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The Synthesis & Characterizes of Nano-Metallic Particles Against Antibiotic Resistant Bacteria, Isolated from Rasoul-e-Akram Hospital's Patients, Tehran, Iran

Alireza Jafari[1], Ali Majidpour[1,5], Roya Safarkar[2], Seyyedeh Masumeh Mirnurollahi[3] & Shahrdad Arastoo[4]

[1]Antimicrobial Resistance Research Center, Rasoul-e-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran

[2]Department of Microbiology, Islamic Azad University, Ardabil Branch, Ardabil, Iran

[3] Department of Biology, Science and Research, Islamic Azad University, Tehran, Iran

[4]Department of Microbiology, Islamic Azad University Qom Branch, Qom, Iran

[5] Department of Infection Disease, School of Medicine

Correspondence: Ali Majidpour, Antimicrobial Resistance Research Center, Rasoul-e-Akram Hospital, Iran University of Medical Sciences, Tehran, Iran. E-mail: alimajidpour@yahoo.com

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Abstract

The emergence of antimicrobial resistance of microorganisms to antibiotics, Also, an increase in nosocomial infections, particularly by *Methicillin Resistant Staphylococcus aureus* (*MRSA*), *Pseudomonas aeruginosa*, the need to discover new antibacterial agents with a mechanism of action different from killing bacteria were more than ever before. The Ag nanoparticles (NPs), ZnO (NPs) and Ag/ZnO (NPs) were synthesized through the thermal decomposition of the precursor of oxalate. Gram-negative antibiotic resistant bacteria and Gram-positive antibiotic resistant bacteria were prepared from the Central laboratory of Rasoul-e-Akram hospital. All of isolates were confirmed by biochemical tests. For determine of antibiotic resistance patterns of isolated, disk diffusion method in accordance with the standard CLSI were used, again. Antibacterial effects of (NPs) against antibiotic resistance bacteria were conducted by MIC and MBC tests. The particles size was less of 50 nm, approximately. Curiously, the silver (NPs) was not exposed the antibacterial properties against all of isolated bacteria. Also, *klebsiella pneumonia* and *MRSA* had greatest sensitivity to the ZnO (NPs). Also, Gram-positive antibiotic resistant bacteria showed high sensitivity to Ag/ZnO (NPs), compared to other bacteria. Interestingly, The MBC for ZnO (NPs) against *Pseudomonas aeruginosa* ≥8192 was observed. The Ag (NPs) had not the ability to inhibit the nosocomial infection. *Klebsiella pneumonia* and *MRSA* had greatest sensitivity to the ZnO (NPs). The Ag/ZnO (NPs) was ability to kill antibiotics resistant bacteria. The antibacterial agents can open a new leaf in our life in the treatment of nosocomial infections.

Keywords: Nano-metallic particles, Antibiotic Resistant Bacteria, Rasoul-e-Akram Hospital

1. Introduction

Antibiotic resistance is a worldwide problem (Roberts et al., 2009). Majority shape of resistance spread with remarkable speed. World health guidance have described antibiotic-resistant microorganisms as "nightmare bacteria" that "pose a catastrophic threat" to people in every country in the world (Roberts et al., 2009). Each year in the United States, at least 2 million people acquire serious infections with bacteria that are resistant to more of the antibiotics designed to treat those infections. Approximately, 23000 people die each year of these antibiotic-resistant infections. Many more die from other conditions that were intricate by an antibiotic-resistant infection. Antibiotic-resistant infections add avoidable costs to the already overburdened U.S. health care system. In another cases, antibiotic-resistant infections require prolonged treatments, extend hospital stays, necessitate additional doctor visits and result in greater disability and death compared with infections that are easily treatable with antibiotics (Roberts et al., 2009).

Nowadays, researchers have suggested the use of nano-metal oxides, specially Silver and Zinc oxide (NPs) as superior disinfectants and antimicrobial agent for nosocomial Infections microorganisms (Blanc, Carrara, Zanetti,

& Francioli, 2005; Reddy et al., 2007; Yu-sen, Vidic, Stout, McCartney, & Victor, 1998). Investigates shown that residual these metal ions may adversely affect human health (Sondi & Salopek-Sondi, 2004). Another word, they report on the toxicity of ZnO (NPs) to gram-negative and gram-positive bacterial systems, *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) and primary human immune cells. Those results shows that ZnO (NPs)may potentially prove useful as antimicrobial agents at selective therapeutic dosing regimens (Reddy et al., 2007). Also, they believed that silver (NPs) is incorporated in the cell membrane, which causes leakage of intracellular substances and eventually causes cell death (Kim et al., 2007; Ruparelia, Chatterjee, Duttagupta, & Mukherji, 2008). Some of the silver (NPs) also penetrate into the cells (Morones et al., 2005).

Jayesh assumed that combination of metal oxide (NPs) may give rise to more complete bactericidal effect against mixed bacterial population (Ghosh, Das, Jena, & Pradhan, 2015). We know that the bactericidal effect of metal (NPs) has been attributed to their small size, photo-catalytic of activity and high surface to volume ratio, which allows them to interact closely with microbial membranes and is not merely due to the release of metal ions in solution (Jafari, Ghane, Sarabi, & Siyavoshifar, 2011). The aim of this work was to synthesize nano-metallic particles with potent antibacterial activity, also with simple and cost-effective method that is capable kill of hospital resistant bacteria. Continue the process of the investigation to the In-vitro, In-vivo and Ex-vivo condition, may be lead to the discovery of nano-drugs with potent antibacterial activity, in the future.

2. Material and Method

2.1 Synthetizes of Nano-Metallic Particles via Oxalate Decomposition

The general reaction for decomposition basic compounds of metal oxalate following:

$$M^{+x}(NO_3)_{x(aq)}.xH_2O + H_4C_2O_4.xH_2O \rightarrow [M(O_4C_2)].4H_2O + HNO_{3(aq)}$$

$$[M(O_4C_2)].4H_2O \rightarrow \text{Metal oxide} + CO_2\uparrow + H_2O\uparrow$$

End products of metal oxides depended to the stability of the crystalline oxide and the metal cautions desired stability which can be M^{+}, M^{2+}, $M^{+8/3}$and the M^{3+}.

2.2 Synthesis of Ag, ZnO, Ag/ZnO Nanoparticles and Characterization

The Ag, ZnO, Ag/ZnO(NPs) were synthesized in Antimicrobial Resistance Research Center (ARRC) of Iran University of Medical Sciences (IUMS), according to Jafari and their Colleague's protocol. To study of the crystal structure of nano-metallic particles, X-ray diffractometer set (XRD, Bruker D8-Advance diffract meter using Cu Kα radiation) in X-Ray Laboratory School of Mining Engineering, University of Tehran, were used. The FT-IR spectrum was record don a Bruker spectrophotometer in KBr pellets in Institute of Materials and Energy (MERC) of Tehran. Surface morphology of product was characterized by using a Scanning Electronic Microscopy (SEM, Cam Scan MV 2300, nano-electronics laboratory, Tehran University) with an accelerating voltage of 30 KV (Dabbagh, Moghimipour, Ameri, & Sayfoddin, 2010; Gan, Liu, Zhong, Liu, & Li, 2004)**.**

2.3 Sampling and Collection of Isolated Bacteria

Klebsiella pneumoniae, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii, Methicillin resistant Staphylococcus aureus (MRSA), had been delivered with respect for the ethical considerations and appropriate licenses to the Central Laboratory of Rasoul-e-Akram Hospital and then was transferred to the Antimicrobial Resistance Research Center laboratory. Sampling and collection of isolated antibiotic-resistant bacteria, since early May 2015 until late June 2015 had been conducted. According to the reports of central laboratory of Rasoul-e-Akram Hospital, Klebsiella pneumonia and Escherichia coli had been isolated from urine samples of patients. Pseudomonas aeruginosa, Staphylococcus epidermidis and Acinetobacter baumannii were isolated from burn wounds and MRSA that was obtained from blood samples.

2.4 Identification & Determination of Antibiotic Resistance Patterns of Bacteria

To confirm the identification of bacteria resistant to antibiotics, biochemical tests were used. For determine of antibiotic resistance patterns of isolated, disk diffusion method (Kirby-Bauer) in accordance with the standard CLSI (Clinical and Laboratory Standards Institute) and the National Committee for clinical laboratory Standards (NCCLS) were used, again. Antibiotic discs for each bacteria isolated were listed in Tables 1, 2, 3, 4.

Table 1. The lists of antibiotic discs that used in *Klebsiella pneumoniae*, *Escherichia coli* & *Pseudomonas aeruginosa*

Antimicrobial Agent	Symbol & Count.	Made in
Cefazolin	CEF10	PADTAN TEB Co. Iran
Gentamycin	GM10	PADTAN TEB Co. Iran
Amikacin	An	PADTAN TEB Co. Iran
Cefepime	FEP30	PADTAN TEB Co. Iran
Cefotaxime	CTX30	PADTAN TEB Co. Iran
Ciprofloxacin	CP10	PADTAN TEB Co. Iran
Trimethoprim sulfa methoxazole	TMP5	PADTAN TEB Co. Iran
Meropenem	MEN10	PADTAN TEB Co. Iran
Ceftazidime	CAZ30	PADTAN TEB Co. Iran
Nitrofurantoin	FM300	PADTAN TEB Co. Iran
Piperacillin Tazobactam	PTZ100/10	PADTAN TEB Co. Iran
Ampicillin	AM30	PADTAN TEB Co. Iran
Imipenem	IPM10	MAST Co. UK
Colistine	CL10	MAST Co. UK
Aztreonam	AZ15	PADTAN TEB Co. Iran

Table 2. The lists of the antibiotic discs that used in *Methicillin Resistance of Staphylococcus aureus (MRSA)*

Antimicrobial Agent	Symbol & Count.	Made in
Nitrofurantoin	FM300	PADTAN TEB Co. Iran
Trimethoprim sulfa meth oxazole	TMP5	PADTAN TEB Co. Iran
Erythromycin	E15	PADTAN TEB Co. Iran
Methicillin	ME5	PADTAN TEB Co. Iran

Table 3. The lists of the antibiotic discs that used in *Acinetobacter baumannii.*

Antimicrobial Agent	Symbol & Count.	Made in
Imipenem	IPM10	PADTAN TEB Co. Iran
Ceftazidime	CT30	PADTAN TEB Co. Iran
Ticarcillin	TIC75	PADTAN TEB Co. Iran
Tobramycin	TOB10	PADTAN TEB Co. Iran
Gentamycin	GM10	PADTAN TEB Co. Iran
Cefotaxime	CTX30	PADTAN TEB Co. Iran
Ciprofloxacin	CP10	PADTAN TEB Co. Iran
Co-trimoxazole	SXT25	PADTAN TEB Co. Iran
Colistine	CL10	PADTAN TEB Co. Iran

Table 4. The lists of the antibiotic discs that used in *Staphylococcus epidermidis*

Antimicrobial Agent	Symbol & Count.	Made in
Cefazolin	CEF10	PADTAN TEB Co. Iran
Rifampicin	RA5	PADTAN TEB Co. Iran
Vancomycin	V30	PADTAN TEB Co. Iran
Clindamycin	CC2	PADTAN TEB Co. Iran
Co-trimoxazole	SXT25	PADTAN TEB Co. Iran
Minocycline	MI30	PADTAN TEB Co. Iran
Linezolid	LZ30	PADTAN TEB Co. Iran
Azithromycin	AZM15	PADTAN TEB Co. Iran
Clarithromycin	CLR15	PADTAN TEB Co. Iran
Oxacillin	OX1	PADTAN TEB Co. Iran

2.5 Supplying of Standard McFarland

In order to providing of 0.5 McFarland concentration, 0.5 ml of pure sulfuric acid and 9.95 ml of barium chloride to the clean test tube were stirred, slowly. The test tube was kept in a dark location away from light and heat.

2.6 Determining the Sensitivity of Bacteria to Ag, ZnO and Ag/ZnO NPs via Disk Diffusion and Cavity Method

First at all, we were poured 0.327 gr of Ag, ZnO and Ag/ZnO (NPs) into the sterile test tubes, containing 20 ml of liquid medium Mueller Hinton broth (MHB) (Merck, Germany). In each of the test tubes, sterile blank discs were placed. Then for 30 min were sonicated at room temperature by ultrasonic waves at room temperature and frequency of 28 KHz (PULSE Co. Germany). Next, all of discs in order to lose of moisture were placed in desiccators at room temperature. Immediately, several colonies of freshly bacteria were injected into test tubes containing 10 ml of sterile saline and equivalent to 0.5 McFarland. Then 100 λof bacterial suspension was culture don MHB. All of discs impregnated with (NPs) was polluted on MHB, also was incubated at 37 °C for at least 18 hours. In order to performance of cavity test, we were drilled several cavity on MHB, also 100 λ of (NPs) is poured into it.

2.7 MIC and MBC Tests

The serial dilution method was used for determine the Minimum Inhibitory Concentration (MIC) of the Ag, ZnO and Ag/ZnO (NPs) (Dabbagh et al., 2010; Jafari, Ghane, Sarabi, et al., 2011). In this way, all of test tubes were filled with 1 ml of the liquid Muller Hinton broth (MHB) medium. Then, all of (NPs) had been sonicated with the culture medium were added and mixed. Subsequently, one ml of the content of test tube number two was added to test tube number 3 and mixed completely and then this process was performed serially to last test tube. Totally, microbial suspensions of *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, MRSA, containing 1.5×10^8 $CFUml^{-1}$ were added to test tubes and were incubated at 37 °C for 24 h. All the experiments were carried out in triplicate (Dabbagh et al., 2010; Jafari, Ghane, Sarabi, et al., 2011).

The minimum bactericidal concentration (MBC), i.e., the lowest concentration of nanoparticles that kills 99.9% of the bacteria was also determined from the batch culture studies. To experiments for bactericidal effect, a loop-full from each test tubes (Specially, negative & positive test tubes) was inoculated on Muller Hinton agar and incubated at 37 °C for 24 h. The nanoparticles concentration illustrating bactericidal effect was picked out based on absence of colonies on the agar plate (Gan et al., 2004; Jafari, Ghane, & Arastoo, 2011; Kim et al., 2007).

3. Result

3.1 The FT-IR Spectra Analysis of Ag, ZnO, Ag/ZnO Nanoparticles

The nanoparticles obtained at a temperature of 550°C, FT-IR spectra were taken. The interpretation is as follows; (Figures 1a, 1b, 1c) shows FT-IR spectra of Ag, ZnO and Ag/ZnO (NPs), respectively. Figures 1a shows that the shoulder at 1428.89 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 875.31 cm^{-1} and 577.35 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (M-O) tremble respectively.

Also, figure 1b that depended to ZnO (NPs) FT-IR spectrum was demonstrated the band at 1428.89 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands at 876.14 cm^{-1} and 551.12 cm^{-1} are presents in the spectrum evidence of (O-C-O) tensional tremble and (Zn-O) tensional tremble respectively[10].

At last, Figure 1c was related on Ag/ZnO (NPs) FT-IR spectrum. Consistent with the results obtained by Jafari et al. the shoulder at 1458.22 cm^{-1} is present in the spectrum evidence of (N-O) tremble and the closely spaced bands 625.36 cm^{-1} are presents in the spectrum evidence of (Ag/ZnO) (NPs) tensional tremble respectively (Dabbagh et al., 2010).

3.2 The XRD spectra, SEM Images Analysis

The XRD patterns of Ag, ZnO and Ag/ZnO (NPs) (Figures 2a, 2b, 2c) were compared and interpreted with standard data of International Centre of Diffraction Data (ICDD). Results of XRD spectra and SEM of Ag, ZnO and Ag/ZnO (NPs) (Figures 3a, 3b, 3c) consistent with the results of Jafari and their colleagues.

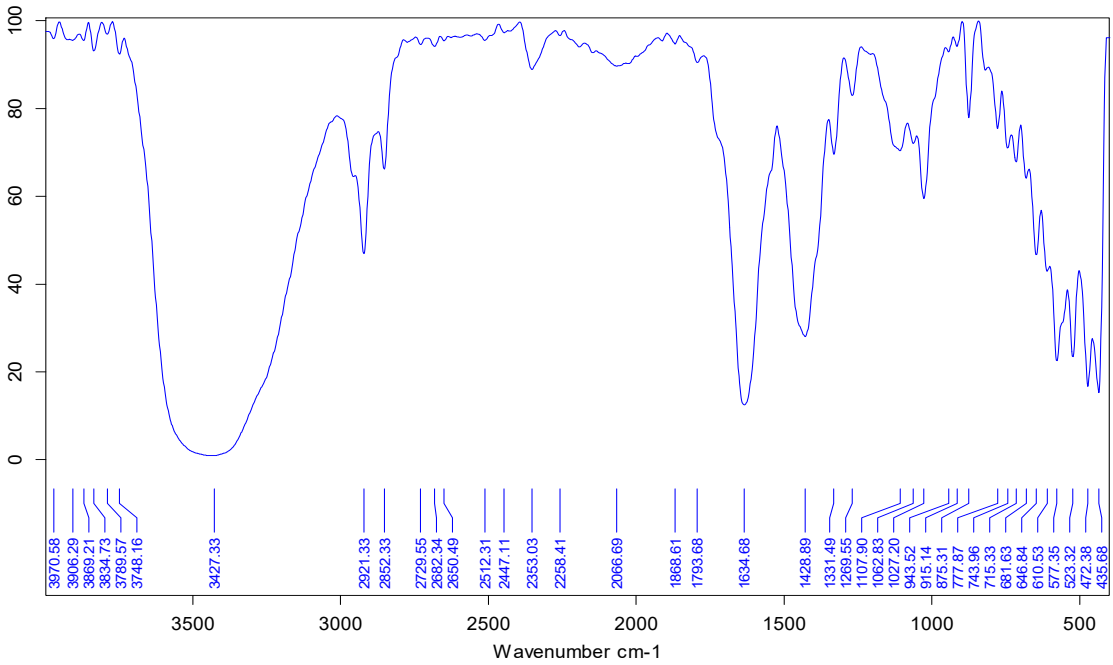

Figure 1a. The FT-IR spectra analysis of Ag NPs

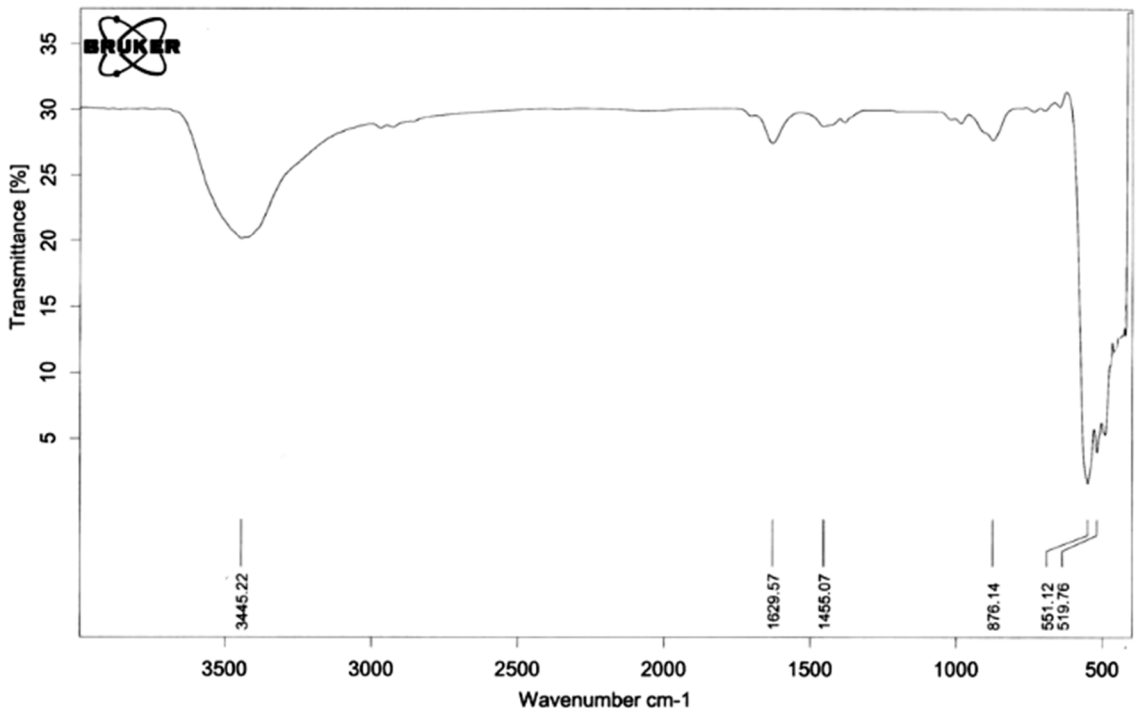

Figure 1b. The FT-IR spectra analysis of ZnO NPs

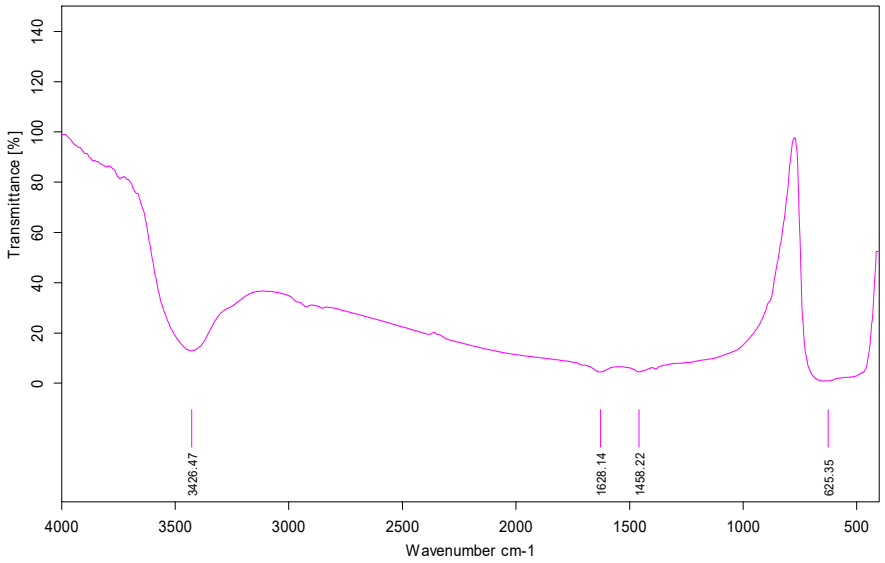

Figure 1c. The FT-IR spectra analysis of Ag/ZnO NPs

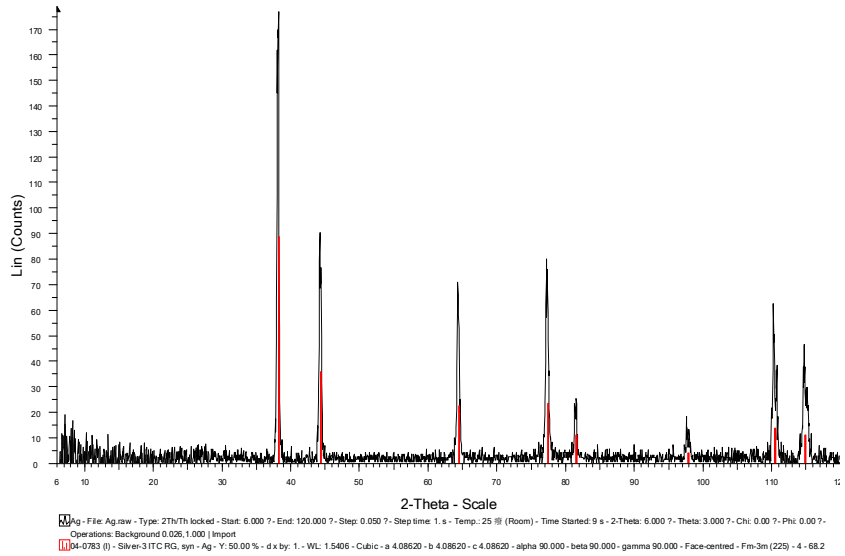

Figure 2a. The XRD patterns of Ag (NPs)

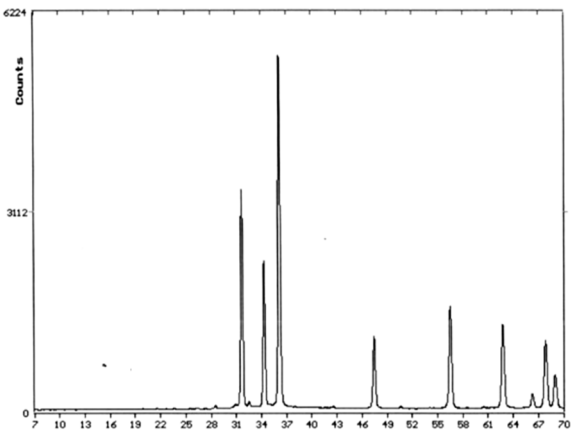

Figure 2b. The XRD patterns of ZnO (NPs)

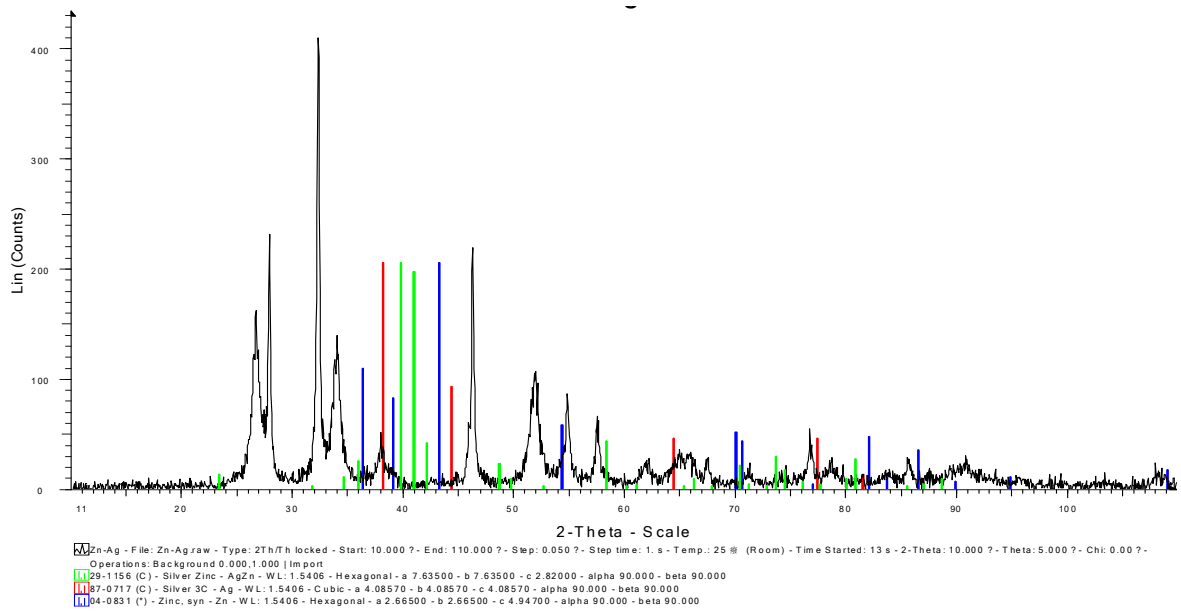

Figure 2c. The XRD patterns of Ag/ZnO (NPs)

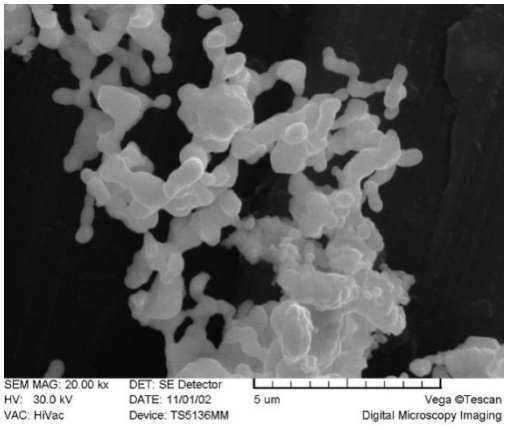

Figure 3a. SEM images analysis of Ag (NPs)

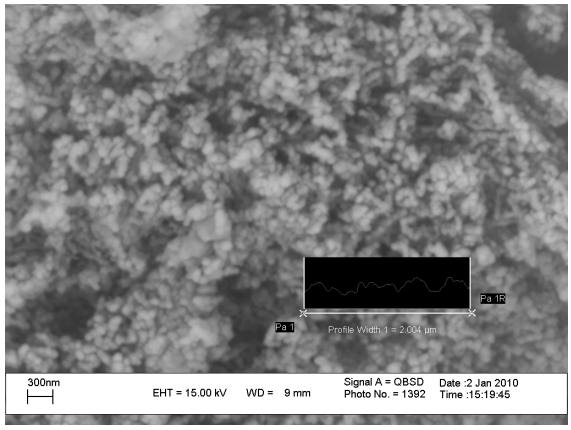

Figure 3b. SEM images analysis of ZnO (NPs)

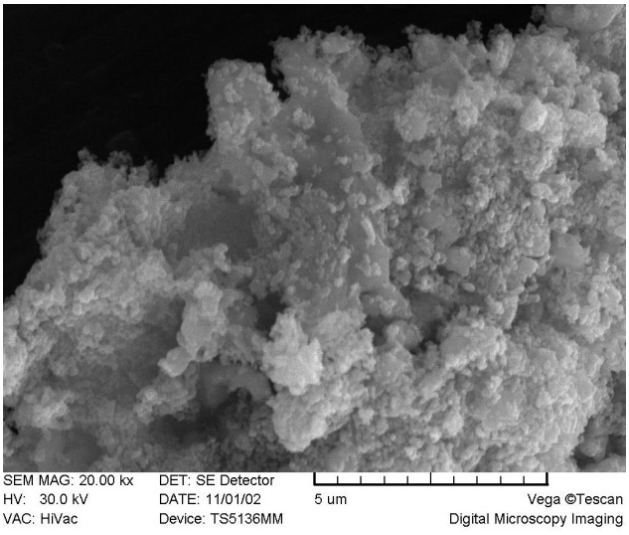

Figure 3c. SEM images analysis of Ag/ZnO (NPs)

3.3 Identification & Determination of Antibiotic Resistance Patterns of Bacteria

The results of biochemical tests for each of the bacteria shown in Table 5. Klebsiella pneumoniae, Staphylococcus epidermidis, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii, Methicillin resistant Staphylococcus aureus (MRSA) were identified by biochemical tests and were confirmed Genus and species. Based on the results, Klebsiella pneumonia was resistant to Cefixime, Ceftriaxoneand Aztreonam. While Nitrofurantoin, Ceftazidime, Imipenem, Amikacin, Cefepime, Ciprofloxacin, Piperacillin, Colistin, Trimethoprim, Cefazolin, Gentamycin and Ampicillin showed sensitivity (Table 6).

Table 5. The results of biochemical tests for each of the bacteria

Escherichia coli

Gram stain	Catalase test	Oxidasetest	TSI	Motile	Urease test	SH_2	Citrate test	MR	VP	Indole test
Negative	Positive	Negative	A/AG	Positive	Negative	Negative	Negative	Positive	Negative	Positive

MRSA

Gram stain	β-hemolysis	Catalase	Coagulase	Motile	Urease	Acetoin	Mannitol	OF Aerobic	OF Anaerobic	Baird Parker agar
Positive	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Positive	Positive

Klebsiella pneumoniae

Gram stain	Lysine	Citrate	Indole	TSI	motile	Ornithine
Negative	Positive	Positive	Negative	Acid/ Acid (with gas)	Negative	Negative

Staphylococcus epidermidis

Gram stain	Coagulase Test	Bacitracin Test	Novobiocin test	Catalase Test	β-hemolysis	Glucose	Urease
Positive	Negative	Resistance	sensitive	Positive	Negative	Positive	Positive

Acinetobacter baumannii

Gram stain	lactose	TSI	Oxidase	OF Glucose	Motile	Catalase	Polymyxin B
Negative	Negative	ALK/ALK	Negative	Positive	Negative	Positive	sensitive

Pseudomonas aeruginosa

Gram stain	Motile	Oxidase	lactose	TSI	SH_2	Catalase	Citrate	OF Aerobic	OF Anaerobic	β-hemolysis
Negative	Positive	Positive	Negative	ALK/ALK	Negative	Positive	Positive	Positive	Negative	Positive

Table 6. Determination of antibiotic resistance patterns of *Klebsiella pneumonia.*

Antimicrobial Agent	*Symbol & Count.*	
Cefazolin	CEF	Sensitive
Gentamycin	GM10	Sensitive
Amikacin	An	Sensitive
Cefepime	FEP30	Sensitive
Cefotaxime	CTX30	Sensitive
Ciprofloxacin	CP10	Sensitive
Trimethoprim sulfa methoxazole	TMP5	Resistance
Meropenem	MEN10	Sensitive
Ceftazidime	CAZ30	Sensitive
Nitrofurantoin	FM300	Sensitive
Piperacillin Tazobactam	PTZ100/10	Sensitive
Ampicillin	AM30	Resistance
Imipenem	IPM10	Sensitive
Colistine	CL10	Sensitive
Aztreonam	AZ	Sensitive
Ceftriaxone	CRO30	Sensitive
Cefixime	CFM5	Sensitive
Nitrofurantoin	FM300	Resistance

Pseudomonas aeruginosa to Gentamicin, Imipenem, Colistin, Ciprofloxacin, Meropenem, Amikacin, Ceftazidime, Cefepime and Cefotaxime was sensitivity. However, Trimethoprim, Ampicillin, Nitrofurantoin, showed resistance (Table 7).

Table 7. Determination of antibiotic resistance patterns of *Pseudomonas aeruginosa*

Antimicrobial Agent	Symbol & Count.	
Cefazolin	CEF	Sensitive
Gentamycin	GM10	Sensitive
Amikacin	An	Sensitive
Cefepime	FEP30	Sensitive
Cefotaxime	CTX30	Sensitive
Ciprofloxacin	CP10	Sensitive
Trimethoprim sulfa methoxazole	TMP5	Sensitive
Meropenem	MEN10	Sensitive
Ceftazidime	CAZ30	Sensitive
Nitrofurantoin	FM300	Sensitive
Piperacillin Tazobactam	PTZ100/10	Sensitive
Ampicillin	AM30	Sensitive
Imipenem	IPM10	Sensitive
Colistine	CL10	Sensitive
Aztreonam	AZ	Resistance
Ceftriaxone	CRO30	Resistance
Cefixime	CFM5	Resistance
Nitrofurantoin	FM300	Sensitive

Escherichia coli to Piperacillin, Nitrofurantoin, Imipenem, Gentamicin, Ampicillin, Amikacin, Ceftazidime, Cefepime and Cefotaxime were sensitive. However, *Trimethoprim* and *Cefazolin*, showed resistance. (Table 8).

Table 8. Determination of antibiotic resistance patterns of *E.coli*

Antimicrobial Agent	Symbol & Count.	
Cefazolin	CEF	Resistance
Gentamycin	GM10	Sensitive
Amikacin	An	Sensitive
Cefepime	FEP30	Sensitive
Cefotaxime	CTX30	Sensitive
Ciprofloxacin	CP10	Sensitive
Trimethoprim sulfa methoxazole	TMP5	Resistance
Meropenem	MEN10	Sensitive
Ceftazidime	CAZ30	Sensitive
Nitrofurantoin	FM300	Sensitive
Piperacillin Tazobactam	PTZ100/10	Sensitive
Ampicillin	AM30	Sensitive
Imipenem	IPM10	Sensitive
Colistine	CL10	Sensitive
Aztreonam	AZ	Sensitive
Ceftriaxone	CRO30	Sensitive
Cefixime	CFM5	Sensitive
Nitrofurantoin	FM300	Sensitive

MRSA to *Nitrofurantoin*, *Trimethoprim* showed sensitivity. But it showed resistance to *Erythromycin* and *Methicillin* (Table IX). *Staphylococcus epidermidis* was resistance to *Cefazolin, Clindamycin, Clarithromycin, Minocycline*, *Azithromycin*, *and Oxacillin*. However, *Staphylococcus epidermidis* was sensitive to *Rifampicin, Vancomycin, co-trimoxazole* and *Linezolid* (Table 9).

Table 9. Determination of antibiotic resistance patterns of *Staphylococcus epidermidis*

Antimicrobial Agent	Symbol & Count.	
cefazolin	CEF	Resistance
Rifampicin	RA5	Sensitive
Vancomycin	V30	Sensitive
Clindamycin	CC2	Resistance
Co-trimoxazole	SXT25	Sensitive
Minocycline	MI30	Resistance
Linezolid	LZ30	Sensitive
Azithromycin	AZM15	Resistance
Clarithromycin	CLR15	Resistance
Oxacillin	OX1	Resistance

Acinetobacter baumannii, isolated from burn wounds was resistance to *Imipenem, Ceftazidime, Cefepime, Ticarcillin, Tobramycin, Gentamycin, Cefotaxime, Ciprofloxacin, Co-trimoxazole* and also it was sensitive to *Colistin* (Table 10).

Table 10. Determination of antibiotic resistance patterns of *Acinetobacter baumannii*

Antimicrobial Agent	Symbol & Count.	
Imipenem	IPM10	Resistance
Ceftazidime	CT30	Resistance
Ticarcillin	TIC75	Resistance
Tobramycin	TOB10	Resistance
Gentamycin	GM10	Resistance
Cefotaxime	CTX30	Resistance
Ciprofloxacin	CP10	Resistance
Co-trimoxazole	SXT25	Resistance
Colistin	CL10	Sensitive

3.4 Disk Diffusion and Cavity Method

According to the results in the Table 12, silver (NPs) after being exposed to ultrasonic waves has no effect on the resistant antibiotics bacteria. *Staphylococcus epidermidis* and *Methicillin resistance Staphylococcus aureus* had most sensitive to ZnO and Ag/ZnO (NPs). Meanwhile, Gram-negative antibiotic-resistant bacteria such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli* were weaker.

3.5 MIC and MBC Tests

According to the results recorded in the Table 13, no one of the bacteria showed any sensitivity to silver (NPs). Compared with Gram-negative bacteria, *Staphylococcus epidermidis* and *Methicillin Resistance Staphylococcus aureus* growth, at concentration slower of ZnO and Ag/ZnO (NPs) had stopped. The greatest resistances to zinc oxide (NPs) were observed in *Pseudomonas aeruginosa* ($\geq$8192 $\mu g.ml^{-1}$).

Meanwhile, with combination of silver and zinc oxide (NPs), the dose of MIC and MBC were decreased. It means that 256 μgml^{-1} and 4096 μgml^{-1}, respectively. The results were evident in the case of other gram-negative bacteria.

Table 12. Disk diffusion and Cavity method for Ag, ZnO, Ag/ZnO (NPs) against *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Methicillin Resistant Staphylococcus aureus* (*MRSA*)

		Ag (NPs)	ZnO (NPs)	Ag/ZnO (NPs)
Klebsiella pneumoniae	Disc Diffusion Test	Negative	8mm	12mm
	Cavity Test	Negative	10mm	14mm
Staphylococcus epidermidis	Disc Diffusion Test	Negative	12mm	18mm
	Cavity Test	Negative	10mm	14mm
Pseudomonas aeruginosa	Disc Diffusion Test	Negative	8mm	10mm
	Cavity Test	Negative	5mm	8mm
Escherichia coli	Disc Diffusion Test	Negative	8mm	12mm
	Cavity Test	Negative	8mm	10mm
Acinetobacter baumannii	Disc Diffusion Test	Negative	8mm	12mm
	Cavity Test	Negative	8mm	10mm
MRSA	Disc Diffusion Test	Negative	14mm	18mm
	Cavity Test	Negative	12mm	20mm

Table 13. MIC and MBC tests for Ag, ZnO, Ag/ZnO (NPs) against *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, *Methicillin Resistant Staphylococcus aureus* (*MRSA*)

		Ag(NPs)	ZnO(NPs)	Ag/ZnO(NPs)
Klebsiella pneumoniae	MIC	≥8192 µg/ml	512 µg/ml	256 µg/ml
	MBC	≥8192 µg/ml	1024 µg/ml	512 µg/ml
Staphylococcus epidermidis	MIC	≥8192 µg/ml	64 µg/ml	128 µg/ml
	MBC	≥8192 µg/ml	512 µg/ml	512 µg/ml
Pseudomon asaeruginosa	MIC	≥8192 µg/ml	512 µg/ml	256 µg/ml
	MBC	≥8192 µg/ml	≥8192 µg/ml	4096 µg/ml
Escherichia coli	MIC	≥8192 µg/ml	256 µg/ml	256 µg/ml
	MBC	≥8192 µg/ml	2048 µg/ml	512 µg/ml
Acinetobacter baumannii	MIC	≥8192 µg/ml	256 µg/ml	128 µg/ml
	MBC	≥8192 µg/ml	1024 µg/ml	512 µg/ml
MRSA	MIC	≥8192 µg/ml	128 µg/ml	128 µg/ml
	MBC	≥8192 µg/ml	256 µg/ml	256 µg/ml

4. Discussion

The MIC and MBC results obtained by Gan and his colleagues, in 2004, showed that the metal oxide (NPs) were able to inhibiting or destroying many pathogenic bacteria (Sondi & Salopek-Sondi, 2004). Regarding this theory, Guogang and Jayesh dispersed suspension of metal oxide (NPs) with the ultrasonic waves. They believed that the antibacterial properties of (NPs) will increase (Avadi et al., 2004; Lok et al., 2006). So far, many studies by researchers around the world in the field of antibacterial properties of silver (NPs) have been carried out (Avadi et al., 2004; Batarseh, 2004; Lok et al., 2006; Sondi & Salopek-Sondi, 2004; Thirumurugan, Shaheedha, & Dhanaraju, 2009). Though, studies of several authors in recent years, confirmed the antibacterial effects of Ag (NPs) (Batarseh, 2004; Lok et al., 2006; Sondi & Salopek-Sondi, 2004).

In the current study, zinc oxide, silver and zinc oxide/silver (NPs), by thermal decomposition of a precursor oxalate, were synthesized. FTIR spectroscopy, X-ray powder diffraction (XRD), scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM) for identification, structure, and surface morphology were used. Analysis of the results obtained in this study was interesting. Regarding that the diameter of inhibition zone (DIZ), reflects the sensitivity of the organism, strains of sensitive, show larger DIZ and the resistant strains show smaller DIZ (Thirumurugan et al., 2009). Results of disc diffusion test of antibiotic resistant *Pseudomonas aeruginosa* showed the least sensitivity to ZnO and Ag/ZnO (NPs). While DIZ obtained from the disc diffusion test, showed the greatest sensitivity to *Staphylococcus epidermidis* and MRSA.

Silver (NPs) have not antibacterial effect against six strains of antibiotic resistant bacteria. Additionally, Silver (NPs) had not bacteriostatic effect on *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter baumannii*, and *Methicillin resistant Staphylococcus aureus* at

concentrations of 8192 μg.ml^{-1} to 0.2μg.ml^{-1}. Whereas, results of the disc diffusion with Ag μg.ml^{-1}, by Thirumurugan against strains of pathogens *E. coli, S. typhi, B. subtilis, S. aureus*, indicated higher sensitivity to silver μg.ml^{-1} which is in contrast with the results of our study (Thirumurugan et al., 2009).

In 2005, Cho studies the MIC of Ag nanoparticles against *Pseudomonas aeruginosa* bacteria in which its growth in concentration of 7.5 μg.ml^{-1} completely inhibited (Cho, Park, Osaka, & Park, 2005). However, antibiotic resistance of *Pseudomonas aeruginosa* used in the current study, was resistance to Ag (NPs). Lowest MIC in *Pseudomonas aeruginosa* observed Ag/ZnO with 256 μgml^{-1} it has the highest inhibitory effect on *Pseudomonas aeruginosa*. In fact, the greatest resistance to zinc oxide (NPs) was seen in *Pseudomonas aeruginosa*(≥8192 μgml^{-1}). In another study, Cho reported the MIC rate of silver (NPs) for *S. aureus*as 12.6 μgml^{-1}, but interestingly, *MRSA* was resistance to the Ag (NPs) completely (Cho et al., 2005). The *MRSA* used in the current study, showed the least and the most sensitivity to silver and Ag/ZnO (NPs), respectively. Actually, the least degree of MIC in *MRSA* was related to combine (NPs) of silver and zinc oxide with concentration 128 μgml^{-1}, this (NPs) had the most growth inhibitory effect in *MRSA*.

In fact, the results of our tests, MIC and MBC, was also interesting. In our study, Silver/Zinc oxide (NPs) had a highest inhibitory effect against, all of the antibiotics resistant bacteria. However, our results had been consistent with Jafari and their colleagues in 2009 (Jafari, Ghane, Sarabi, et al., 2011). Jayesh recorded the MIC rate ranged 40-180 μgml^{-1} using tests of determining the sensitivity of silver (NPs) against different strains of *Escherichia coli* (Jafari, Ghane, Sarabi, et al., 2011). Kim studied the gram negative bacteria *E. coli* and gram positive *S. aureus,* also reported that the antibacterial silver (NPs) mostly affects the *E. coli,* which is due to the difference between cell wall of gram negative & positive microorganisms (Kim et al., 2007). Reddy were worked on the toxicity of the ZnO (NPs) in gram negative & positive bacteria (Reddy et al., 2007). They found that this (NPs) are able to completely inhibit the growth of *E. coli.* Also, Reddy and colleagues found ZnO (NPs) do not have any toxicity against eukaryotic cells. Actually, Ling Yang believed that photo catalytic ability of ZnO (NPs) plus silver (NPs) improves and also increases its oxidation and reduction abilities, while suppressing bacteria growth (Yang et al., 2006).

However, silver ions, eventually release during sterilization and kill bacteria due to their high antibacterial activation. They theorized that silver ions release following bacteria death and colloid with other bacteria and repeat their sterilization behavior. It was also mentioned that silver covered in the surface of ZnO (NPs) has the ability to involve the electrons produced through photo catalytic reactions of ZnO (NPs) which increases electron isolation and makes gaps in cell membrane, so increase its antimicrobial activity. Regarding studies of these authors, antibacterial property of silver and zinc oxide (NPs) improves with their combination. Based on the results obtained in this study, it was found that the composition of the metallic nanoparticle(NPs), silver and zinc oxide (NPs), it increases the antibacterial properties.

5. Conclusion

Silver (NPs) had not the ability to inhibit the antibiotic resistant bacteria. Also, we shows that Gram-positive antibiotic resistant bacteria such as MRSA, *Staphylococcus epidermidis* and also, *klebsiella pneumonia*, showed high sensitivity to Ag/ZnO and ZnO (NPs), compared to other bacteria.

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