Antibacterial Activity of Nickel Nanoparticles Against Pathogenic Bacteria

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Antibiotic resistance among bacteria can be regarded as becoming more topical in the medical field. Here we studied the effects of (Ni NPs) on antimicrobial agents with pathogenic bacteria. The synthesis of nickel nanoparticles by a cheaper route and physicochemical characterization were carried out using Atomic force microscopy (AFM) analysis. Of the tested Ni NPs, five are active against the Gram-positive strains E. coli and S. aureus, as well as the Gram-negative P. aeruginosa. method of putting the bacterial culture of Pseudomonas aeruginosa on solid petri dishes is a good diffusion method. The discovered results indicated the tolerable antibacterial activity shown by the Ni NPs to all bacteria strains applied. The inhibition zones were within 18, 24, and 15 mm ranges. The minimum inhibitory concentration of the Ni NPs was also determined and it was discovered to be inside the range of 20, 10, 40, 50, and 100 Along this, the data from the time-kill assay, also showed that the bacteria were interfered with, by the Ni NPs, as within the first four hours, the bacterial count was reduced by 99%. Therefore, our results conclude that nickel nanoparticles might be utilized as an antibacterial medium with proven efficiency against pathogenic bacteria.

Keywords: Nanoparticles, Pathogenic Bacteria, Antibacterial, Atomic Force Microscopy

1. Theoretical Background.

The compelling trouble involving antibiotic-resistant bacteria causes the deepest problem that threatens all the inhabitants of the world, and it requires the greatest effort as soon as possible [1]. Anti-microbial resistance is similar to the tangled web of society, which is now being catalyzed by the misuse or overuse of antibiotics escalating fast, evidently leading to a very complicated situation for the prevention and treatment of infectious diseases. This, therefore, is a huge call on innovative and experienced scientists to work hard and become creative builders of improved non-antibiotic drugs that can skillfully maneuver the intricate barrier of

antibiotic resistance [2]. Concerning the emerging trend in the alternative antimicrobial strategy, metal nanoparticles are one of the most dynamic and potential applications because of their unique and numerous chemical and physical properties, [3, 4, 5]. However, among the metal nanoparticles, nickel nanoparticles (Ni NPs) have shown significant and effective antimicrobial activity to microorganisms, the occurrence of Bacterial strains, that is even pathogenic kinds of bacterial species, as well as industries [6]. The nanoscale NPs spearhead the intricate world with three distinctive properties featuring predominant small size, colossal surface area, and high reactivity [7, 8]. Their capability to let through delicate Ni NPs counter their membrane is how the latter creates a detailed and intimate interplay by interacting with intracellular elements that are eventually a cause of their death [9]. Along with it, the larger surface area of Ni NPs means more points of contact with bacterial cells, so it boosts and strengthens their antimicrobial capability [10]. Moreover, the overreactive nature of Ni NPs because of the unpaired electrons on their surface underscores the intricate interactions with cellular components of bacteria, which in turn disrupt the metabolic pathways and result in the death of the bacteria [11, 12]. Although still unclear as to the accurate and detailed mechanisms used to make Ni NPs antibacterial, various paths have been suggested. What is remarkable here is that the Ni NPs can also cause oxidative stress in bacteria which is a significant mechanism of their antimicrobial action. For instance, bacterial cell membrane destruction which leads to cell content release and cell death is also a vital option [14]. In a nutshell, Ni NPs as anti-bacterial agents against pathogenic bacteria could be a promising and fascinating trail, which can reshape the treatment of infections [15]. The unique and variegated physicochemical properties of Ni NPs make them not only alternatives but highly competitive to conventional antibiotics, further opening a new horizon in which such agents can fight against bacterial infections [16]. Although the situation is quite complex, a robust research agenda should be the next critical step, not only to clarify the mechanisms of action but also to scrutinize the safety and effectiveness of Ni NPs when applied in vivo [17]. This complex knowledge gap related to antibacterial strategies requires urgent attention, not only due to the need to deepen our fundamental understanding, but also to inspire the development of robust, reliable, and ethical therapeutic approaches to ensure the effective management of bacterial infections in the ever-evolving landscape of global health.

Bio Inhibition Of The Substance Under Investigation By The Mcfarland Method:

The McFarland method, renowned for its enormous application, serves as a quintessential approach for precisely figuring out microorganism concentrations in suspensions, imparting a standardized and strong method to assess bacterial density [18]. In this meticulous process, a standardized turbidity of 0.5 McFarland units is established as a baseline, in opposition to which the turbidity of the investigated bacterial suspension is intricately in comparison. Employing spectrophotometry at a particular wavelength (625 nm), the turbidity of the bacterial suspension is quantified, and this value is systematically correlated with the usual turbidity using a nephelometer, furnishing a solid basis for calculating the bacterial concentration inside the sample. Beyond its utility in quantification, the McFarland technique assumes prominence in bioinhibition studies, emerging as a familiar approach for comparing the efficacy of diverse antimicrobial agents, which include cutting-edge substances like nanoparticles [19]. In the complex web of these investigations, the substance under scrutiny is meticulously introduced into the bacterial suspension at a specific concentration, and subjected

to a carefully delineated incubation duration. Post-incubation, the turbidity of the suspension undergoes a scrupulous evaluation through the McFarland method, dropping mild at the inhibitory dynamics of the substance on bacterial growth. This no longer only exhibits the extent of antimicrobial efficacy but additionally unravels nuanced components of the substance's bioinhibitory properties. Nanoparticles, propelled via their diminutive size and expansive surface area, stand as promising contenders for antimicrobial programs. Within this context, the McFarland method emerges as a powerful tool, specifically while scrutinizing the inhibitory outcomes of nanoparticles on bacterial increase. It assumes a pivotal position in elucidating the Minimum Inhibitory Concentration (MIC) of nanoparticles - the concentration threshold in which their efficacy against the target bacteria becomes conspicuous. This approach no longer handiest enriches our understanding of their antimicrobial capacity however also lays the foundation for progressive antimicrobial agent development and the refinement of current strategies. The McFarland approach transcends its foundational role as a quantification tool, evolving into an indispensable device for a nuanced and complete evaluation of antimicrobial substances, showcasing incredible versatility, specifically inside the evolving panorama of nanoparticle studies [18, 19]. By enabling researchers to delineate the MIC of a substance, this method not only contributes critical data for the development of antimicrobial agents but also stands as a cornerstone in the perpetual quest for advanced strategies to combat microbial challenges. Expanding on the McFarland method's applications underscores its adaptability, positioning it as a pivotal player in the continual pursuit of precision and innovation within the realm of antimicrobial research.

Minimum Inhibitory Concentration (MIC):

The Minimum Inhibitory Concentration (MIC) designates an important quantifier that expresses the lowest concentration of an antimicrobial agent able to impede the visible growth of the microorganism within a predetermined incubation period. MIC determination is a complicated procedure that requires using different methods, with broth dilution method and agar dilution method being the most spread ones [20].

In the broth dilution technique, a close-knit series of dilutions of the antimicrobial agent is put into a bacterial suspension in a liquid growth medium. Next, the MIC shows the minimum concentration of an antimicrobial agent at which no observable bacterial growth occurs upon completion of an incubation period. On the other side, the disc diffusion method adds the antibiotic onto the agar medium, followed by streaking of a bacterial suspension on the agar plate. Following an incubation period, MIC represents the minimum antimicrobial agent concentration that completely inhibits the bacterial growth on the agar surface, thus capturing how such an antibiotic mechanism operates.

The decision about MIC is not only technical but more importantly about the overall development and assessment of new antimicrobial drugs. This metric provides crucial information about killing the microorganisms and the concentration required for inhibiting their growth. Apart from this, MIC values are useful in creating breakpoints that are limit values that indicate the susceptibility of microorganisms to certain antimicrobial agents. These checkpoints, rigorously set by authoritative bodies such as the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), form the basis for guidelines that are universally accepted both clinically

and in research.

The fine-tuning of the MIC thus serves a pivotal function in a holistic assessment of the antimicrobial potency of the material. It exceeds the mere numeric presentation providing deep information about the material substance, its exact potency, and its exact efficacy against certain microorganisms. Such a wealth of information not only helps in the development of new antimicrobial agents but also directs the judicious use of these agents in clinical applications. Acknowledging the significance of MIC as a milestone parameter unveils the multi-sided context of antimicrobial research which leads to a recurring process of improvement and innovation aimed at tackling a range of microbial hazards.

Experimental Method:

A set of dilutions of nickel NPs (20, 10, 40, 50, and 100 µg/mL) were made using distilled water and liquid nutrient media where the measurements were conducted. For each of the aforementioned dilutions, 0.1 mL of a bacterial suspension containing 1.5×108 (cells/mL) was added to an individual tube, which was then incubated for 24 hours at 37°C. The Minimum Inhibitory Concentration (MIC) was the lowest concentration of the inhibiting agent that had no visible turbidity, discernible by the naked eye. Then, 0.1 mL extracted from tubes without turbidity was spread on the surface of the nutrient agar, followed by incubation at 37°C for 24 hours. Data were obtained according to the presence of growth with the lowest number of colonies. The Minimum Bactericidal Concentration (MBC) was defined as the lowest concentration of the inhibitory substance that decreased the growth by 99.9%. MBC was decided by transferring 0.1 mL from tubes exceeding the MIC value onto the surface of nutrient agar, followed by incubation at 37°C for 24 hours. Results were counted as present or absent of growth.

2. Results and Discussion:

The average size of the sample in µm as well as the Standard Deviation (SD) is measured at different points. An Atomic Force Microscopy (AFM) is carried out and the results appear in figure (1). This figure represents an AFM test, which is a type of microscopy, that uses a cantilever with a sharp tip to scan the topography of the sample for a high-resolution topographic image. This figure is an important detail for the sample size and variation involved in AFM testing. A mean size of 0.156 µm is presented with a low SD of 0.0053 µm. On the other hand, several measurements were done at different points, 0.0555 µm to 0.474 µm, that signify different degrees of sample consistency.

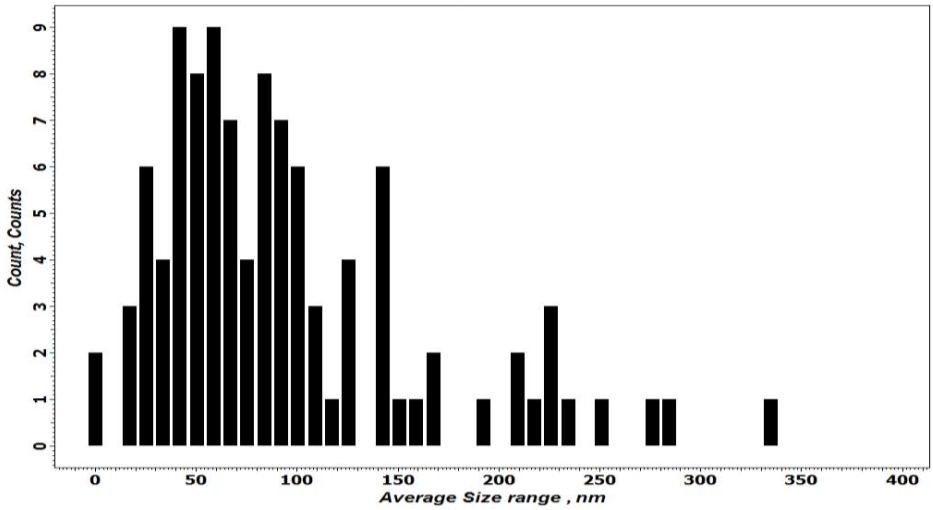

Fig.1. The average size of a sample in units of micrometers (μm), along with the Standard Deviation

In the AFM test, it should be remembered that the size and dispersion of the sample play a role in the resolution and accuracy of the generated images. With the help of the measurements obtained from Figure (1), one can get a better idea of the sample and hence can fine-tune the parameters of the AFM test as shown in Figure (2).

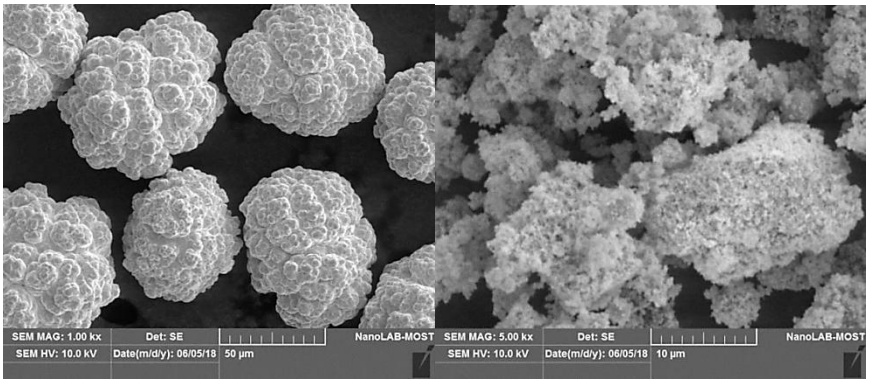

Fig.2. Atomic-Force-Microscopy (AFM) test, production of a high-resolution topographic image.

Table 1. Shows the average inhibition diameters of the silver nitrate nanoparticles.

Pathogenic Bacteria	Staph aureus	E. coli	Pseudoscience
Inhibition Diameter (mm)	18	24	15

The inhibition diameters show the size of the clear zone surrounding every bacterial colony, which means the area is not affected by silver nitrate nanoparticles. Table (1) presents the data of the inhibitory diameter rate (in mm) for each of the infectious bacteria, E. coli, P. aeruginosa, and S. aureus. The inhibitory diameter rate denotes the degree to which the

substance investigated reduces bacterial growth. The outcomes show that P. aeruginosa gives the biggest inhibitory diameter rate of 24 mm, followed by E. coli which is 18 mm and S. aureus is the lowest with an inhibitory diameter rate of 15 mm. Therefore, we can conclude that the investigated substance is more effective in inhibiting the growth of P. aeruginosa and E. coli compared to S. aureus. However, the inhibition zone diameter rate is only one of the many dimensions of the inhibitory potential of this compound and more research is needed to completely grasp its effectiveness against these pathogenic bacterial strains.

Table 2. Shows the concentrations of nanoparticles used against pathogenic bacteria.

Pathogenic Bacteria	10 µg /mL	20 µg/mL	40 µg/mL	50 µg/mL	100 µg/mL
Staph aureus	++++	+++	++	+	-
E. coli	+++	+++	++	+	-
Pseud. aeruginosa	+++	+++	+++	+++	+++

The symbols used here are a demonstration of the level of inhibition for each concentration of nickel nanoparticles. The symbols are as follows: "+" stands for partial inhibition, "++" represents moderate inhibition, "+++" shows good inhibition, and "++++" suggests complete inhibition. The "-" symbol shows no inhibition.

Table (2) illustrates the inhibitory effect of different concentrations of nickel nanoparticles on three types of pathogenic bacteria: Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. For Staphylococcus aureus, the highest inhibition was obtained in the lowest concentration of 10 µg/mL and it showed complete inhibition (++++) followed by good inhibition (+++) at 20 µg/mL, moderate inhibition (++) at 40 µg/mL, partial inhibition (+) at 50 µg, and no inhibition (-) at 100 µg/mL. In the case of Escherichia coli, the inhibitory activity was a little less than that observed concerning Staphylococcus aureus. The maximum inhibitory activity was observed at 10 µg/mL, where partial inhibition was recorded (+++), followed by moderate inhibition (++) at 20 µg/mL, partial inhibition (+) at 40 µg/mL, no inhibition (-) at 50 µg/mL, and no inhibition (-) at 100 µg/mL.

For Pseudomonas aeruginosa, the highest inhibition was observed at the highest concentration of 100 µg/mL, where good inhibition (+++) was observed, followed by good inhibition (+++) at 50 µg/mL, good inhibition (+++) at 40 µg/mL, good inhibition (+++) at 20 µg/mL, and good inhibition (+++) at 10 µg/mL. Overall, the outcomes reveal that the three types of pathological bacteria are inhibited by nickel nanoparticles to a certain extent. The inhibition effect is concentration-dependent; the lower the concentration, the lower the degree of inhibition. These findings have wide-ranging implications concerning the possible utilization of nickel nanoparticles as antimicrobial agents.

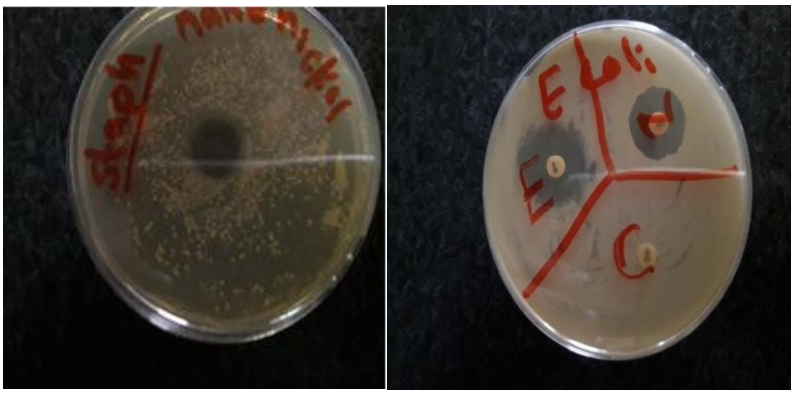

Fig.3. Shows the areas of bacterial inhibition of the material under examination.

It is far evident that nickel nanoparticles synthesized in this look at established substantial antibacterial pastime towards three extraordinary lines of pathogenic microorganisms, specifically E. Coli, S. Aureus, and P. Aeruginosa. The inhibition zones ranged from 15 to 24 mm, and the Minimum Inhibitory Concentration (MIC) of the Ni NPs was discovered to be in the range of 10 to one hundred µg/mL. When comparing these effects to comparable studies, consistent and divergent findings can be found. For example, a study published in the Journal of Biomedical Materials Research Part B: Applied Biomaterials said that silver nanoparticles (AgNPs) synthesized by using an inexperienced method exhibited antibacterial activity against E. Coli and S. Aureus, with inhibition zones ranging from eleven to 18 mm and MIC values ranging from 1.56 to 50 µg/mL. These outcomes align with the findings of the present observation concerning the antibacterial pastime towards E. Coli and S. Aureus.

On the opposite hand, a look at published in the Journal of Materials Science: Materials in Medicine located that copper nanoparticles (CuNPs) synthesized by using a chemical reduction approach exhibited antibacterial hobby against E. Coli and S. Aureus, with inhibition zones starting from nine to 18 mm, and MIC values ranging from 25 to 100 µg/mL. These results are barely one of a kind from the findings of the existing examination, as the inhibition zones for Ni NPs have been better than the ones said for CuNPs.

3. Summary and conclusions:

The findings of the present study align with some previous studies regarding antibacterial activity against certain strains of bacteria and while they diverge from others in terms of the magnitude of the inhibitory effects. However further research is necessary to draw definitive conclusions regarding the comparative effectiveness of different antibacterial materials. Based on the results of our study and the material under examination nickel nanoparticles (Ni NPs) and exhibited significant antibacterial activity against pathogenic bacteria. The findings suggest that Ni NPs hold potential for use as an effective antibacterial agent in various applications. One potential application could be in the field of medicine where the emergence of antibiotic-resistant bacteria has become a growing concern. Ni NPs could serve as an alternative to traditional antibiotics and as they have demonstrated effectiveness against pathogenic bacteria. Additionally, the use of Ni NPs as an antibacterial agent could potentially mitigate the development of antibiotic-resistant bacteria.

Other potential applications could include the utilization of Ni NPs in food packaging preservation where bacterial growth can result in spoilage and contamination. Ni NPs could also find application in water treatment systems and where they could aid in preventin the proliferation of harmful bacteria in drinking water. In summary, our findings suggest that the employment of nickel nanoparticles as an antibacterial agent holds promising potential in various industries fields including medicine food packaging preservation, and water treatment. Nonetheless and further studies are required to comprehensively explore the potential applications of Ni NPs an to ensure their safety for human use.

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