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ORIGINAL STUDY

Nano Zinc Oxide Against Bacteria Isolated From Burn Infection

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Abstract

Aim of study: **This study aims to evaluate the antibacterial and antibiofilm activity of Zinc Oxide Nanoparticles (ZnO NPs) against many bacteria isolated from burn infection.**

***Pseudomonas aeruginosa* is a type of bacteria that is found commonly in the environment, like in soil and in water. This bacteria can cause infections in the blood, lungs (pneumonia), or other parts of the body after surgery. These bacteria are constantly finding new ways to avoid the effects of the antibiotics used to treat the infections they cause. Zinc oxide nanoparticles' (ZnO-NPs') or Nano zinc oxide antibacterial properties have drawn a lot of attention from around the world, especially since nanotechnology has been used to create particles in the nanometer range. Because of their larger specific surface area and improved particle surface reactivity as a result of their smaller particle size, ZnO-NPs have appealing antibacterial qualities. ZnO is a bio-safe substance that affects chemical and biological species through photo-oxidizing and photocatalysis. The Aim of study is to evaluate the antibacterial activity and antibiofilm of Zinc Oxide Nanoparticles (ZnO NPs) against many bacteria isolated from burn infection include *P. aeruginosa* and *S. aureus* and *Klebsiella*. The tests in this study focusing on antibacterial and antibiofilm activity of zinc oxide nanoparticles. The results showed a moderate antibacterial activity by nano zinc oxide where 40 % of *P. aeruginosa* isolates as inhibited, *Klebsiella* was unaffected, and *S. aureus* was inhibited. On the other hand, it was exhibit a good antibiofilm activity produced by *Pseudomonas aeruginosa* and *Klebsiella*, so the nano zinc oxide shows promise as a potent antibiofilm agent.**

Keywords: Nano zinc oxide, Nanoparticles, ZnO NPs, Antibacterial, Burn infection, Anti-biofilm

1. Introduction

The development of antibiotic resistance among many bacterial isolates is considered a big problem; it leads to high morbidity and mortality worldwide [1]. Bacterial infections are considered severe health issues that attend internationally due to their significant impact on public health, social system, and economic burden [2]. The increasing of antibiotics resistance bacteria due to many causes, including genetic mutation, leads to become global health risks especially children, as increased hospital-associated infections, that increase in outbreaks and infections of pathogenic strains [3]. Thus, increases the interest in the development of alternative treatments rather than antibiotics [4]. Among these alternative treatments is the use of nanotechnology to generate nanoparticles that are used as antibacterial due to many characteristics of the nanoparticles, including a larger specific surface area because the smaller particles have a higher surface reactivity [3]. The transformation of any particles to nanoparticles leads to changes in their characteristics, including morphological, mechanical, chemical, electrical, etc. These alterations facilitate the entrance of the

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nanoparticles into the inner structures of the cells in a special physical manner [3,5]. Many particles have a unique properties by improving their physicochemical and biological properties in addition to their nanoscale size, these are used in this technology [6]. The nanoscale size increases the surface area of the particles that facilitate their adsorption [3,7]. The above characteristics enhance the uses of nanoparticles in many fields, including nanomedicine and nanotechnology [8,9].

One of these nanoparticles is zinc oxide nanoparticles (ZnO-NPs) or nano zinc oxide, which has many properties to use in the microbiology field as an antibacterial. From these properties, its larger surface area is due to its smaller particle size, which improves its surface reactivity [10]. In addition, it is considered biosafe because its effectivity on bacteria species through photo oxidizing and photocatalysis, mainly on the production of reactive oxygen species (ROS) as hydrogen peroxide (H_2O_2), hydroxyl radicals (OH), and peroxide (O_2^{2-}), that lead to bactericidal or bacteriostatic [11–13]. Many studies showed that ROS mechanisms that lead to bacterial damage occur as a result of many processes, including the uptake of hazardous dissolved zinc ions, the entrance of nanoparticles, increasing membrane permeability and destruction of cell wall. All that leads to intracellular outflow and an increase in the expression of oxidative stress genes that lead to cell growth inhibition and death [14]. ZnO NPs were used as antibacterial in the food packaging industry to reduce foodborne illnesses. When ZnO-NPs are properly included into packaging materials, they can interact with foodborne pathogens and release NPs onto food surfaces, where they can interact with harmful bacteria and either kill or suppress them [15]. Numerous studies have studied the antibacterial effectivity of zinc oxide nanoparticles against various bacterial species [16–22]. Zinc oxide, when transformed into nanoparticles, leads to an increase in its antibacterial effectivity by interacting with bacterial surfaces and components that exhibit unique bactericidal processes [23]. On the other hand, ZnO NPs are not harmful to human cells and exhibit a good antibacterial activity, so it can be considered a good alternative to antibiotics [24]. Even if a number of procedures have been presented and adopted, the specific ones are still up for debate. The mechanisms and phenomena of nanoparticles can benefit from studies on antibacterial nanomaterials, primarily ZnO-NPs. Although ZnO-NPs' antibacterial activity has disadvantages including its toxicity is remain unclear and up for debate since some of the questions within the antibacterial activity spectrum need in-depth answers. Several distinct mechanisms have been proposed, including the generation of ROS [25–28], the release of antimicrobial ions, primarily Zn^{2+} ions [29–31], and direct interaction of ZnO-NPs with cell walls, that lead to bacterial cell destruction [32–34].

2. Materials and methods

2.1. Bacterial isolates

In this study, three types of bacteria were isolated from skin burns infection, including 5 isolates of *P. aeruginosa,* 1 isolate of *S. aureus* and 4 isolates of *Klebsiella*. They were diagnosed using traditional methods and then using Vitec2 systems to confirm their diagnosis [35].

2.2. Zinc oxide nano particles (ZnO NPs) preparation

Zinc oxide nano particles (ZnO NPs) powder, (ZnO, 99+%, 10-30 nm) Stock #:US3590,CAS#: 1314-13-12 from US Research Nanomaterials, Inc. TX77084, USA), were prepared as manufacture instructions by dispersing 10 mg of ZnO NPs in 10 ml of distilled water using sonication for 20 min to obtain a homogenous suspension magnetic stirring at 100 °C for 30 min to provide heat and agitation, aiding in the dissolution.

2.3. ZnO NPs as antibacterial

After that investigation, its antibacterial activity against all isolated bacteria was measured by well diffusion methods with a standardized conditions on Muller Hinton agar using zinc oxide nanoparticles prepared in four concentrations (100 %, 50 %, 25 % and 12.5 %) according to Aldujaili et al. (2020) [36].

2.4. ZnO NPs as antibiofilm

Also, the antibiofilm activity of zinc oxide nanoparticles was investigated on the above isolated bacteria by studying the inhibitory effect and destructive ability of ZnO NPs on biofilm before and after biofilm formation respectively. Firstly, studying the destructive ability of ZnO NPson biofilm formation after its formation by inoculated 10 µL of the activated bacteria in sugar-containing BHI medium on ELIZA plate with 200 µL of sugar-free BHI to each well and incubate for 48 h followed by adding ZnO NPs and incubate for 48 h, then processes by sodium acetate to stabilize the biofilm in the wells, which act as biological membranes, for

30 min and staining the remaining biofilm by crystal violet and read the absorbance using ELIZA reader. Secondly study the ability of ZnO NPs to inhibit biofilm before its formation by adding ZnO NPs before inoculating the bacteria on the ELIZA plate and proceeding with the remaining steps mentioned above. The procedures were performed according to Ref. [37].

2.5. Statistical analysis

The study aimed to evaluate the feasibility and decide the direction rather than giving statistical conclusions, so it was restricted to descriptive.

3. Results and discussion

3.1. Results

The results of the sensitivity test at different concentrations showed that ZnO NPs at the concentration 100 % and 50 % inhibit the growth of two from five isolates of *Pseudomonas aeruginosa*, the inhibition of isolate No.1 was higher than isolate No.2, and inhibit the growth of *S. aureus* isolate but in lesser effect. While there is no inhibition was observed in any of the *Klebsiella* isolates as shown in Table 1.

Table 1. Inhibition of Nano zinc oxide against different bacterial isolates.

Type of bacteria	Concentration of ZnO NPs	Inhibition Zone diameter
P. aeruginosa Isolate No. 1	100 %	2.3 cm
	50 %	2 cm
	25 %	1.5 cm
	12.5 %	None
P. aeruginosa Isolate No. 2	100 %	1.1 cm
	50 %	0.85 cm
	25 %	None
P. aeruginosa Isolate No. 3	100 %	None
P. aeruginosa Isolate No. 4	100 %	None
P. aeruginosa Isolate No. 5	100 %	None
Klebsiella Isolate No. 1	100 %	None
Klebsiella Isolate No. 2	100 %	None
Klebsiella Isolate No. 3	100 %	None
Klebsiella Isolate No. 4	100 %	None
S. aureus	100 %	0.75 cm
	50 %	0.7 cm
	25 %	None

3.1.1. Nano zinc oxide particles as antibiofilm

The results of current study show that only two isolates (Klebsiella isolates No. 3 and *P. aeruginosa* isolate no.2) were able to form biofilm so the affectivity of ZnO NPs against biofilm was tested on these two isolates as shown in Fig. 1.

The biofilm formation among different bacterial strains is due to many causes, mainly of this variation depends on genetic variability between the different strains as psl, pel and algD genes in *P. aeruginosa* [38], icaADBC in *S. aureus*, and mrkA, fimbrial genes in Klebsiella [39]. On the other side, the clinical origin of bacterial isolates also affects biofilm formation (i.e. isolates that are taken from acute infection (as burn infection) form biofilm less than isolates taken from chronic infection or device associated infection [40].

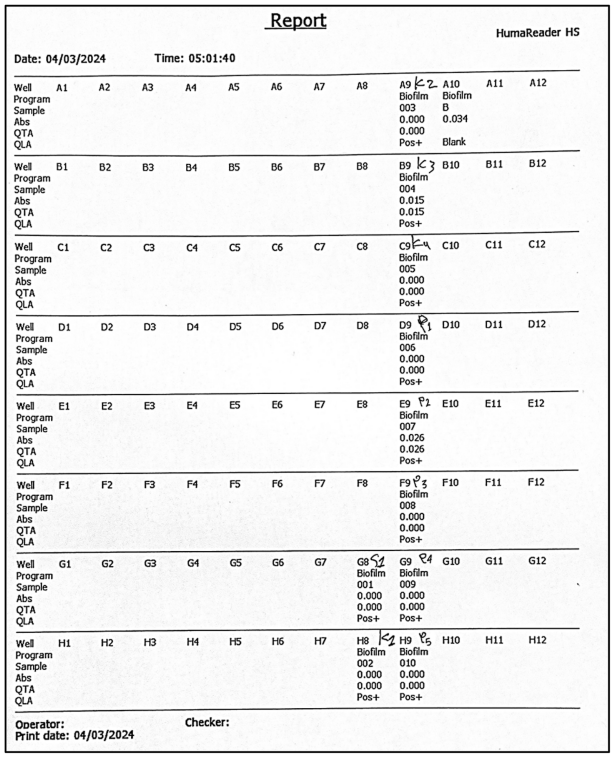
After study the effectivity of ZnO NPs in preventing and destruction of biofilm formation in the tested bacteria by adding it before and after biofilm formation respectively in different wells, the results found that they no longer formed a biofilm in the two wells that indicate its prevention of biofilm formation in the first well and destructed the biofilm in the second well as shown in Fig. 2. After the destruction of biofilm, growing the bacteria on culture media indicates that the bacteria still survive and confirms the effectiveness of ZnO NPs in the biofilm destruction rather than killing the bacteria.

4. Discussion

In this study, zinc oxide nanoparticles were used as antibacterial and antibiofilm (prevention and destruction) on three types of bacteria that were isolated from burn infection (*P. aeruginosa, S. aureus,* and *K. pneumoniae).*

However, the nano zinc oxide results showed the inhibition of two isolated strains of *P. aeruginosa* and one isolated strain of *S. aureus*, while the four isolated strains of Klebsiella were unaffected.

Nano zinc oxide (ZnO) exhibits moderate antibacterial activity due to several factors, Nano ZnO can lead to cell death by interacting with cell membranes leading to membrane damage. This type of interaction differs among different types of bacteria, which makes Nano zinc oxide (ZnO) have moderate effects against bacteria [41]. Also, other factors can affect the activity of nanoparticles in vitro as it is affected by PH changes and others, where the low acidic PH can increase the antibacterial activity of this nanoparticle [42]. Several factors may contribute to this variation as mentioned above, and the response of different bacteria varies,



Report

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	1	2	3	4	5	6	7	8	9	10	11	12
A									K2 Biofilm 003 0.000 0.000 Pos+	Biofilm B 0.034 Blank		
B									K3 Biofilm 004 0.015 0.015 Pos+			
C									Biofilm 005 0.000 0.000 Pos+			
D									Biofilm 006 0.000 0.000 Pos+			
E									Biofilm 007 0.026 0.026 Pos+			
F									Biofilm 008 0.000 0.000 Pos+			
G								Biofilm 001 0.000 0.000 Pos+	Biofilm 009 0.000 0.000 Pos+			
H								Biofilm 002 0.000 0.000 Pos+	Biofilm 010 0.000 0.000 Pos+			

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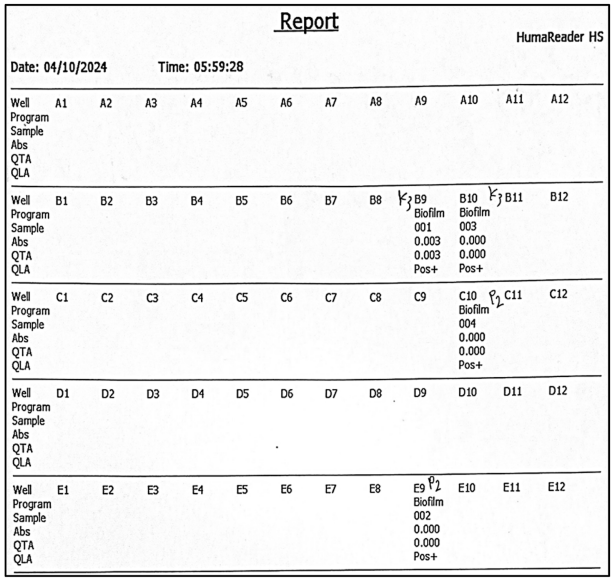
Fig. 1. ELIZA reader reports show the biofilm formation of the tested bacteria. (In figure well no. G8: represents S. aureus*, H8, A9, B9, and C9: represent* Klebsiella *Isolates No.1, 2, 3, and 4 respectively while D9, E9, F9, G9, and H9: represent* P. aeruginosa *Isolates No.1, 2, 3, 4, 5, respectively.)*



Report

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	1	2	3	4	5	6	7	8	9	10	11	12
A												
B									K3 Biofilm 001 0.003 0.003 Pos+	B10 Biofilm 003 0.000 0.000 Pos+	K3 B11	
C										C10 Biofilm 004 0.000 0.000 Pos+	P2 C11	
D												
E									P1 Biofilm 002 0.000 0.000 Pos+			

Fig. 2. ELIZA reader reports show the prevention of biofilm formation by ZnO NPs in the two tested bacteria (B9 & E9 for Klebsiella *isolate No.3 &* P. aeruginosa *isolate no.2) and show the destruction of biofilm after formation in the same bacteria (B10 & C10 for* Klebsiella *isolate No.3 &* P. aeruginosa *isolate no.2).*

some bacterial strains can be affected more than other strains and some are more resistant to it [43]. The variation between different species of bacteria used in the present study results from many variations in phenotypic and genotypic characteristics between them. *S. aureus* is gram-positive, so it has thick peptidoglycan without an outer membrane that may facilitate the entrance and binding of ZnO-NP with the cell membrane, which leads to its damage [44,45]. The intermediate sensitivity of ZnO NPs against *P. aeruginosa*, which inhibits some strains from returning to genetic variation, as some strains possess a virulence mechanism (e.g efflux pump) rather than others [46]. While the lack of ZnO NPs effect on all *Klebsiella* isolates is attributed to presence of polysaccharide capsule in addition to other virulence factors as efflux pump which prevent passing of nanoparticles to the cell membrane [45,47].

On the other hand, the results of this study showed good activity of zinc oxide nanoparticles (ZnO-NP) in the destruction of biofilm after formation and in preventing biofilm formation in the tested bacteria, these results are consistent with Wahab R. *et.al.* study [48]. Zinc oxide nanoparticles affect bacterial cells and biofilm formation in many ways including their interaction with cell membrane which leads to membrane damage and cell destruction. Additionally, ZnO-NP may damage bacterial cells by generating reactive oxygen species (ROS) [11,12]. ZnO-NP can prevent biofilm formation by inhibiting the initial attachment of bacteria to the surfaces, the first step of biofilm formation, on the other hand, it can destroy biofilm after formation by destabilizing them that facilitate the removal of biofilm [11]. ZnO-NP nanoscale size increases their interaction with biofilms on one side and with bacteria on the other side due to a high surface area/volume ratio [10]. Other studies showed that ZnO-NP can disrupt the metabolic process of bacteria by releasing zinc ions [49] and others showed that ZnO-NP ability to disrupt bacterial signaling pathways [50] or interfering with bacterial enzyme activity [11]. Overall, all these factors contribute to the effectivity of ZnO-NP against bacteria and biofilm.

5. Conclusion

Zinc oxide nanoparticles show moderate antibacterial activity, while they show a good result in preventing biofilm formation and biofilm destruction. Therefore, Zinc oxide nanoparticles demonstrated potential antibiofilm activity that may be considered a promising anti-biofilm.

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Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Ethical Approval

This study was conducted entirely in laboratory settings and did not involve human participants, human data, or human biological samples. Therefore, ethical approval and informed consent were not required.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

- Conceptualization: Mohammed Jaafar Al-Anssari
- Methodology: Hayder Razzaq AL-Ameedee and Hawraa Majid Mahdi
- Data Collection: Mohammed Sahib AL-Hamami and Hayder Razzaq AL-Ameedee
- Formal Analysis: Mohammed Sahib AL-Hamami and Rabab Abdul Razzaq Kasema
- Writing – Original Draft: Rabab Abdul Razzaq Kasema and Hawraa Majid Mahdi
- Writing – Review & Editing: Mohammed Jaafar Al-Anssari
- Supervision: Mohammed Jaafar Al-Anssari

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