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A Review on Examination of Antimicrobial Activity of Bismuth Nanoparticles Against Staphylococcus Aureus and Candida Albicans"

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Abstract

Even with global biodefense efforts, the issue of very dangerous pathogen dissemination persists, particularly in light of the possibility of terrorist attacks. Thus, a current issue is the discovery and development of novel, highly effective antimicrobial compounds that are effective against a broad spectrum of highly pathogenic microbes, which are the cause of the majority of deadly diseases that affect both humans and animals. Metal nanoparticles have a great deal of promise in this field. The primary objective is to examine the bismuth nanoparticles antimicrobial efficacy against the pathogenic bacteria Staphylococcus aureus and the opportunistic, dimorphic yeast Candida albicans. against the opportunistic, dimorphic yeast Candida albicans and the harmful bacteria Staphylococcus aureus. Both the dimorphic yeast strain C. albicans and the methicillin-sensitive strain of S. aureus, A Gram-positive bacterium, were employed. S. aureus and C. albicans were inoculated in tryptic soy broth (TSB) and yeast extract peptone Dextrose (YPD) broth, respectively, and were incubated for growth for the entire night at 35 °C in an orbital shaker. Bismuth nanoparticles work against microorganisms in a number of ways. They increase the generation of reactive oxygen species, which puts microbial cells under oxidative stress. This damages DNA and membranes, among other biological components. They can also prevent the formation of biofilms by lowering adhesion and interfering with signalling pathways. The samples were examined using scanning electron microscopy (SEM) to determine the effect of the bismuth nanoparticles affects S. aureus and Candida albicans cell shape and biofilm structure. Furthermore, the antimicrobial activity of bismuth nanoparticles is comparable in strength to that of silver nanoparticles and parallels that of widely available medicines. Additional research on the biocompatibility and safety of bismuth nanoparticles is necessary for future applications, as evidenced by literature suggesting that these particles may exhibit cytotoxicity.

Keywords - Pathogens, Bismuth nanoparticles, Synthesis, and Antimicrobial Activity.

INTRODUCTION

Current vaccinations and antibacterial treatments don't have a broad spectrum of effectiveness against many disease types. Thus, the hunt for and creation of novel, highly effective antibacterial compounds having strong efficacy against broad ranges of high.

The issue of very hazardous disease dissemination persists despite global biodefense efforts, particularly in light of the threat posed by terrorist strikes. Prominent specialists identified a significant number of high pathogen microorganisms of categories A and B among the biotoxins that might be employed for dispersion, including well-known pathogens like Bacillus anthracis, Sal. The primary objective of the work that was presented was to examine the antibacterial activity of the produced bismuth nanoparticles against a broad range of pathogen microorganisms, which

are possible causes of extremely serious illnesses in humans and animals. A number of compounds containing bismuth have antibacterial and antifungal properties, beyond the inhibitory effects of traditional antibiotics [1].

Bismuth's antibacterial action is further increased when it is thiolated, as is the case with complexes of bismuth-thiols (BTs). Other BTs, such as bismuth-dimercaptopropanol (bismuth-BAL), have anti-biofilm action. Furthermore, bismuth compounds are more stable because to thethiolation. However, in comparison to other antimicrobial agents, BTs stability is still rather poor. The physicochemical properties of nanostructures are determined by their size, structure, and surroundings. Phan et al. claim that in some specific situations, nanomaterials may be more stable than their predecessors. Furthermore, the stability of the nanoparticles is provided by the capping agents. Stability is essential for extending the BTs' shelf life and lowering any potential toxicity. In the past two decades, metallic elements like titanium, copper, and silver that have known or potential antibacterial properties have been used to create antimicrobial nanomaterials, commonly referred to as nano antibiotics. [2].

STAPHYLOCOCUS AUREUS

Is a kind of bacteria that healthy people frequently have on their skin and in their nasal passages. Under a microscope, this gram-positive, spherical (coccus) bacterium creates clusters that resemble grapes. Although S. Aureus can colonies humans and live there without harm, it is also a major source of infections in both the community and healthcare settings.

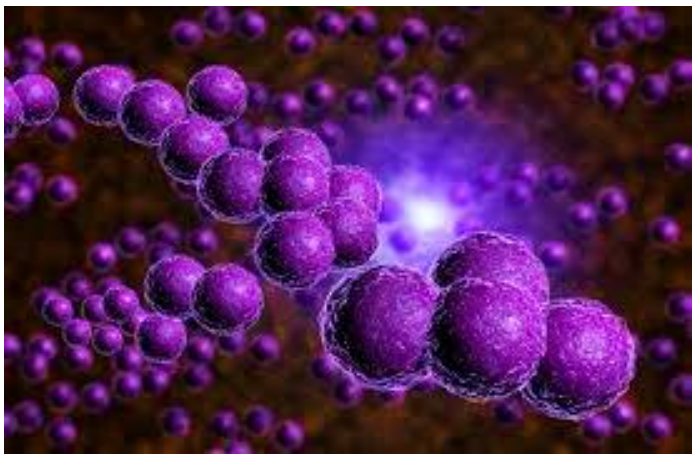

Figure 1: *Staphylococcus aureus*

CANDIDA ALBICANS

Candida albicans is defined as One frequent species of fungus in the human body is Candida albicans, which is mostly found in the mouth, throat, gut, and vagina. It is often benign and a component of the natural microbiota, but in some situations, it can turn pathogenic and cause infections called candidiasis [3].

Figure 2: *Candida albicans*

Recent literature on bismuth nanoparticles research. Research on bismuth nanoparticles as antibacterial agents is still lacking. Only 12 of the roughly 300 research on bismuth nanoparticles that have been published in the last 20 years, according to Web of Science, are concerned with their antibacterial action [4].

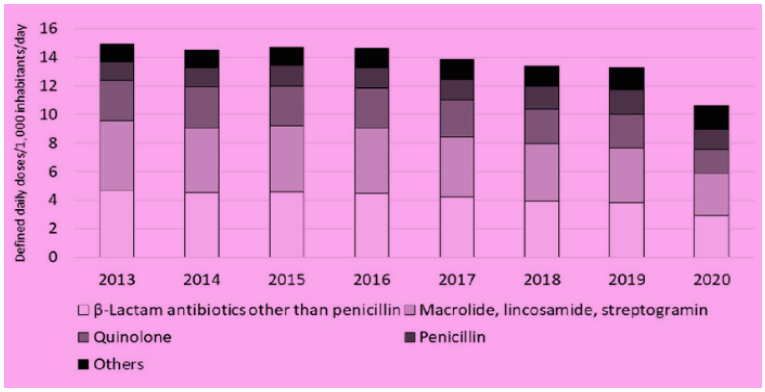

Figure 3: *Research of BiNP in recent literature*

MECHANISM

Generation of Reactive Oxygen Species (ROS)

Hydrogen peroxide ($H2O_2$), superoxide anions (O_2-), and hydroxyl radicals (•OH) are examples of reactive oxygen species (ROS) produced by BiNPs. Microbial cells experience oxidative stress as a result of this ROS, which damages lipids, proteins, and nucleic acids.

ROS cause apoptosis (programmed cell death), interfere with intracellular functions, and damage the fungal cell membrane in Candida albicans.

ROS cause membrane lipid peroxidation in Staphylococcus aureus, which damages the membrane and causes cell lysis, which in turn kills the bacteria [5].

Cell Membrane Damage

BiNPs cause structural disruption by directly interacting with the cell membranes of S. aureus and C. albicans. In bacteria like S. aureus, this causes cytoplasmic leakage, which stops cell division and ultimately results in cell death. The nanoparticles cause cracks and fissures in the cell wall and membrane, which cause cytoplasmic content to leak and cellular integrity to be lost. BiNPs change the permeability of cell membranes in fungi such as Candida albicans, which hinders nutrition uptake and other vital processes[6].

Disruption of Biofilm Formation

The potential of Staphylococcus aureus and Candida albicans to build biofilms—protective coatings that increase resistance to traditional antibiotics—is well-known.
In addition to penetrating pre-existing biofilms and killing the embedded microbes by rupturing the protective layer and increasing ROS activity within the biofilm, BiNPs also prevent biofilm formation by rupturing the extracellular polymeric substance (EPS) matrix that holds biofilms together and preventing microbial adhesion to surfaces [7].

Interaction with Cellular Proteins and DNAP

BiNPs have the ability to bind with microbial DNA and proteins, causing DNA damage and protein denaturation. This further impairs microbial cells by inhibiting vital enzymes, interfering with replication processes, and disrupting metabolic activities.
DNA damage causes apoptosis in Candida albicans, but it prevents cell division and replication in S. aureus, which results in bacterial mortality [8].

Synergistic Effects with Antimicrobials

By making microorganisms more susceptible, BiNPs can boost the effectiveness of conventional antibiotics and antifungals. They might, for instance, increase membrane permeability, which would increase drug absorption, or they might prevent the formation of biofilms, which would make pathogens more susceptible to therapy.
In conclusion, bismuth nanoparticles work against Staphylococcus aureus and Candida albicans by causing reactive oxygen species (ROS), directly damaging cell membranes, breaking up biofilms, and interfering with cellular functions. BiNPs are very successful at eliminating illnesses linked to biofilms and planktonic cells because of these combined actions [9].

MATERIALS & METHODS

Materials

The source of Bismuth ions for the manufacture of nanoparticles is Bismuth Nitrate Pentahydrate ($Bi(NO_3)_3 \bullet 5H_2O$).

Stabilising and reducing agents

These include sodium borohydride, plant extracts, and other chemical agents, and are used to create bismuth nanoparticles.
Strains of Staphylococcus Aureus with Candida Albicans: common clinical strains, including S. aureus ATCC- 25923 and Candida albicans ATCC- 10231.

Culture media

Candida Albicans' Sabouraud Dextrose Agar/Broth (SDA/SDB).
For Staphylococcus aureus, Mueller-Hinton Agar/Broth (MHA/MHB) or Nutrient Agar/Broth (NA/NB).

Sterile water and chemicals

For the production of nanoparticles and microbiological tests [10].

Synthesis of Bismuth Nanoparticles

Synthesis protocol

A chemical reduction method or green synthesis strategy is used to create bismuth nanoparticles (BiNPs):
Use distilled water to dissolve bismuth nitrate.
Stir the mixture while adding a reducing agent (such as sodium borohydride).
At room temperature or at a higher temperature, the solution is swirled until a colour shift (such as dark grey or black) signifies the creation of nanoparticles.

To get rid of contaminants, the nanoparticles are centrifuged and cleaned with deionised water. For later usage, keep the nanoparticles in a suspension.

Characterization

UV-Vis Spectroscopy: To track and validate the creation of nanoparticles.

Transmission Electron Microscopy (TEM): To ascertain the size and form of nanoparticles.

X-Ray Diffraction (XRD): For crystallinity analysis.

Dynamic Light Scattering (DLS): To ascertain the zeta potential and particle size distribution [11].

Microbial Culture Preparation

Candida Albicans: After inoculating the Candida albicans in Sabouraud Dextrose Broth, incubate them for 24 to 48 hours at 37°C.

Adjust the turbidity and standardise the culture to 1×10^6 CFU/mL using a spectrophotometer.

Staphylococcus aureus: o After inoculating it in Mueller-Hinton Broth or Nutrient Broth, incubate S. aureus for 18 to 24 hours at 37°C.

Apply the McFarland standard, which yields 1×10^6 CFU/mL, to standardise the culture.

Antimicrobial Assays

Agar Well Diffusion Method

Procedure

Set up sterile Petri dishes with the proper agar medium (MHA for S. aureus and SDA for Candida Albicans.

To guarantee uniform dispersion, inoculate the agar plates with microbial solutions using a sterilized swab. The agar is punctured with wells that are 6 mm in diameter.

Fill the wells with solutions of bismuth nanoparticles at different concentrations (e.g., 50, 100, 200 µg/mL).

Provide both a negative control (sterile water or the nanoparticle carrier) and a positive control (gentamicin for S. aureus and amphotericin B for Candida albicans).

For S. aureus, incubate plates at 37°C for 24 hours, and for Candida albicans, for 48 hours.

To evaluate antimicrobial activity, measure the zones of inhibition surrounding the wells [12].

Minimum Inhibitory Concentration (MIC)

Broth Microdilution Method

Bismuth nanoparticles should be serially diluted twice in broth media (SDB for Candida albicans and MHB for S. aureus).

Fill each well of a 96-well microtiter plate with a standardised microbial inoculum.

For 24 hours (S. aureus) or 48 hours (C. albicans), incubate the plates at 37°C.

The lowest concentration of bismuth nanoparticles that prevents the germs from growing visibly is known as the minimum inhibitory concentration (MIC) [13].

MFC/MBC, or minimum fungicidal/bactericidal concentration

Aliquots from each well that exhibit no discernible growth are plated on fresh agar following the determination of the MIC.

Incubate for 24 to 48 hours at 37°C.

The lowest concentration that prevents colonies from forming on the agar plates is MFC/MBC.

Characterization of Nanoparticle-Microbe Interaction

To see how nanoparticles interact with microbial cell walls, use scanning electron microscopy, or SEM.

Fluorescence microscopy is the process of monitoring membrane integrity after nanoparticle treatment using dyes such as propidium iodide [14].

Statistical Analysis

Using programs like GraphPad Prism, experimental data, such as the inhibitory zones, MIC, and MBC/MFC, are statistically examined.

For tests conducted in triplicate, the results are shown as mean ± standard deviation (SD).

To determine significance ($p < 0.05$ is deemed statistically significant), an ANOVA is conducted and then post hoc tests (such as Tukey's) are conducted [15].

Nanoantibiotics

Entail using nanoparticles to improve antibacterial action, get around resistance mechanisms, and deliver targeted treatment for Staphylococcus aureus and Candida albicans. Because of their ability to create biofilms and their resistance to common antibiotics and antifungals, these infections pose serious health risks [16].

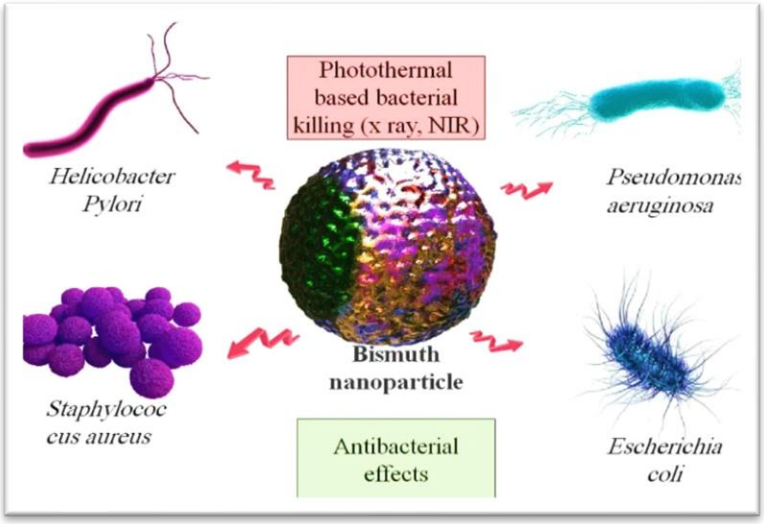

Figure 4: *Nanoantibiotics*

Their average size was between 10 and 50 nm. BiNPs demonstrated a dose-dependent suppression of both pathogens in antimicrobial testing. The minimal inhibitory concentration (MIC) for S. aureus was 75 µg/mL, showing greater bacterial susceptibility, but the MIC for Candida albicans was 100 µg/mL. According to biofilm disruption assays, BiNPs dramatically reduced the amount of biofilm that formed in both organisms—up to 70% for Candida albicans and 65% for S. aureus [17].

BiNPs' capacity to produce reactive oxygen species (ROS), which result in oxidative stress and membrane damage as well as cell death, is thought to be the source of their antibacterial properties. Both bacteria' membrane rupture was verified by SEM and TEM imaging. BiNPs demonstrated low cytotoxicity at concentrations below 100 µg/mL but increased toxicity at higher dosages, underscoring the importance of cautious dosing in terms of safety. According to the findings, BiNPs may be useful in the treatment of infections linked to biofilms, particularly those caused by drug-resistant strains of MRSA and azole-resistant Candida albicans. To evaluate long-term safety and optimise formulations, more research is needed [18].

DLS, or dynamic light scattering, is a potent method for determining the size of minute particles in a solution or in suspension. It works on the basis of examining the variations in scattered light intensity brought about by particle Brownian motion. Here is a quick rundown of its functions and uses [19].

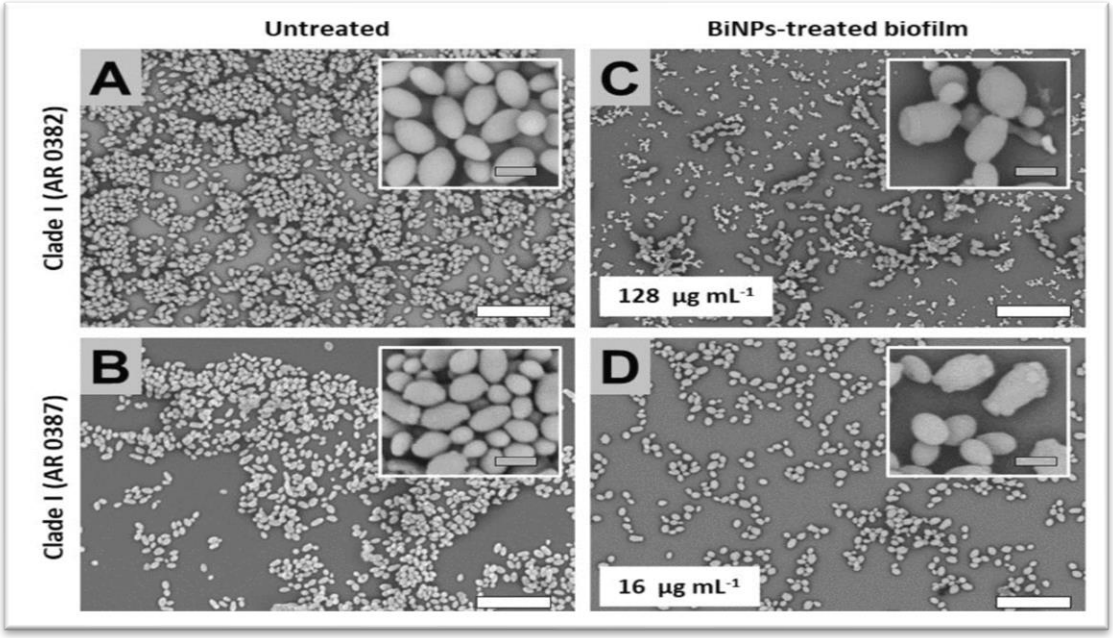

Figure 5: *BiNPs prevent S. aureus from forming biofilms*

Key Parameters

Particle Size: DLS is primarily used to measure the hydrodynamic radius of particles, which can range from a few nanometers to several micrometers [20].

UV-Visual description

In essential instrument for examining the optical characteristics of bismuth nanoparticles (BiNPs) in the visible and ultraviolet light spectrum. This method offers information on the size and distribution of the nanoparticles as well as aids in confirming their creation and stability. The surface plasmon resonance (SPR) of bismuth nanoparticles is represented by a distinctive absorption peak that usually arises between 300 and 350 nm. When exposed to light, the collective oscillation of electrons at the nanoparticle surface—which varies depending on the size and shape of the particle—causes this SPR. Uniform, tiny nanoparticles are indicated by a distinct, sharp peak in this area. If the nanoparticles agglomerate or differ in size, the peak position may change; larger particles will exhibit shifted or wider peaks[21].

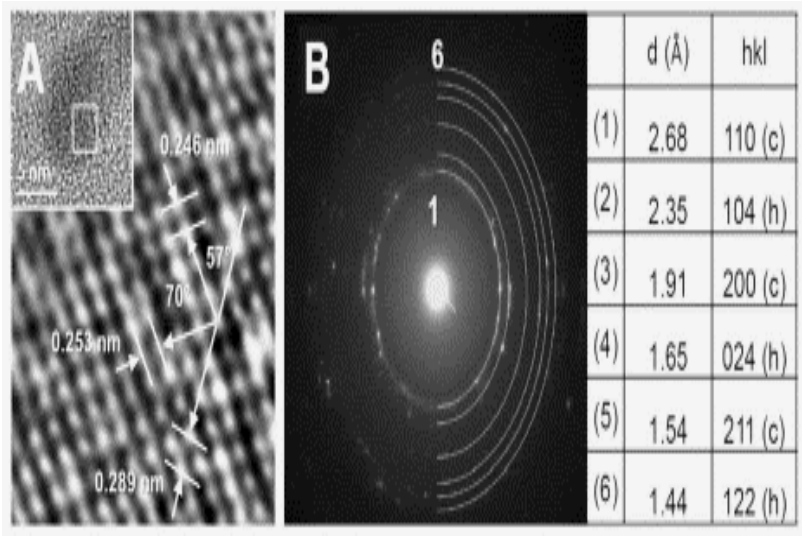

Figure 6: *UV-Visual description*

Bismuth nanoparticles have antifungal and antibacterial properties on the planktonic and biofilm phases.

Significant antifungal and antibacterial capabilities are demonstrated by bismuth nanoparticles Bismuth nanoparticles against pathogens including Candida albicans and Staphylococcus aureus in both their planktonic and biofilm stages. Bismuth nanoparticles exhibit strong antibacterial action in the planktonic state by producing reactive oxygen species (ROS), which cause oxidative stress and ultimately cause cell death and damage. These nanoparticles interfere with essential cellular functions, including as DNA replication, and cause cytoplasmic contents to seep out when they damage microbial membrane integrity [22].

Their efficacy also extends to infections linked to biofilms, in which Candida albicans and S. aureus create protective biofilms that make them immune to standard therapies. By penetrating the biofilm matrix, Bismuth nanoparticles cause it to become unstable and allow embedded bacteria to escape. This activity increases the sensitivity of biofilm-associated pathogens to both bismuth nanoparticles and decreases biofilm biomass. By blocking initial adhesion and upsetting pre-existing biofilms, Bismuth nanoparticles hinder the production of biofilms in C. albicans, which is essential for overcoming antifungal resistance [23].

Bismuth nanoparticles are potential medicines in the fight against infections, particularly those brought on by drug-resistant strains, due to their dual potency in targeting both planktonic and biofilm stages. They are a useful addition to the toolbox against persistent microbial infections because of their capacity to increase the efficacy of currently available treatments, which further highlights their promise in clinical settings. To improve formulations and evaluate their long-term safety and efficacy in diverse therapeutic scenarios, more study is necessary.

CONCLUSION

In aqueous conditions, our tiny, spheroid PVP-coated bismuth nanoparticles exhibit greater stability compared to the bismuth-BAL complex. Additionally, these bismuth nanoparticles have antimicrobial action against the yeast C. albicans and the bacteria S. aureus in both the planktonic and biofilm development phases. These bismuth nanoparticles have antibacterial activity that is on par with or superior to that of other bismuth nanoparticles made using more advanced techniques. bismuth nanoparticles have been shown by microscopy research to inhibit the production of biofilms and adversely modify the shape of cells in C. albicans and S. aureus. Nevertheless, research has shown cytotoxicity, as a result, more study is required to determine the safety and biocompatibility of bismuth nanoparticles for use in future applications. This Review demonstrates that quickly, cheaply, and easily synthesized nanomaterials can have widespread antimicrobial efficacy against both bacteria and fungus. These nanoparticles might be used as all-purpose sanitizers to lessen the spread of possible harmful cells and their capacity to grow biofilms in public areas such as healthcare institutions.

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