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Investigating the potential of Bismuth vanadate to combat multidrug-resistant *Acinetobacter baumannii* and methicillin-resistant *Staphylococcus aureus*

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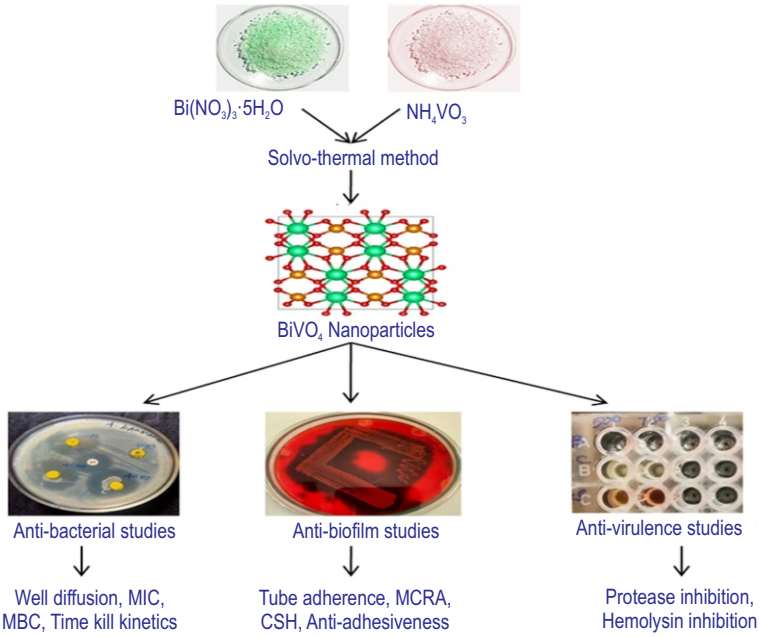
Abstract

Aim: This study investigated the efficiency of $BiVO_4$ nanoparticles ($BiVO_4$NPs) against drug-resistant bacteria, *Acinetobacter baumannii* and Methicillin-resistant *Staphylococcus aureus* (MRSA).

Methodology: The study employed a multifaceted approach, evaluating the ablity of $BiVO_4$ NPs to inhibit bacterial growth, reduce viable cell counts over time, and hinder biofilm formation. Furthermore, the investigation explored the NPs impact on virulence factors using protease and hemolysin inhibition assays.

Results: The findings showed the potent efficacy of $BiVO_4$ NPs against *A. baumannii* and MRSA. Notable results included the formation of clear inhibition zones up to 19 and 15 mm, indicating the antimicrobial activity against both pathogens. $BiVO_4$ NPs also effectively inhibited biofilm formation, reduced bacterial adherence, and inhibited virulence factors, underscoring their potential as effective agents against drug-resistant bacteria and suggesting promising applications in medical implants and anti-microbial research.

Interpretation: Overall, this study provides compelling evidence supporting the potential use of $BiVO_4$ Nps as an antimicrobial agent and a promising candidate for addressing various human health concerns.



Key words: Antimicrobial, Antibiofilm, Anti-adhesive, Bismuth vanadate, Nanoparticles

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Introduction

The emergence of antimicrobial resistant microbes poses a serious threat to the existence of humankind. The spread of resistant strains from hospitals to community settings has caught immediate attention from policy makers, healthcare providers, researchers and public due to their tremendous negative impact on general public health. The indecisive use of antibiotics, along with other determinants like lack of infection control in hospitals, poor hygiene, use of antibiotic drugs in food and agricultural industries etc., has accelerated the development of resistant genes in microbes (Tang *et al.,* 2023). In 2017, WHO published a priority list of harmfulbiofilm forming and drug resilient microbes as ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) which are the major cause for medical device associated and hospital acquired infections. Owing to the increase in the number of deaths arising from infections caused by resistant strains, the WHO has also listed antimicrobial resistance as one among the top ten threats to global health. Multiple factors are attributed to the emergence of Multi drug resistant (MDR) strains. Mutational resistance, transfer of resistant genes through horizontal gene transfer, alterations in the cell wall components, quorum sensing and biofilm formation are the most common mechanisms adopted by bacteria to survive the harsh environment created by antibiotics (Munita *et al.,* 2016;Oliveria *et al.,* 2024).

The search for novel antimicrobials and new therapies to combat these resistant mechanisms leading to MDR strains hold great importance in microbiological research. Within the realm of nanomedicine, NPs have emerged as a versatile platform for combatting microbial infections. They can function in two primary capacities; as independent inherent therapeutic agents, or as nano-carriers for established antimicrobial drugs. In their inherent therapeutic role, certain NPs possess intrinsic antimicrobial properties like titanium dioxide (TiO_2) and zinc oxide (ZnO), which have demonstrated efficacy against various pathogens. Similarly, copper, nickel, seleniumand silver NPs have also exhibited promising antimicrobial activity. Alternatively, NPs can also serve as efficient delivery vehicles for conventional antimicrobial agents. This approach, termed "nano-biotics," offers distinct advantages such as enhanced drug solubility, targeted delivery to infection sites, and potentially reduced systemic side effects (Hetta *et al.,* 2023).

Nanoparticles exhibit a broad spectrum of antimicrobial mechanisms, including disruption of bacterial cell wall, induction of diverse stress responses (both reactive oxygen species-dependent and independent), inhibition of protein synthesis and direct DNA damage. This multi-pronged approach offers a significant advantage over traditional antibiotics, which may struggle to penetrate complex biofilms composed of multiple bacterial species. Furthermore, NPs demonstrate prolonged residence time within biofilms, enhancing their effectiveness against these persistent infections (Chakraborty *et al.,* 2022).

Bismuth-containing NPs (BiNPs) are emerging as a promising class of biomaterials due to their unique properties. These include high stability, surface area, diamagnetism, electrical and magnetic manipulation potential, catalytic activity, low cost and biocompatibility. Notably, BiNPs demonstrate low toxicity compared to other heavy metal NPs, making them acceptable for *in-vivo* applications. Additionally, the ability to control their size and shape during synthesis opens doors for further clinical development. These combined advantages position BiNPs as a potentially superior alternative to conventional metal-based nanoparticles in biomedicine. There are researches suggesting BiNPs hold promise in combating biofilm producing species like *E. faecalis, S. aureus, P. aeruginosa* and *Proteus mirabilis* (Azad *et al.,* 2020; Shakibaie *et al.,* 2019). However detailed studies on the antimicrobial and antibiofilm potential of $BiVO_4$ NPs against biofilm forming species remains largely under explored. The present study fills the void in this area by detailed investigation on the antimicrobial and antibiofilm potential of $BiVO_4$ NPs against *A. baumannii* (MTCC 1425) and MRSA (MTCC 1430) revealing the strong antimicrobial, antibiofilm and antiadhesion properties of $BiVO_4$ NPs against resistant microbes. This suggests $BiVO_4$ NPs holds future in potential biological applications, such as coating medical implants and combating biofilm-related infections.

Materials and Methods

Synthesis and characterization of $BiVO_4$ nanoparticles: $BiVO_4$ NPs measuring15 nm were procured from the Department of Applied Physics, Karunya Institute of Technology and Sciences, Coimbatore, India. The NPs were synthesized by solvo-thermal method following the modified protocol of Longchin *et al.* (2016) for $BiVO_4$ NP synthesis. The process involved dissolving 0.97g of bismuth nitrate pentahydrate ($Bi(NO_3)3•5H_2O$) in a solvent mixture of acetic acid, ethanol and water (1:1:3 ratio) termed as Sol A. Simultaneously, ammonium metavanadate (NH_4VO_3) was dissolved in a solvent mixture of ammonia and water (3:1 ratio) termed as Sol B. Sol B was then added to Sol A under continuous stirring for 30 min maintaining a pH of 7. The resulting mixture was autoclaved at 80°C for 3 hrs, followed by cooling at room temperature, filtration and washing. The filtrate obtained was subsequently dried overnight at 80°C in an oven, yielding the final product of $BiVO_4$ NPs. The dimensions and morphological characteristics of the produced $BiVO_4$ NPs were analyzed by scanning electron microscopy (SEM) and X-ray diffraction (XRD) analysis (Longchain *et al.,* 2016).

Test microorganisms: Microorganisms, *A. baumannii* (MTCC1425) and MRSA (MTCC 1430) selected as the test microorganisms were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh India. The microbial samples were inoculated into nutrient broth and incubated at 37°C for 24 hr. to activate them. Subsequently, the overnight cultures of these test strains, adjusted to a 0.5 McFarland value were used for further investigations (CLSI, 2008).

Antibiotic sensitivity test: The antibiotic susceptibility of *A. baumannii* and MRSA was evaluated by disk diffusion method as per CLSI Guideline (2018) for disk susceptibility tests. After spreading liquid cultures of each bacterium on separate Mueller Hinton agar plates, ceftazidime disks (240-0.01µg concentration) were placed for *A. baumannii* and ampicillin (2.048-0.016 µg) disks for MRSA to assess drug resistance across a concentration gradient. Following incubation at 37°C for 24 hrs, the clear zones around each disk were measured. The size of these inhibition zones indicated the bacteria's susceptibility to the antibiotic at specific concentration. This experiment was designed to determine the minimum inhibitory concentration required to cease the growth of each microorganism (CLSI, 2018).

Antimicrobial assay with $BiVO_4$ nanoparticles: The well diffusion assay was conducted in accordance with the method described by Smania *et al.* (1999). Liquid cultures of *A. baumannii* and MRSA strains (20 µl each) were evenly spread onto Mueller hinton agar plates, allowing them to settle undisturbed for 30 min. Subsequently, wells of 6 mm diameter were punctured onto agar and various concentrations (10, 20, 30 and 40 µg m^{-1}) of $BiVO_4$ NPs dissolved in dimethyl sulfoxide were introduced into these wells. The plates were then incubated at 37°C for 24 hr. Clear areas of inhibition around each well served as a sign of antibacterial activity, and these zones were assessed and documented. Additionally, Amikacin (30 µg) for *A. baumannii* and Mupirocin (10 µg) for MRSA were used as reference disks to compare the results (Smania *et al.,* 1999).

Assessment of Minimum Inhibitory Concentration (MIC): The antibacterial activity of $BiVO_4$ NP was assessed using microtiter plate method as per CLSI Guideline (2015). Sterile 96-well plates were prepared with 100 µl of Mueller-hinton broth containing concentrations of $BiVO_4$ from 75-375 µg ml^{-1}. Each well was thereafter inoculated with 50 µl of bacterial suspension. The plates were incubated at 37°C for 24 hrs. Bacterial growth was measured by reading the observance at 560 nm. Wells with only Mueller-hinton broth (without $BiVO_4$ NPs or bacteria) served as negative controls, while wells with untreated bacteria in Mueller-hinton broth served as positive controls. The MIC of $BiVO_4$ NPs, defined as the lowest concentration that visibly inhibited the bacterial growth compared to the positive control, was determined based on the OD_{560} values (CLSI, 2015).

Assessment of Minimum Bactericidal Concentration (MBC): The Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of a substance that is bactericidal. MBC was established following CLSI Guidelines (1999) with some modifications. Following the determination of MIC, the samples from wells showing no visible growth were re-inoculated onto Mueller-hinton agar plates and incubatedat 37°Cfor 24 hr. To ascertain the minimum concentration of $BiVO_4$ NPs required to effectively eradicate both test microorganisms, the microbial cell counts were conducted using the plate count method.

Time-kill kinetics assay: This experiment was conducted following the protocol of Verma *et al.* (2017) with some modifications. $BiVO_4$ NP (500 µg ml^{-1}) was introduced on to *A. baumannii* and MRSA culture in tryptic soy broth and incubated at 37°C for 24 hr. Viability counts were performed at specific time intervals (0, 2, 4, 6, 8, 10 and 12 hrs), by plating 0.1ml aliquots from these samples on to tryptic soy agar plates. A control experiment was conducted without $BiVO_4$ NPs. The final results were plotted on a graph, showing the viable bacterial count against time.

Determination of cell surface hydrophobicity: Bacterial Adherence to Hydrocarbons (BATH) assay was employed to evaluate the hydrophobicity of bacterial cell surfaces in *A. baumannii* and MRSA (Rosenberg 1984). Both bacterial strains were cultured in 5 ml of nutrient broth at 37°C until they reached a stationary phase. After three washes with phosphate buffer, the bacterial cells were collected by centrifugation at 3000 rpm for 15 min to achieve an optical density of 0.5 at 500 nm. In a glass tube, 1 ml of each culture was mixed with 100 µl of toluene and $BiVO_4$ NPs at 100 µg ml^{-1} and 250 µg ml^{-1} concentrations. The mixture was vigorously shaken for one minute. After allowing the mixture to settle for 30 to 60 min, the liquid phase was carefully separated using a micropipette, and the absorbance was readat 500 nm on a UV-Visible spectrophotometer. The affinity of bacteria was calculated by the formula given by Bellon *et al.* (1996).

Protease inhibition assay: Protease inhibition was assessed by the method of Anantha *et al.* (1956) with slight modifications. *A. baumannii* and MRSA were cultured in tryptic soy broth with 250 µg $m\,l^{-1}$ of $BiVO_4$ NP. After incubation on a shaker at 150 rpm for 24 hr at 37°C, the cultures were centrifuged at 6000 rpm for 20 min at 4°C. The supernatant, containing exoproteins, was separated and stored in a refrigerator. Approximately, 100 µl of exoprotein was added to 6 mm wells on pre-prepared skim milk agar plates and incubated at 37°C for 24 hr. After incubation, the clear zones of proteolysis around each well, indicating casein hydrolysis were measured. A control set, without $BiVO_4$ NPs, was used for comparing the results.

Total hemolysin inhibition assay: The hemolytic activity of *A. baumannii* and MRSA was initially assessed using blood agar plates. A buffer solution containing Tris, NaCl, and $CaCl_2$ was adjusted to pH 7.4 with hydrochloric acid. This buffer was then mixed with a solution of 2% sheep erythrocytes prepared in 1x Phosphate-Buffered saline. Specifically, red blood cells of sheep (2%) was combined with extracellular products from *A. baumannii* and MRSA. The mixture was incubated at 37°C for 30 min. Post-incubation, the mixture was centrifuged at 12,000 rpm for 20 min at 4°C on ice. The absorbance of hemoglobin released into the supernatant was read at 530 nm wavelength to quantify hemolysis (Cooper *et al.,* 1964). The hemolysis inhibition percent was calculated by comparing the test strains (treated with $BiVO_4$ nanoparticles) with the control strains (untreated with $BiVO_4$ NPs).

Antibiofilm assay: The tissue culture plate method was employed to evaluate the antibiofilm activity of $BiVO_4$ Nps (O' Toole *et al.*, 1999). In the test group, TSB broth was added to each

well, followed by inoculation with bacterial strain and $BiVO_4$ NPs, resulting in a total volume of 200 µl. The plate was then incubated for 48 hr at 37°C. After incubation, the non-adherent cells were gently washed away with PBS, while adherent cells were fixed with 2% sodium acetate. The adherent cells were subsequently stained with crystal violet, decolorized with an ethanol-acetone mixture, and the optical density was read at 560 nm. Control wells without addition of $BiVO_4$ NPs were also added for comparison of the result.

Statistical analysis: The statistical significance of the obtained data was assessed using Student's t-test.

Results and Discussion

The rise of multi drug resistant pathogens, such as *A. baumannii* and MRSA, is a growing concern worldwide in both healthcare centresand communities. Previous studies have investigated the antimicrobial efficacy of various NPs; however, their high toxicity and cost-effectiveness remainsas a significant barrier for its practical application in the healthcare industry (Azad *et al*., 2020). Bismuth, being biocompatible and less toxic is used single and in combination with other NPs, for the diagnosis and treatment of various diseases such as ulcers, syphilis, respiratory and gastrointestinal disorders (Graham *et al.,* 2015; Liu *et al.,* 2016).

The present study utilized $BiVO_4$ NPs (15nm) synthesized through solvo-thermal method. The SEM micrographs of $BiVO_4$ NPs (Fig. 1) showed a distinct acicular sheath structure characteristic of $BiVO_4$. The micrographs showcased the uniform distribution of particle sizes and shapes, providing detailed insights into the structural features of $BiVO_4$. X ray diffraction analysis (Fig. 2) revealed the diffraction pattern of the prepared sample. The pattern showed a monoclinic scheelite structure, confirmed by JCPDS card number 14-0688, affirming the crystalline nature of the sample. The absence of secondary peaks corresponding to impurities suggested the purity of sample, with the crystallite size measuring 15.39 nm as determined by the Scherrer equation. The study results correspond to that of Dong *et al.* (2014), as they synthesized $BiVO_4$ nanostructures having a unique 3D acicular sheaf shape with XRD results confirming its crystalline nature, indicating monoclinic scheelite phase.

Antimicrobial susceptibility tests using standard antibiotics Ceftazidime and Ampicillin was performed to confirm the drug-resistant nature of *A. baumannii* and MRSA respectively. The results demonstrated complete resistance to the antibiotic disks across all tested concentrations, 240-0.01 µg of Ceftazidime against *A. baumannii* and 2.048-0.016 µg of Ampicillin against MRSA, underscoring high resistance of these microorganisms to standard antibiotics (Fig. 3). These findings are consistent with the findings of Guddeti *et al.* (2023), who studied the antibiotic resistance patterns in *A. baumannii* isolates from a tertiary care hospital in Central India. Their study revealed high levels of resistance to various antibiotics, including Cefotaxime, Ceftazidime, Cefepime, Imipenem and Meropenem, with lower resistance to Doxycycline and Polymyxin B. Similarly, Adhikari *et al.* (2023) studied MRSA in a tertiary care teaching hospital in Nepal andfound a significantly higher prevalence of multidrug resistance (MDR) in MRSA strains, further highlighting the challenge of antimicrobial resistance.

The antimicrobial activity of $BiVO_4$ NPs was assessed by agar well diffusion method. The presence of clear zones of inhibition around each well, where bacterial growth was absent, demonstrated the efficacy of $BiVO_4$ NPs in inhibiting bacterial growth, as shown in Fig. 4. The diameter of the inhibition zones for various concentrations of $BiVO_4$ NPs, are presented in Table 1. The smallest inhibition zone was observed at a concentration of 10 µg ml^{-1}, while the largest inhibition zone was observed at 40 µg ml^{-1} in both *A. baumannii* and MRSA. The findings of this study

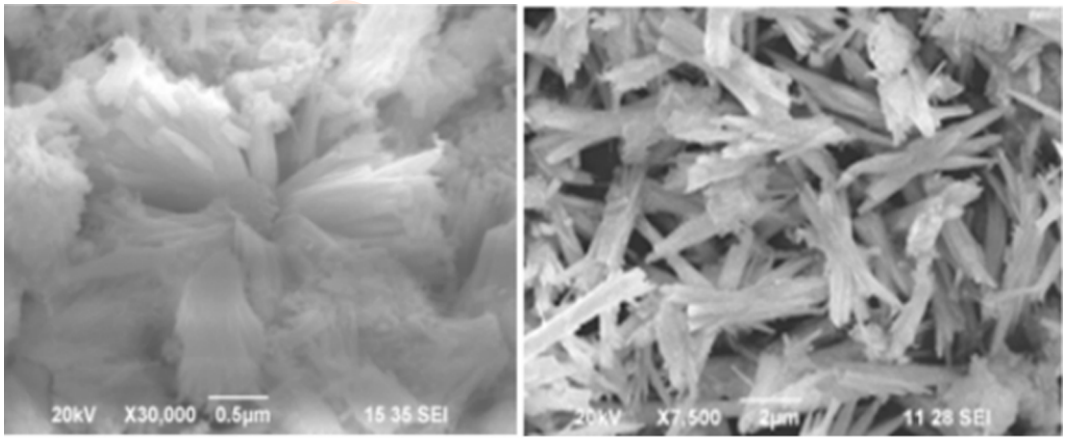

Fig. 1: SEM Micrographs of monoclinic $BiVO_4$ prepared by solvo-thermal technique.

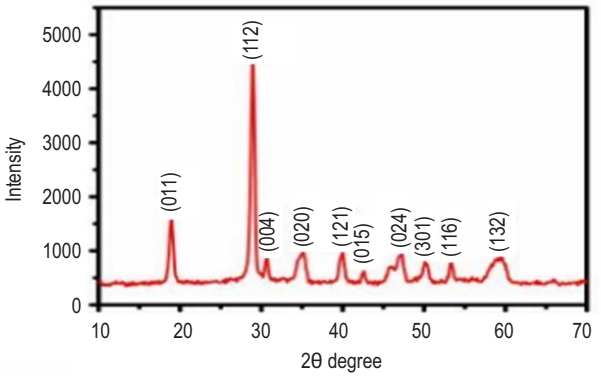

Fig. 2: XRD pattern of $BiVO_4$ NP prepared via solvo-thermal method.

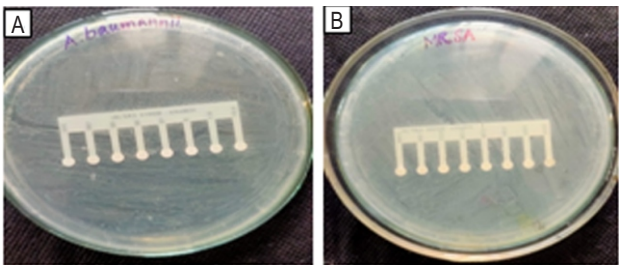

Fig. 3: (A) *Acinetobacter baumannii* exposed to ceftazidime and (B) MRSA exposed to ampicillin showing no zones of inhibition, indicating antimicrobial resistance to the respective antibiotics.

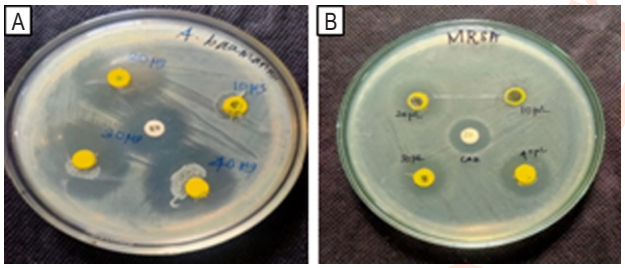

Fig. 4: Inhibition zones of (A) *A. baumannii* and (B) MRSA following treatment with $BiVO_4$ nanoparticles of various concentrations

corroborate with Ganeshbabu *et al.* (2020), who synthesized $BiVO_4$ NPs bychemical precipitation method. Their bactericidal studies demonstrated that $BiVO_4$ NPs, calcined at 400°C, exhibit potent inhibitory effects against both Gram-negative bacterium *P. aeruginosa* within 24 hr and Gram-positive bacterium *S. aureus* within 48 hr. This alignment between the results of the current study and those of Ganeshbabu *et al.* (2020) underscores the antimicrobial efficacy of $BiVO_4$ NPs across different bacterial strains, including *A. baumannii* and MRSA.

The ability of $BiVO_4$ NPs to inhibit the growth of these clinically significant pathogens highlights their potential as

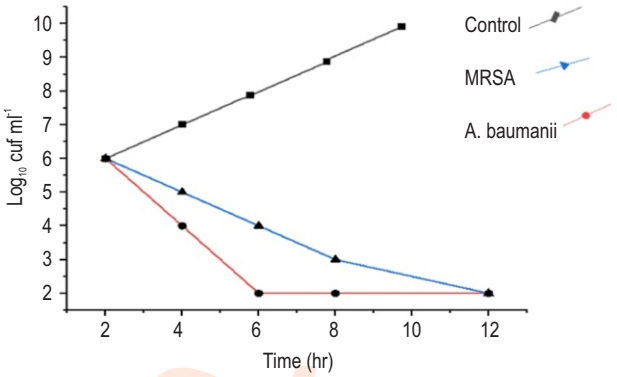

Fig. 5: Time-Kill kinetics of *A. baumannii* and MRSA treated with $BiVO_4$ nanoparticles (100 µg ml^{-1}).

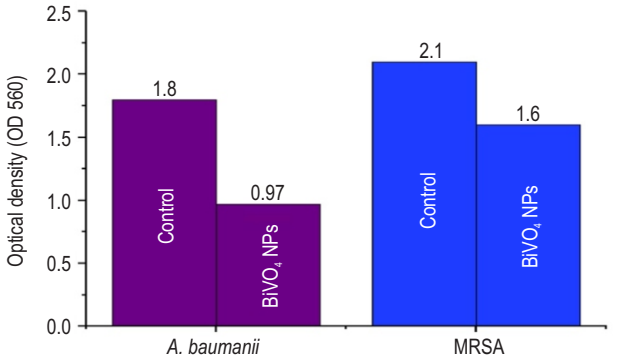

Fig. 6: Optical density values indicating biofilm formation in *A. baumannii* and MRSA:Control vs. $BiVO_4$ nanoparticles treatment.

promising antimicrobial agents for combating drug-resistant bacteria in various healthcare and environmental settings. The Minimum inhibitory concentration determination was established by testing varying concentrations of $BiVO_4$ NPs against the respective microorganisms in a 96-well microtiter plate. The minimum concentrations at which the least growth was observed was 225 µg ml^{-1} (96 % cell death) for *A. baumannii* and 375 µg ml^{-1} for MRSA (97 % cell death), as shown in Table 2. MBC results provided further evidence of the strong antimicrobial activity of $BiVO_4$ NPs against both strains. The MBC values, determined to be 500 µg ml^{-1} for both *A. baumannii* and MRSA, indicate the concentration at which $BiVO_4$ NPs effectively eliminated bacterial growth on nutrient agar, resulting in no visible growth. The percentage of cell death observed in MIC determination along with MBC values revealed the dose-dependent antimicrobial activity of $BiVO_4$ NPs against *A. baumannii* and MRSA. Interestingly, the MBC values of $BiVO_4$ NPs against *Enterococcus faecalis,* ranged between 1.25 µg ml^{-1} and 40 µg ml^{-1}. This underscores the potent antimicrobial activity of $BiVO_4$ NPs across different bacterial species, including clinically relevant pathogens such as *A. baumannii,* MRSA and *E. faecalis* (Azad *et al.,* 2020).

Table 1: Inhibition zones exhibited by varying concentrations of $BiVO_4$ NPs in the agar well diffusion assay

Concentration of $BiVO_4$ nanoparticles (µg m l^{-1})	Inhibition zones (mm)	
	A. baumannii	MRSA
10	8	8
20	12	11
30	17	14
40	19	15
Amikacin/ Mupirocin	11	5

Table 2: Cell death observed in broth microdilution with varying concentration of $BiVO_4$ NPs against *A. baumannii* and MRSA

Concentrations of $BiVO_4$ NPs (µg m l^{-1})	% of cell death	
	A. baumannii	MRSA
75	87	75
100	90	80
150	91	81
200	95	87
225	96	92
300	96	95
375	93	97

Table 3: Effect of $BiVO_4$ NPs on cell surface hydrophobicity of *A. baumannii* and MRSA

Microorganisms	Cell surface hydrophobicity		
	$BiVO_4$ NPs (µg ml^{-1})		
	Control	100	250
A. baumannii	35.45	30.85	24.61
MRSA	44.23	39.56	32.21

Table 4: Proteolysis zone diameters Indicating casein hydrolysis *A. baumannii* and MRSA treated with $BiVO_4$ Nps

Microorganisms	Clear zone indicating casein hydrolysis (mm)	
	Control	$BiVO_4$ NP
A. baumannii	11	8
MRSA	17	13

The time-kill profile of $BiVO_4$ NP against *A. baumannii* and MRSA at a concentration of 100 µg ml^{-1} showed a decrease in the number of viable cells over time. The antimicrobial action of $BiVO_4$ NPs appeared to be both concentration-dependent and bacteriostatic. The area under the curve analysis for $BiVO_4$ NPs against *A. baumannii* and MRSA at the studied concentrations demonstrated a significant reduction in the number of cells compared to the control group (Fig. 5). $BiVO_4$ NP in 100 and 250 µg ml^{-1} also influenced the cell surface hydrophobicity of *A. baumannii* and MRSA with higher concentrations correlating with decreased cell surface hydrophobocity in both strains (Table 3). The anti-adhesive effects of $BiVO_4$ NPs directly hinders the biofilm formation inhibiting bacterias to attach onto biomedical surfaces. Notably, $BiVO_4$ NPs exhibited high efficacy against *A. baumannii* compared to MRSA.

Furthermore, $BiVO_4$ NPs inhibited proteolytic activity and hemolysis in both organisms, as evidenced by smaller zones of casein hydrolysis and hemolysis on agar plates treated with $BiVO_4$ demonstrating the strong ability of $BiVO_4$ NPs to reduce virulence factors in *A. baumannii* and MRSA. In the protease inhibition assay, the clear zones on skim milk agar plates correspond to casein hydrolysis by bacterial exoenzymes. The reduced zones of proteolysis with the addition of $BiVO_4$ NPs indicated the antivirulence activity (Table 4). Similarly, the hemolytic activity of exoproteins from *A. baumannii* and MRSA assessed with and without $BiVO_4$ NPs, showed a significant decrease in the percentage of hemolysis upon treatment. Upon addition of 250 µg $ml^{-1}$of $BiVO_4$ NP, the hemolytic activity of *A. baumannii* was reduced by 80%, MRSA by 75%. This result indicates that $BiVO_4$ NPs effectively reduce hemolysin-like exotoxins, which disrupt the membrane of red blood cells (RBCs). These assays highlight the antivirulence activity of $BiVO_4$ NPs against the test organisms. In antibiofilm assay, the control samples of *A. baumannii* and MRSA exhibited OD values ≥1, indicating robust biofilm formation by both strains. There observed a great reduction in biofilm formation upon the addition of $BiVO_4$ NPs (Fig. 6) which demonstrates their significant antibiofilm potential. Shakibaie *et al.* (2019) has also published similar results on the antibiofilm potential of Bi NPs against clinical isolates of *S. aureus, P. aeruginosa* and *P. mirabilis.* This suggests that $BiVO_4$ NPs are promising candidates for combating

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drug-resistant biofilm-forming species. However, further studies involving a broader range of drug-resistant microbes are necessary to validate their efficacy as antimicrobial agents.

While there is extensive research on silver and copper NPs, studies on $BiVO_4$ NPs are comparatively fewer. However, recent studies have highlighted the photocatalytic bactericidal properties of $BiVO_4$ NPs, particularly in combination with Ag/ $BiVO_4$ nanocomposites and polydopamine-reduced Graphene oxide/ $BiVO_4$ composites, demonstrating effective bacterial deactivation mechanisms (Li *et al.,* 2022; Guan *et al.,* 2020; Regmi *et al.,* 2018). Several studies have also explored the green synthesis of $BiVO_4$ NPs, producing NPs with reduced cytotoxicity, showing promising results in anti-cancer and antibacterial applications. Green synthesis methods, utilizing plant phytochemicals, have emerged as viable alternatives to chemical synthesis for producing $BiVO_4$ NPs, particularly for medical applications. These green-synthesized $BiVO_4$ NPs have shown promising results in both anti-cancerous and antibacterial research (Pramila *et al.,* 2020; Mallikarjunaswamy *et al.,* 2021). The research data was assessed for its statistical significance using a student'- t test, revealing a noteworthy decrease ($p < 0.05$) in microbial activity when compared with the control group.

The present study demonstrates that $BiVO_4$ NPs effectively reduced the growth, biofilm formation, and virulence factors in *A. baumannii* and MRSA, suggesting potential control over MDR strains. Looking ahead, this research can be extended to evaluate the potential of $BiVO_4$ NPs as a surface coating for medical implantable devices, such as dental implants (aligners, crowns, screws, caps) and orthopedic-related implants (plates, screws, bolts). Additionally, exploring its impact on cell lines for potential anti-cancer properties is essential. These efforts will pave the way for developing nanotherapeutic compounds designed to combat multi-drug and extensively drug-resistant pathogens, offering hope for improved healthcare outcomes.

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Authors' contribution: V. Aswathy: Contributed by writing the research article and conducting research work; **S. Sajith:** Played a role in writing the research article and conducting research work; **R. Shakila:** Contributed to the research article through writing and conducting research work; **S. Vijayanand:** Provided the initial research idea and instructions; **B. Vidhya:** Contributed to the research idea, coordinated the research, and finalized corrections for the paper; **S.Murugan:** Contributed to the research idea, assisted in coordination, and participated in the final correction of the paper.

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Ethical approval: The present research does not involve the use of Plants/ animals/ humans. For the use of microorganisms, approval was sought from IBSC (Institutional Biosafety Commitee), Karunya Institute of Technology and Sciences, Coimbatore- 641114.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: Not applicable.

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