance showed only Ampicill's sensitivity to sulbactam (SAM) and Getamycin (GN), and its antimicrobial effect against Silver (AgNPs) and Zinc oxide nanoparticles (ZnONPs) was studied. It is very important not to form an inhibition zone against AgNPs at a concentration of only 16 µg/disc from nanomolecules prepared at a concentration of (1.024-16 µg/disc). The effect of ZnO-NPs was determined as 1.024 µg/disc. NPs with important applications in biomedicine provide hope for the development of effective antimicrobial agents in the future. However, it is a known fact that it has toxic effects on human and animal health. In order to reduce these effects, concrete methods should be developed by expert authors in the first place.
Keywords: Nanoparticle; A. baumannii; Multi Drug Resistance.

1. Introduction

Acinetobacter baumannii has been identified as a nosocomial red alert pathogen contributing to increased morbidity and mortality (1-3). *A. baumannii* is a pathogen resistant to many classes of antibiotics, causing many infections including hospital-acquired pneumonia, respiratory tract infection, urinary tract infections, surgical site and bloodstream infections (4, 5). Worldwide, due to *A. baumannii*, especially intensive care units (ICUs); Outbreaks have occurred in surgical wards, burn units, and general medical wards (6-11). As a result, active surveillance studies have been initiated in high-risk patients to prevent the spread

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of *A. baumannii* (12). In addition, the "super bacteria" resulting from the unconscious and misuse of antibiotics have developed resistance to almost all known antibiotics. The level of antibiotic resistance they show is attributed to the presence of a super-resistant gene called 'New Delhi metallo-beta-lactamase 1' (13). With its enzymes capable of breaking down antimicrobial agents such as aminoglycosides and beta-lactamase, many develop resistance to antimicrobials through many mechanisms, such as the carbapenemases enzyme, ribosomal mutations, pump systems (14). With all these conditions, factors such as limited approval of antibiotics have drawn attention to alternative antimicrobials. Nanoparticles (NPs) have found a place in the medical field with their use in cancer treatments and their ability to inhibit the formation of advanced glycation end products (16). Thanks to these molecules that we have combined, the treatment can be reduced in dose and antimicrobial activities can be increased. Combined use of conjugated antimicrobial agents and NPs improves their ability to kill isolates that develop antimicrobial resistance, increases antimicrobial concentrations at bacteria-antibiotic interaction sites, and helps to bind antimicrobial agents to bacteria (17). Today, many studies have been carried out with NPs and its antimicrobial effect and mechanism have been investigated. Silver (Ag) shows a broad spectrum antimicrobial effect against bacteria, fungi and viruses. This effect is called oligodynamic activity. Ag and its compounds interact with bioactive Ag+, proteins and amino acids by ionizing in body fluids or water. Microorganisms are highly sensitive to the toxic effects of Ag+ and Ag compounds. It has been determined that Ag NPs have mechanisms of action such as affecting the cell membrane, disrupting DNA damage and electron transport, superior antimicrobial activities mediated by the synthesis of reactive oxygen species (ROS) and killing biofilm-forming isolates. They provide all these effects by having a larger surface-to-volume ratio. Thus, they interact more with the cell membrane and penetrate the cell easily (18, 19). ZnO (Zinc Oxide) NPs, on the other hand, have been reported to have antimicrobial properties such as disrupting the cell membrane of pathogens, accumulating in the cell and producing toxic H_2O_2 (hydrogen peroxide) (20). In the light of this information presented to the literature, we planned to examine the antimicrobial effect of Ag NPs and ZnONPs against *A. baumannii*, which has multi-drug resistance.

2. Material and Methods

2.1. Sample Collection and Colony Identification

The blood sample of a patient treated in the intensive care unit was taken under aseptic conditions and placed in the VItek II device. After 48 hours, the sample, which we obtained with the button indicating growth, was cultivated on 5% sheep blood agar and McConkey agar media and incubated at 37 °C under aerobic conditions. The growth characteristics of the media were examined according to their macroscopic appearance, colony and gram staining characteristics. Microorganism was identified by conventional methods. Oxidase negative colonies morphologically similar to Acinetobacter were identified using Vitek II (bioMerieux; Durham, NC) (21).

Determination of Antibiotic Resistance Gene: The resistance gene of *A.baumannnii* was investigated using the Multiplex PCR technique. For the PCR reaction, the method kit consisting of Taq PCR master mix (New England Biolabs, Beverly, MA), sterile RNase-free water, primer and DNA template was made according to the method. And it was examined in a 2% agarose gel (22, 23).

2.2. Ethics Committee Approval

Since this sample was collected as part of routine infection control surveillance, individual informed consent and ethics committee report were not obtained prior to including the sample in the study.

2.3. Sensitivity Tests

Antibiogram tests were performed using Disk diffusion method and E-tests (bioMerieux; Durham, NC) and were interpreted according to the current Clinical and Laboratory Standards Institute (CLSI) criteria (24). Acinetobacter isolates were tested against imipenem, doripenem, meropenem, ertapenem, sulfamethoxazole-trimethoprim, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, cefepime, ciprofloxacin, amikacin, gentamicin, polymycline, and tigesycline. Multidrug resistance was defined as susceptible to two or less antibiotics without polymyxin B and tigecycline (25).

2.4. AgNPs Preparation

AgNPs were purchased without sigma. Nanoparticles in distilled water (ddH2O). a stock suspension was prepared by resuspension. AgNPs were absorbed in 100 µl on 6mm sized disks from the final solution (1-1.024 µg/disk).

2.5. Preparation of ZnONPs

The nanoparticle was purchased from sigma. The final concentration of the suspended solution was prepared. 100 µl of the final solution (1-1.024 µg/disk) was impregnated onto 6mm disks.

2.6. NPs by Disk Diffusion Method

The bacterial suspension was adjusted to the turbidity of McFarland standard solution 0.5 and an inoculum containing approximately 1×108 CFU/mL was prepared.

Table 1: Multi-drug resistant A. baumannii profile: [30 µg/disc].

IPM	R
DORI	R
MER	R
ERT	R
SXT	R
SAM	S
AMK	R
GEN	S
TZP	R
CIP	R
CAZ	R
FEP	I
CT	R

IPM Imipenem, DORI Doripenem, MER Meropenem, ERT Ertapenem, SXT Sulfamethoxazole-Trimethoprim, SAM Ampicillin-Sulbactam, Amk Amikacin, GENgentamicin, TZP Piperacillin Tazobactam, CIP Ciprofloxacin, CAZ Ceftazidime, FEP Cefepime, CT Colistin, MIC Minimum Inhibitory Concentration, R Resistant, S Sensitive, no NS Sensitization, I Medium Susceptible.

It was inoculated on the whole surface of Müller Hinton Agar medium. 6 mm sized discs impregnated with nanoparticles, which we previously recalled and sterilized, were placed in the medium. Microorganism petri dishes and nanodisks were incubated at 37 °C for 24 hours. The antimicrobial activity of the nanodisks was determined by measuring the zone of inhibition around each disc (mm). Each test was repeated 2 times.

3. Results

Antibiotic Resistance Gene: DNA bands in *A. baumannii* 2% agarose gel analyzed with multiplex pcr were separated using the CHEF DR III system (Bio-Rad, Nazareth, Belgium). And the resistance gene was determined to be blaOXA-51.

3.1. Antimicrobial Activity

AgNPs showed an antibacterial effect against the agent. The zone diameters formed by the molecule prepared in different concentrations are shown in Table 2.

Table 2: Zone diameters obtained with different AgNPs concentrations against *A. baumannii* strain.

Zone of inhibition (mm)	AgNPs (µg/disc)
16	1.024
15	512
13	256
12	128
11	64
8	32
NI	16

NI: no inhibition, AgNPs: Silver nanoparticle. All experiments were repeated twice. Standard deviations were not significant.

The zone diameters formed by the molecule prepared in different concentrations against the ZnONPs agent are shown in Table 3.

Table 3: Zone diameters obtained with different concentrations of ZnONPs against *A. baumannii* strain.

Zone of inhibition (mm)	ZnONPs (µg/disc)
4	1.024
2	512
1	256
NI	128
NI	64
NI	32
NI	16

ZnONPs: Zinc Oxide Nanoparticle, NI: No Inhibition. All experiments were repeated twice. Standard deviations were not significant.

4. Discussion

A. baumannii is a Gram-negative microorganism with the ability to develop and accumulate multidrug resistance. The leading factor in hospital infections is very capable of causing morbidity and mortality (26). Alternative searches have begun to eradicate this isolate, which has the property of escaping the mechanism of action of most drugs. The major concern with the development of multidrug resistance is the spread of resistant organisms. In this respect, the idea of replacing traditional antimicrobials with new technology has emerged to prevent antimicrobial resistance. Nanotechnology-driven innovations are starting to show promise for patients and practitioners to tackle the problem of drug resistance. Ultimately, biocides that were in harmony with the ecosystem attracted attention (27). Among these, a bactericidal effect of nanomolecules with very small fragments on microorganisms was determined. Zinc oxide 12 nm, silver (5, 9, 10, 12, and 13.5 nm) shows the highest antibacterial activity (28). The use of silver is very common, especially in wound healing and burns. Bactericidal properties due to high surface areas shows (29). In our study, we examined AgNPs in different concentrations (1.024-16 µg) by disk diffusion method. Silvernano molecules (30), which have a greater effect on the gram-negative cell wall structure, did not form a zone diameter only at a concentration of 16 µg/disc. It has been reported that its effect on Gram-negative microorganisms is related to the thinness of the peptidoglycan, which is a wall component (31). These zone diameters we saw in our study give hope that AgNPs molecules will be an alternative in isolates with multi-drug resistance and especially in *A. baumannii* factor. ZnO-NPs show bactericidal effects due to reasons such as destruction of cell integrity and release of antimicrobial ions, Zn^{2+} ions. Zinc, which has many activities, is capable of producing ROS, hydrogen peroxide (H2O2) and superoxide ions ($O_2^{\bullet-}$) used to target microorganisms when exposed to UV radiation in aqueous solution and prepared an aqueous solution (31, 32). In a study by Navale et al., ZnO-NPs have been shown to have strong bactericidal and antifungal

properties against *S. aureus, S. typhimurium*, and *Aspergillus flavus* and *fumigatus* pathogens (33). In another study conducted with ZnO-NPs, it was reported that Campylobacter jejuni showed a bactericidal effect by disrupting the cell membrane structure. And with *E. coli* O157: H7 it has been reported to show antimicrobial effects against *Salmonella enterica* serotype Enteritidis (34). In our study, the effect of ZnO-NPs on multi-drug resistant *A. baumannii* isolate was determined as 1.024 µg/disk. We hope that an anti-mulrabial effect will occur with the nanparticle concentration we have determined at higher dose ranges. Morones J.R. at all. In a study (0, 25, 50, 75 and 100 µg/ml-1), AgNPs determined in concentration ranges were *E. coli, P. aeruginosa, V. cholera, S. typhus, Acinetobacter baumannii, Enterococcus faecalis, Klebsiella pneumoniae, Listeria. monocytogenes, Micrococcus luteus, Proteus mirabilis, Salmonella typhi, Enterobacter aerogenes, Bacillus subtilis, Brucella abortus, Moraxella catarrhalis, Proteus mirabilis, Streptococcus viridans, Streptococcus pneumonia, Streptococulans, Streptococcus mucosa*. In our study, *A. baumanni* did not create an inhibition zone only at a concentration of 16 (µg/disc). The AgNPs particle, which has a bactericidal effect against S. mutans, is used in dental treatments (36). In addition, many studies showing that it is effective in invasive fungal species in immunosuppressive patients have been presented in the literature (37-40). All these studies show that Ag particles are effective in most microorganisms in different sizes and concentrations. Our study reflects results consistent with this information presented in the literature. The antibacterial effect against our multi-drug resistant isolate gives us hope in our future studies. We believe that it will be an alternative antimicrobial by revealing the MIC (Minimal Inhibition Concentracion) and MBC (Minimal bactericial Concentracion) values. In addition, we hope that a response will be obtained with much lower doses and times with antibiotics and nanoformulation in order to prevent this drain. The US Food and Drug Association has listed the ZnO 'nanoparticle as "generally recognized as safe" (GRAS) (41). This has enabled the use of these particles, which are in harmony with the ecosystem, in nanomedicine. Most Gram-positive and Gram-negative bacteria, and especially foodborne pathogens, are susceptible to ZnONPs (42). In a study with ZnONPs, antibacterial activity was reported against E. coli, Listeria monocytogenes, Salmonella and Staphylococcus aureus (43). Pati et al. In his study, he talked about its antimicrobial activity against S. aureus (44). Reddy et al. He reported that for E. coli (~13 nm) it produced inhibition by ZnONPs at a concentration 3.4 mM and for *S. aureus* at a concentration 1 mM it was completely inhibited (45). Also in another study, the MIC of ZnONPs for Campylobacter jejuni was reported at a concentration of 0.05 to 0.025 mg/mL (46).

5. Conclusions

In our study, it was determined as ≥1.024 µg/disk. We believe that ZnONP will have an effect against *A. baumannii* in higher concentrations.

Limits of the study and Perspectives

We believe that it will be more comprehensive to work with more than one nanoparticle and compare it with each other. In addition, our next goal will be to determine the MIC (Minimum Inhibition Concentration), MBC (Minimum Bactericidal Concentration) values and the effective duration of all these particles. In addition, we aim to conduct antimicrobial studies of plant and nanomolecular compounds (bio-nano) and antibiotic nanocompounds (antimicro-nano).

Meanwhile, although nanomedicine creates a new alternative field, we are also aware of the existence of toxic effects and negative processes. Our only wish is to create guidelines that will overcome all these negativities and to work on new antimicrobials.

Conflict of Interests

There is no conflict of interest between authors.

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There is no financial support.

Author Contributions

DÇ: Writing, analysis and statistics, ÖÇ: Literature review, laboratory analysis and statistics

Ethical Approval

Since this sample was collected as part of routine infection control surveillance, individual informed consent and ethics committee report were not obtained prior to including the sample in the study

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