RESEARCH ARTICLE

Antibiotic Profile and Antibacterial Activity of Copper Oxide Nanoparticles against *Pseudomonas Fluorescens* Isolated from Wound Infection

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ABSTRACT

Fifty specimens were collected from wound patients who visited Al-Hilla Teaching Hospital. The samples were grown on Blood and MacConkey agar for 24-48 hour at 37ºC. The bacterial isolates, which achieved as a pure and predominant growth from clinical samples as *Pseudomonas fluorescens,* were identified using morphological properties and Vitek2 system.

The antibacterial activity of copper oxide nanoparticles (CuO NPs) against was tested by (disk diffusion assay) using dilutions of (400, 200, 100, 50, 25, and 12.5µg/mL). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each isolate was determined. The CuO NPs shows wide spectrum antibacterial activity against tested bacteria with rising zone of inhibition diameter that is proportionate with the increase in nanoparticle concentration. The MIC of CuO NPs extended from 100-200µg/mL, and the MBC ranged from 200-400µg/ml. The antibiotic profile was determined by Viteck 2 compact system (Biomérieux).

CuO NPs found highly effective and safe in *P. fluorescens* wounds infections comparing with used antibiotics.

Keywords: Antibacterial activity, CuO, Nanoparticles, *Pseudomonas fluorescens.*

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INTRODUCTION

Pseudomonas fluorescens are motile, aerobic gramnegative rods bacteria 2–3µm in size; they frequently inhabit wastewater and wounds.[1] It is glucose fermentation negative, (chemoorganotrophic) and best grow at pH 4–8. Peptide rich media such as nutrient and tryptic soy broth/agar are the best to grow *P. fluorescens* species.[2] Selective and pigment improving media that are lacking iron but have other compounds like K, Mg and cetrimide, enable careful growth of *P. fluorescens* species where Cetrimide inhibits the growth of other bacteria and suitable for pigment production.[3,4] The pyocyanin pigment is produced by *P. aeruginosa* strains that is usually not made by of *P. fluorescens*.[5] The excretion of a fluorescent pigment (fluorescein), is what conveys *P. fluorescens* under UV light.[6]

Diverse kinds of metal and metal oxide nanoparticles that showed antimicrobial properties have been studied especially metal nanoparticles containing oxides of (magnesium, silver, copper, zinc, iron, and nickel) have been reported with broad antimicrobial possessions.[7,8] NPs mention to spherical particles in diameter extending from 1–100 nm.[9] They have a great surface to volume ratio in contrast to the particles established of similar material but not at the nanoscale. Therefore, NPs are more reactive.[10] The union of nanotechnology and biology is used to resolve numerous biomedical difficulties and can be practiced effectually and safely in the arena of health as specific NPs were widely investigated, and they showed capable antibacterial activity.[11] Among several metal oxide NPs, CuO has appealed more attention due to its safety among other members of the copper compounds family..[12]

Air, water, and soil microbial contamination owed to diverse kinds of microorganisms adding difficulties in living circumstances and is a severe case in health care issues. The emergence of antibiotic-resistant strains that cause serious infections encouraged research on alternative antimicrobial agents that have received much considerations.[13]

MATERIALS AND METHODS

CuO NPs (50nm) was acquired from (Zhengzhou Dongyao Nano Materials Co., LTD, China). Standardized media of (MacConkey's, Blood, and Muller's Hinton agar) were obtained from (HIMEDIA, India).

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Isolation and Characterization

The bacterial isolates were collected from the patients who stay the Teaching Hospital in Hillah, Babylon province, Iraq using Ames transport medium. All samples were cultured on blood and MacConkey's agar and incubated for 24–48 hours at 37ºC. All *Pseudomonas fluorescence* isolates and antibiotic profile tests were confirmed by Viteck 2 compact system (Biomérieux).[17]

Antibacterial activity of CuO NPs

Antimicrobial activity of the CuO NPs was tested against *Pseudomonas fluorescence*. The antimicrobial activity was conducted as described by the Clinical and Laboratory Standards Institute (2015) using a disk diffusion assay. Triplicates of CuO NPs were used in dilutions of (400, 200, 100, 50, 25, and 12.5µg/mL) in sterile deionized water. The plates were incubated for 15min at 4ºC then incubated at 37ºC overnight. Inhibition zone diameter was measured using a digital vernier caliper.[14]

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) determination

The bacterial isolates were incubated at 37ºC overnight, which was used to make 0.5 McFarland. The whole of the 10mL tube nutrient broth medium was ready then each sample was inoculated (aseptically) with 1mL of the bacterial suspension (about 108 CFU/mL).

Five dilutions of CuO NPs were set (200, 100, 50, 25, and 12.5µg/mL) in sterile deionized water, and negative control (without CuO NPs) was used. Tests were made in triplicates for each isolate. The inoculated groups were incubated at 37ºC overnight. After (incubation period), the visible turbidity in all tubes were examined. The lowermost concentration with no turbidity is represented as the MIC for the tested strain. Tubes displayed no turbidity were cultured on nutrient agar and incubated at 37ºC overnight. Bacterial colonies' growth was checked, and the concentration that shows no growth is represented as the MBC.[15,16]

Table 1: Antibiotic Sensitivity Tests by Viteck 2 compact system.

Antimicrobial	*MIC*	*Interpretation*
Ampicillin	= <32	R
Ampicillin/Sulbactam	= <32	R
Gentamicin	> =4	S
Cefazolin	= <32	R
Cefoxitin	= <32	R
Ceftazidime	= <4	S
Ceftriaxone	=<32	R
Cefepime	8	R
Meropenem	= <16	R
Imipenem	= <8	R
Amikacin	= <4	S
Ciprofloxacin	<= 0.25	S
Levofloxacin	4	I
Tigecycline	<= 0.5	S

RESULTS AND DISCUSSION

Seven isolates of *P. fluorescens* 1–7 were isolated and identified with conventional methods and also for further confirmation with Viteck 2 compact system. The isolates showed positive results with (PyrA, dGLU, dMNE, ProA, CMT, and O129R) tests and negative results with all other tests.

Sensitivity test results by Viteck 2 compact system showed resistance for antibiotic Ampicillin, Ampicillin/Sulbactam, Cefazolin, Cefoxitin, Ceftriaxone, Cefepime, Meropenem, Imipenem, but sensitive to Gentamicin, Ceftazidime, Amikacin, Ciprofloxacin, and showed intermediate sensitivity to Levofloxacin as shown in Table 1.

Antibacterial activity of CuO NPs

CuO NPs shows powerful broad-spectrum antibacterial activity against tested bacteria. The result in Figure 1 and Table 2 showed an increase in inhibition zone diameter proportional to the nanoparticle concentration. 400µg/mL concentration showed the highest zone of inhibition. CuO NPs cause a sudden decline in bacterial cell membrane integrity in addition to the release of reactive oxygen species (ROS) where superoxide

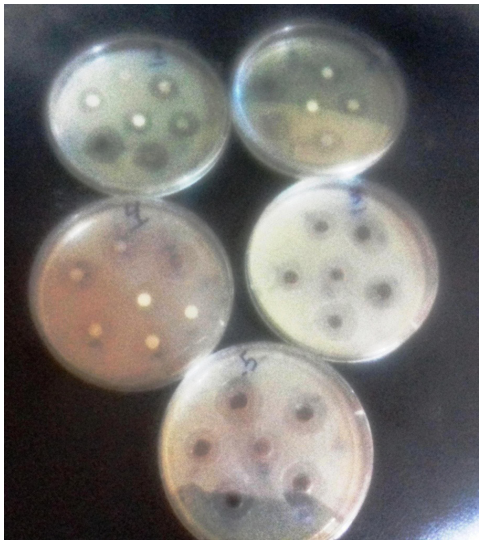

Figure 1: Antibacterial activity of CuO NPs against *P. fluorescens*

Table 2: Antibacterial activity of CuO NPs aganist *P. fluorescens.*

	Inhibition zone diameter average (mm)						
CuO NPs concentration	*Isolate(1)*	*Isolate(2)*	*Isolate(3)*	*Isolate(4)*	*Isolate(5)*	*Isolate(6)*	*Isolate(7)*
400µg/mL	20	19	20	18	19.5	20	19
200µg/mL	17	16	15.5	16	17	18	17
100µg/mL	14.5	13	12	13	12	13	12
50µg/mL	12	10	9	10	11.5	11	10
25µg/mL	9	7.5	7	8	9	9	8
12.5µg/mL	6	5	4	5.5	5	6	5

Table 3: MIC and MBC of CuO NPs for *P. fluorescens*

Bacteria isolate NO	*MIC*	*MBC*
1	100µg/mL	200µg/mL
2	100µg/mL	200µg/mL
3	100µg/mL	200µg/mL
4	200µg/mL	400µg/mL
5	200µg/mL	400µg/mL
6	100µg/mL	200µg/mL
7	200µg/mL	400µg/mL

species are generated and contributing in the degradation of biomolecules.[15]

Minimal Inhibitory Concentration (MIC) and Minimal bactericidal concentration (MBC)

Table 3 shows that the MIC of CuO NPs ranged from 100-200µg/ml, and the MBC ranged from 200-400µg/ml.

CONCLUSION

CuO NPs showed a good inhibitory and antibacterial effect on *P. fluorescens* that agrees with this.[15] It is recommended using CuO NPs as an alternative antibacterial agent, particularly for ectopic infections devoid of taking the risk of emerging bacterial strains resistant to antibiotics.

ETHICAL CLEARANCE

The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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