JOURNAL OF KUFA–PHYSICS
journal.uokufa.edu.iq/index.php/jkp/index | ISSN: 2077–5830

Characterization of Antimicrobial Activity of NiO Nanoparticles Against Pathogenic Bacteria Isolated from Biofilm of Water Lines in Medical Devices

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ARTICLE INF.

Article history:
Received: 16 MAY., 2023
Revised: 19 JAN., 2024
Accepted: 30 MAR., 2024
Available Online: 29 DEC., 2024

Keywords:

NiO NPs, nanomaterial, *S. aureus*, *P. aeruginosa*, Biofilm, MDR

ABSTRACT

The major goal of this research was to determine how well NiO nanoparticles (NPs) inhibited the growth of harmful bacteria that were separated from biofilm layers that had developed inside of medical equipment used on a regular basis in hospitals and dental offices. Chia seed extract was previously used in the preparation and physical characterization of NiO nanoparticles. Thirty isolates of *Pseudomonas aeruginosa* (n=16) and Staphylococcus aureus (n=14) were found in separate swab samples taken from dental office bottles, syringe tubes, spittoons, ventilation machine tubes, and emergency room bottles. Using conventional bacteriological techniques, moist swab samples were inoculated and identified on culture medium, and subsequently antibiotic susceptibility was assessed in accordance with NCCLs. Using two different techniques—well diffusion and microdilution, respectively—the antibacterial activity of NiO nanoparticles against contaminated bacteria was assessed both qualitatively and quantitatively. The antibacterial susceptibility of suggested antibiotics was determined using a Muller Hinton agar (MHA) plate. Antibacterial susceptibility of bacterial isolates revealed considerable resistance to the antibiotics utilized in the current investigation. According to these results, NiO nanoparticles showed the greatest effectiveness when used at a concentration of 30 mg/mL in well diffusion, and the mean inhibition zones for P. aeruginosa and S. aureus isolates ranged from 16 to 20 mm, as opposed to 16 mm for the positive control (ofloxacin disk). In this work, the inhibition zone diameters of NiO nanoparticles were within the region of high susceptibility of positive control (16 mm/ Ofloxacin disk breakpoint). However, the minimum inhibitory concentration (MIC) of NiO nanoparticles was 1.875 mg/ml for 57.1% of S. aureus isolates and 0.468 mg/ml for 31.2% of P. aeruginosa isolates, with no isolates inhibited at high doses. The MIC findings revealed that the lowest concentration of NiO nanoparticles had in vitro efficiency and proved to be a good antibacterial agent against contaminated bacteria.

DOI: https://doi.org/10.31257/2018/JKP/2024/v16.i02.12091

تشخيص النشاط المضاد للميكروبات لجسيمات أوكسيد النيكل النانوية ضد البكتيريا الممرضة المعزولة من الأغشية الحيوية في خطوط المياه بالأجهزة الطبية

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الـــخُـــلاصـــة

الهدف الرئيسي من هذا البحث هو تحديد مدى قدرة جسيمات اوكسيد النيكل النانوية (NiO NPs) على منع نمو البكتيريا الضارة المعزولة من طبقات الأغشية الحيوية التي تكونت داخل المعدات الطبية المستخدمة بشكل منتظم في المستشفيات وعيادات الأسنان. تم استخدام مستخلص بذور الشيا في تحضير وتوصيف الجسيمات النانوية لأكسيد النيكل من الناحية الفيزيائية. تم العثور على ثلاثين عزلة من بكتيريا (*Pseudomonas aeruginosa* عدد = 16) و(*Staphylococcus aureus* عدد = 14) في عينات مسح مأخوذة من زجاجات عيادات الأسنان، وأنابيب الحقن، وأحواض البصاق، وأنابيب أجهزة التهوية، وزجاجات غرف الطوارئ. باستخدام التقنيات البكتريولوجية التقليدية، تم استنبات عينات المسحات الرطبة وتحديدها على وسط استنبات ثم تم تقييم الحساسية للمضادات الحيوية وفقًا لمعايير NCCLs .تم تقييم النشاط المضاد للبكتيريا للجسيمات النانوية لأكسيد النيكل ضد البكتيريا الملوثة باستخدام تقنيتين مختلفتين، هما تقنية انتشار الآبار وتقنية التخفيف الميكروي، لتحديد النشاط النوعي والكمي. تم تحديد حساسية العزلات البكتيرية للمضادات الحيوية المقترحة باستخدام وسط أجار مولر هينتون (MHA) .أظهرت الحساسية المضادة للبكتيريا للعزلات البكتيرية مقاومة كبيرة للمضادات الحيوية المستخدمة في الدراسة الحالية. ووفقًا لهذه النتائج، أظهرت الجسيمات النانوية لأكسيد النيكل أعلى فعالية عند استخدامها بتركيز 30 ملغم/مل في تقنية انتشار الآبار، حيث تراوحت متوسط أقطار مناطق التثبيط لعزلات *P. aeruginosa* و*S. aureus* بين 16 و20 ملم، مقارنة بـ 16 ملم للتحكم الإيجابي (قرص أوفلوكساسين). في هذه الدراسة، كانت أقطار مناطق التثبيط للجسيمات النانوية لأكسيد النيكل ضمن نطاق الحساسية العالية للتحكم الإيجابي (16 ملم/ قرص أوفلوكساسين). ومع ذلك، كان أقل تركيز مثبط (MIC) للجسيمات النانوية لأكسيد النيكل 1.875 ملغم/مل لـ 57.1% من عزلات *S. aureus* و0.468 ملغم/مل لـ 31.2% من عزلات *P. aeruginosa*، ولم تُظهر أي عزلات تثبيطًا عند الجرعات العالية. كشفت نتائج MIC أن أقل تركيز للجسيمات النانوية لأكسيد النيكل كان له فعالية **(في المختبر)** وأثبت أنه عامل مضاد للبكتيريا فعال ضد البكتيريا الملوثة.

الكلمات المفتاحية:

NiO NPs, المواد النانوية, *S. aureus*, *P. aeruginosa*, الاغشية الحيوية, MDR

1. INTRODUCTION

Nowadays, the world is paying significant attention to the use of nanoparticles comprising metal oxide NPs [1]. Nanoparticles have diverse biological properties. Metal oxide nanoparticles are often used in biomedical sciences, drug delivery, optoelectronic devices, biosensing, catalysis, antimicrobial characteristics, and chemical sensors [2]. These NPs have been used as antibacterial and antifungal agents. NiO NPs have been demonstrated to produce Ni2+ ions, which injure bacterial cells by

producing oxidative stress and cell death. The effectiveness of the NPs to destroy infections is reliant on the synthesis medium. In terms of antibacterial action, NiO-NPs are effective against both Gram-positive and Gram-negative bacteria [3].

According to Slavin [4], the scientific community has recently been compelled to look for novel antimicrobial drugs due to the rising prevalence of antibiotic resistance among common bacterial strains. Antibiotic-resistant bacteria typically appear out of nowhere and may even pose a threat to recently launched antibiotics [5]. P. aeruginosa and S. aureus, two nosocomial infections with a high prevalence of multidrug resistance, are two examples. Nanoparticles have demonstrated strong antibacterial action and are among the most promising novel antimicrobial agents [6]. When hospitalized individuals with weakened immune systems are colonized and infected, *Pseudomonas aeruginosa* and S. aureus are thought to be a prominent pathogen. This bacterium can cause life-threatening infections in the skin, ear, urethra, gastrointestinal system, lung, brain wound, and burns [7].

In addition to P. aeruginosa and S. aureus, other major surface adhesion proteins include fibrinogen binding protein, surface binding protein A, cell wall-anchored proteins, and clumping factors. These proteins mediate the bacterial binding to host cells. Additionally, these microbes play a crucial part in the development of biofilms, which serve as both a host defense mechanism and a mechanism for resistance to antibiotics [8]. A lot of bacteria are present on surfaces in aqueous settings. Urban water pipes have been found to contain biofilms, according to reports [9]. An enormous number of medical equipment and tools, such as intravenous catheters, injection needles, urine catheters, intrauterine devices, and cardiac pacemakers are susceptible to biofilms, which represent a significant medical issue in hospitals across the world. As a result of their antibiotic resistance, bacteria biofilms on medical equipment and devices serve as a source of recurring infections [10]. According to the American Dental Association (ADA), the amount of bacteria in dental unit water lines shouldn't be more than 200 CFUs/ml up until the year 2000 [11].

The biofilm, which is created by bacteria in the entering water and is naturally resistant to most biocides, subsequently serves as the main source of ongoing contamination of the system. Dental water may become severely polluted with opportunistic respiratory infections like Legionella and Mycobacterium spp. Nevertheless, infection management aims to reduce the risk of exposure to possible infections and to establish a secure working environment for treating patients [12].

Dental unit waterlines, such as the plastic tubing used to supply water to high-speed handpieces, air/water syringes, and ultrasonic scales, encourage the growth of bacteria and the formation of biofilm because they have long, narrow bore tubing, fluctuating flow rates, and the potential to retract oral fluids [13].The

antimicrobial properties of metal oxide nanoparticles were therefore established and improved the coating of plastic film and infused with internal surface layers in tubes of medical equipment and water lines. Commercial products and devices are now available that can improve the quality of materials used in dental units and any medical equipment. According to the aforementioned, this study succeeds in achieving all of its goals related to evaluating NiO NPs against contamination bacteria in medical facilities, and it will attempt to use nanotechnology in the future to produce new auto-sterilizing medical devices in order to prevent contamination and the indirect transmission of microbial infections.

2. MATERIAL AND METHODS

Study of Antibacterial activity

2.1. Isolation and Identification of Bacterial Isolates

Both species of bacteria were isolated and identified using blood agar, MacConkey agar, and Mannitol salt agar (Difco, USA). Cultures which were cultured at 37 0C for 18-24 hours. Identification processes based on Bergeys Manual [14] [15]. Muller-Hinton (MH) agar (Oxoid, UK) was employed in the disk diffusion technique to determine antibiotic susceptibility of P. aeruginosa and S. aureus isolates. As conventional antibiotics, amoxicillin, ampicillin, cloxacillin, oxacillin, clindamycin, carbenicillin, cefotaxime, ciprofloxacin, furadantine, tetracycline, rifampicin, and ofloxacin disks (Himedia, India) were used. MacFarland tube number 0.5 was utilized as a reference for inoculum production, which contained about 1108 CFU/mL. According to Kirby-Baure [16] [17]. Zones of inhibition were measured and analyzed in accordance with NCCL recommendations published in 2010[18]. Escherichia coli (ATCC 25922) was utilized in this investigation as the reference strain for assessing antimicrobial susceptibility.

2.2. Antimicrobial Activity of NiO nanoparticles

The evaluation of activity of NiO nanoparticles against *P. aeruginosa* and *S. aureus* isolates were achieved as following methods:

A. Agar-well diffusion method: This procedure was carried out in accordance with [19]. a suspension of bacteria that has been made in sterile normal saline and calibrated to 1 108 CFU/ml. After applying the inoculums to the Mueller Hinton agar surface with a sterile cotton swab, the cultured agar plates were punctured with 8mm holes after 15 minutes. NiO nanoparticles (30 g/ml) were pipetted into wells using a micropipette. Inhibition zones were assessed as a measure of the antibacterial activity after plates were incubated at 37 °C overnight. As a positive control, ofloxacin disk was employed.

B. Broth microdilution technique: According to Azam [20], microbroth dilution was used to determine the minimum inhibitory concentration (MIC). A 96-well micro titer plate was

filled with 100 L of serially diluted NiO nanoparticles (30–0.234) g/ml using a pipette, and 100 L of MH broth was poured to each well. They were made in three copies. The medium was then combined with 100 L of bacterial suspension (108 CFU/ml). To avoid dehydration, the plates were covered, and they were then incubated at 37 °C for 18–24 hours. the growth's turbidity as determined visually. The MIC value, which prevents bacterial growth, was determined to be the lowest concentration at which turbidity changed. As a control, standard antibiotics were used.

2. RESULT AND DISCUSSION

Thirty bacterial isolates of Gram positive (Staphylococcus aureus 14 isolates) and Gram-negative (*Pseudomonas aeruginosa* 16 isolates) bacterial isolates (Figure 1 A and B) were collected from water lines of medical instruments used in dental clinics and hospitals, and all isolates were identified in advance laboratory of basic science department in Dentistry college at University of Kufa between November 2022 and February 2023.

Figure 1: Morphological colonies growth on bacteriological selective medium. A: pure colonies of *Pseudomonas aeruginosa* with biocyanin coloring (green hue) and B: purified colonies of Staphylococcus aureus on Mannitol salt agar, all cultures incubated at 370C for 18-24 hours.

The antimicrobial susceptibility test of bacterial isolates using disk diffusion test showed high percentage of resistance for almost antibiotics (Figure 2).

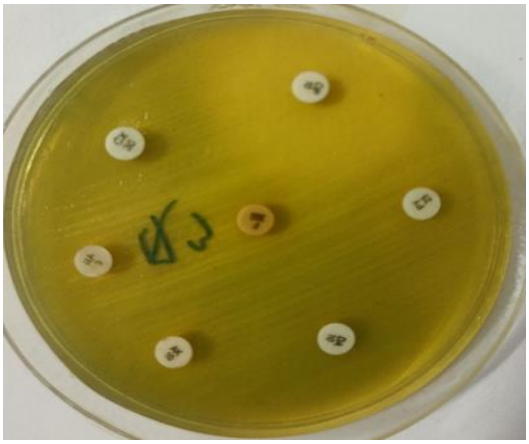
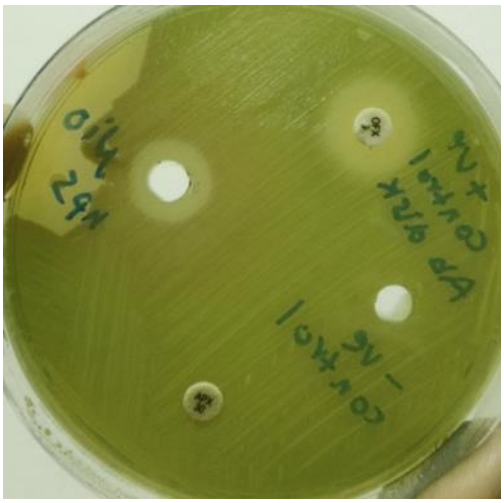


Figure 2: The antimicrobial susceptibility of bacterial isolates using disk diffusion test. The figure shows resistant of bacteria to almost antibiotics.

The findings of this study revealed that P. aeruginosa and S. aureus strains are strictly pathogens that are commonly found in hospital environments as well as medical equipment, and they were resistant to

most clinically relevant drugs. The isolates were classified as multidrug-resistant (MDR) due to their intrinsic resistance to more than three antibiotic classes and their ability to easily acquire antibiotic resistance determinants. Furthermore, in the absence of an adequate treatment method for hospital devices, resistant bacteria are discharged directly into the public and healthy people, and they might serve as possible vectors for the development of antibiotic resistance [21].

The NiO nanoparticles were examined at a concentration of 30 mg/ml and had already been produced and purified in the Physics Department at the Faculty of Science in the same university using certain physical techniques. The two most effective approaches for evaluating nanoparticle compounds were the well diffusion method (Figure 3).



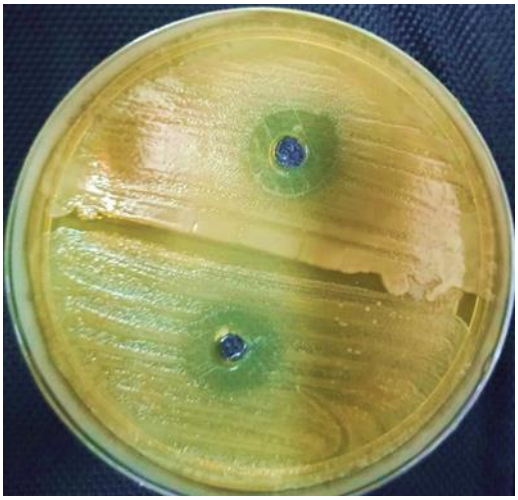


Figure 3: The antimicrobial activity of NiO nanoparticles against A: *Pseudomonas aeruginosa*and B: *Staphylococcus aureus*using well diffusion test. Ofloxacin disk used as control positive and the ampicillin/clavulanic acid as negative control.

The mean of inhibition zone diameters of NiO nanoparticles were approximately 16.5 mm against 16 isolates of *P. aeruginosa* in compare with positive control (16 mm) and 20 mm against 14 isolates of S. *aureus* in compare with positive control (18 mm). Table 1 shows that NiO nanoparticles have high activity against multidrug-resistant isolates in this study and the inhibition zone diameters of NiO nanoparticles within the range of high susceptibility of positive control (≥ 16 mm/ Ofloxacin disk breakpoint) according to guidelines of NCCLs [18].

Table 1: The mean of inhibition zones of NiO nanoparticles against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in compare with breakpoints of antibiotic controls

Compounds	Description	Mean of inhibition zones (mm)	
		***Pseudomonas aeruginosa* (n=16)**	***Staphylococcus aureus* (n=14)**
NiO Nanoparticles (Crud concentration)	200 µl/ well (30 mg/ml)	16.5	20
Ofloxacin disk (5 µg) (Positive control)	Diameter breakpoint ≥ 16 mm (susceptible) 13-15 mm (intermediate) ≤ 12mm (resistant)	16	18
Ampicillin/clavulanic acid disk (30 µg) (Negative control)	Diameter breakpoint ≥ 18 mm (susceptible) 13-17 mm (intermediate) ≤ 13 mm (resistant)	6.0 - 10	6.0- 12

NiO nanoparticles have been used as a suitable nanomaterial for the manufacture of tools that are used in medical devices to prevent contamination by pathogenic bacteria and the formation of biofilm because they had a significant impact on bacteria that were isolated from waterlines of dental units and ventilator machines. Since lbeigi [22] found that NiO nanoparticles have inhibitory activity against both Gram-positive and Gram-negative bacteria, these findings are somewhat similar to Shanaj [23] who found that NiO nanoparticles have excellent selectivity against Gram positive bacteria. Basically, the nickel ions produced from NiO nanoparticles are also responsible for the antibacterial action of nanoparticles. The ions produced in turn might make the membrane more permeable and encourage oxidative stress, which would then activate the celldeath pathways [22].

The minimum inhibitory concentration (MIC) was employed in the current work to quantitatively assess the antibacterial activity of NiO nanoparticles against the test microorganisms using the two-fold dilution technique [18]. The MIC measurements for NiO NPs in the range

of 0.234–30 mg/ml are shown in figures (4 and 5), and ofloxacin (100 mg/ml) was used as the control for comparison. The findings demonstrate that all bacterial strains were most sensitive to low concentrations of NiO nanoparticles, and P. aeruginosa isolates were most affected by low concentrations (0.468 mg/ml), with 2 isolates (12.5%) being inhibited by high concentrations of NiO NPs (15 mg/ml).

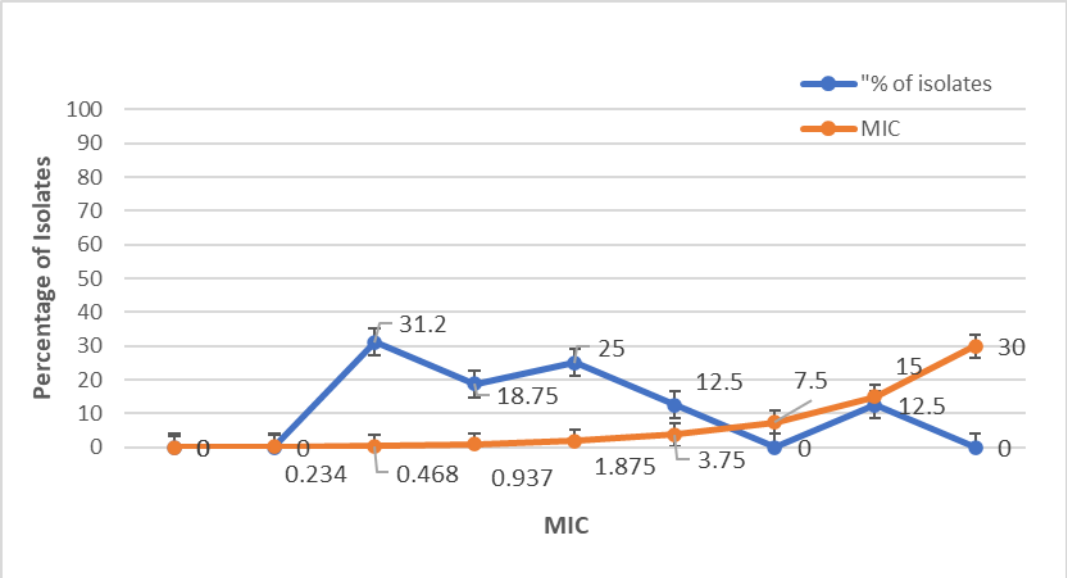


Figure 4: The association between percentage of *Pseudomonas aeuroginosa* Isolates and MIC of NiO NPs

P. aeruginosa is regarded as a pathogen that is strictly extremely resistant because to the poor permeability of its outer membrane, development of several efflux pumps, and synthesis of enzymes that inactivate antibiotics [7]. The outcomes showed higher NiO nanoparticle activity as compared to standard antibacterial screening.

The MIC of NiO and against Gram-positive Bacteria *S. aureus* was shown to range between 0.937 and 3.75 mg/ml (Figure 5), with 1.875 mg/ml inhibiting the most germs (57.1%). Additionally, no isolates are inhibited by high NiO NPs concentrations (7.5, 12, or 30 mg/ml). That indicates that *S. aureus* is more susceptible to being affected by NiO NPs than *P. aeruginosa*. The Central Public Health Laboratory in the UK found that 61% of nosocomial *S. aureus* infections in the 96 hospitals studied were methicillin resistant (MRAS). This appears to be related to thickening of the cell wall peptidoglycan and due to an increase in modified target for the antibiotics in the cell [24].

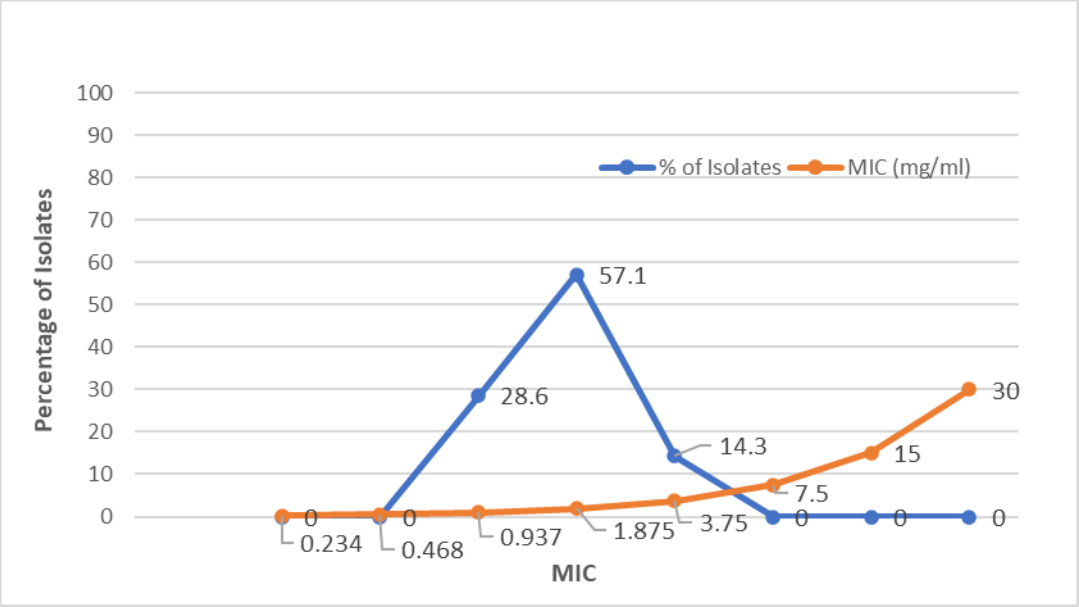


Figure 5: The association of percentage of *Staphylococcus Aureus* Isolates and MIC of NiO NPs

But NiO nanoparticles have stronger antibacterial effects against some strains of S. aureus. Gram-positive bacteria typically have a single thick layer of peptidoglycan in their cell walls, whereas Gram-negative bacteria have an envelope made up of three layers, the first of which is a lipopolysaccharide outer membrane and the second of which is a rigid exoskeleton called peptidoglycan [25]. This difference in cell wall composition may explain why Gram-positive bacteria generally have more effective antibacterial activity than Gram-negative bacteria [26]. Gram-negative bacteria can modify the outer membrane in any way, for as by mutating porins or other proteins or altering its hydrophobic characteristics [27]. Due to the presence of this crucial

layer in Gram-negative bacteria, Gram-positive bacteria are less susceptible to antimicrobial drugs [28]. The NiO nanoparticles have high antimicrobial activities against S. aureus, as demonstrated in this study. This may be because of their physical characteristics, such as their small particle size, which may make them easier to penetrate bacterial cell membranes and surface functionalization, which may increase their dispensability. In addition, heavy metals have a stronger affinity for protein molecules, which binds them to functional groups of proteins, resulting in protein. These results support the idea of utilizing NiO nanoparticles as a metal oxide to interfere with the inner surfaces of medical equipment' water lines for cleaning and long-term prevention of biofilm formation by Gram positive and Gram negative pathogenic multidrug-resistant bacteria.

4. CONCLUSIONS

NiO NPs are an effective nanomaterial that may be used as an antimicrobial agent, particularly as a highly antimicrobial active product for

industrial tools and medical equipment to prevent contamination by harmful bacteria. NiO NPs were reported to have more antibacterial action against gram positive S. aureus bacteria than gram negative P. aeruginosa, with considerable effectiveness even at low NiO NP concentrations.

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