Nanoparticle-Mediated Plasmid Curing in Combating Antibiotic Resistance in Pathogenic Bacteria

Muntaha R. Ibraheem [1*], Dhafar N. Al-Ugaili [2]

Abstract

Background: Nanotechnology has emerged as a pivotal domain in material science research with extensive applications across various sectors including biotechnology and medicine. Nanoparticles offer unique properties facilitating advancements in nanobiotechnology, particularly in nanomedicine, to combat bacterial infections and antibiotic resistance. This study aimed to determine the application of nanoparticles, specifically nano-TiO2, in treating plasmid-mediated antibiotic resistance in both Gram-negative and Gram-positive bacteria. Method: We evaluated antibiotic and nanomaterial sensitivity through disc diffusion and broth microdilution assays. Plasmid curing experiments were conducted using varying concentrations of nano-TiO2 and SDS as curing agents, followed by plasmid isolation and DNA extraction. The efficacy of nano-TiO2 in plasmid curing and DNA extraction was assessed, alongside the impact on bacterial growth and antibiotic resistance. Results: Results showed successful plasmid elimination with nano-TiO2 treatment, evidenced by the loss of plasmid DNA bands. Additionally, nano-TiO2 substantially enhanced DNA extraction efficiency and quality. The study indicated nano-TiO2's potential in combating antibiotic resistance by targeting plasmids, thereby presenting a novel approach in molecular biology techniques. Conclusion: In conclusion, this study underscores the promising role of nanoparticles in addressing bacterial infections and combating antibiotic resistance. Nano-TiO2 emerges as a valuable tool in DNA purification and plasmid curing, offering new avenues in molecular biology and antibiotic resistance research. However, further investigations are warranted to elucidate the broader implications of nanoparticles across diverse bacterial species and strains. These findings represent a significant step towards harnessing the potential of nanotechnology in combating antimicrobial resistance and advancing healthcare paradigms.

Keywords: Toxoplasmosis, MCP-1, MIP-1α, Immune response, Chemokines

Significance | Novel approach fights antibiotic resistance. Enhances DNA extraction efficiency. Eliminates resistance plasmids, aiding biotechnology. Highlights research needs for nanoparticle optimization and mechanism understanding.

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Introduction

Nanotechnology is currently one of the most active areas of research in material science, offering immense promise across various domains such as material science, biotechnology, and medicine. Nanoparticles, characterized by their unique properties, have a wide range of applications, spanning from enhancing consumer products like detergents and toothpaste to revolutionizing medical diagnostics and therapies. Nanobiotechnology, a fascinating branch of nanotechnology, utilizes nanoscale materials and devices to

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interface with biological systems, exploiting the fact that many biological processes occur at the nanoscale (Haley et al., 2015).

Nanomedicine, in particular, has attracted significant attention for its potential to revolutionize healthcare. By precisely controlling the size and properties of nanoparticles, researchers can design targeted drug delivery systems, minimizing side effects and enhancing therapeutic outcomes. Additionally, nanotechnology facilitates the development of advanced imaging techniques for early disease detection, thereby enabling more effective treatments (Ruobing & Yu-Qing, 2018).

Noble metal nanoparticles, including gold, silver, platinum, and zinc oxide, find widespread use in products such as detergents, cosmetics, and toothpaste, as well as in scientific and medicinal applications. Nanobiotechnology encompasses diverse programs, ranging from enhancing computing speed to detecting cancerous tumors beyond the resolution of the human eye (Warawan, 2018). The organic length scale at which nanobiotechnology operates is a key factor contributing to its potential, considering that biological molecules, microbes, and individual cells are all nanoscale entities.

Engineered nanoparticles tailored to specific sizes and functions, such as drug delivery to targeted tissues or blood circulation, exemplify the emerging field of nanomedicine. Plasmid curing, the loss of plasmids, is a significant aspect of molecular biology and biotechnology, aiding in genetic engineering (Rezak et al., 2018). Plasmids, circular DNA molecules capable of independent replication, play a crucial role in genetic research and biotechnology applications.

The increasing demand for rapid, efficient genetic testing methods in molecular biology and biotechnology necessitates the development of streamlined techniques that can yield results within hours or minutes, rather than days or weeks. However, the ethical and safety implications of nanobiotechnology, particularly concerning the interaction between nanoparticles and living organisms, must be thoroughly studied and addressed to mitigate potential risks (Belaynehe et al., 2018).

However, the convergence of nanotechnology and biotechnology has ushered in a revolution across various scientific fields, from healthcare to genetic engineering. This interdisciplinary approach holds immense promise for addressing contemporary challenges and advancing scientific understanding and technological innovation.

Recent advancements in healthcare have been hindered by the emergence of pathogenic bacteria resistant to multiple antibiotics, presenting a significant challenge to modern medicine. Overuse and misuse of antibiotics have accelerated the evolution of multidrug-resistant microorganisms, including superbugs resistant to nearly all available antibiotics (Yi-Yun Liu et al., 2016). Among these, bacteria carrying the NDM-1 gene, conferring resistance to multiple antibiotics, pose a particularly concerning threat.

Traditional antibiotic research has focused on targeting specific bacterial processes, such as cell wall synthesis, protein translation, and DNA processing. However, bacterial mechanisms, such as efflux pumps that expel antibiotics from bacterial cells, have thwarted these efforts. Nanoparticles (NPs) have emerged as a promising alternative to traditional antibiotics due to their ability to directly interact with bacterial cell walls, thereby reducing antibiotic resistance (Yi-Yun Liu et al., 2016).

Investigations into the antibacterial properties of nanoparticle-based materials have highlighted their potential to compete effectively with antibiotic-resistant bacteria (Mohammad et al., 2012). NPs exhibit broad-spectrum effects against both Gram-positive and Gram-negative bacteria, offering a promising avenue for combating bacterial infections (Solmaz et al., 2014). Mechanisms such as the disruption of bacterial membranes and the generation of reactive oxygen species contribute to the antibacterial properties of nanoparticles, making them a viable option for combating a wide range of bacterial infections (Solmaz et al., 2014). Furthermore, engineering nanomaterials allows for the efficient enhancement of their antibacterial properties while minimizing side effects. Manipulating variables such as size, shape, and surface characteristics enables nanoparticles to target specific bacterial strains or infections (Fidel et al., 2013). Despite promising results, further research is needed to fully understand the behavior of nanoparticles and elucidate the mechanisms by which they enhance efficacy against bacterial infections (Fidel et al., 2013).

Therefore, nanoparticles offer a novel and promising strategy for addressing bacterial infections, providing broad-spectrum activity to overcome antibiotic resistance. Our current study aims to investigate the application of nanoparticles in treating plasmid-mediated antibiotic resistance in both Gram-negative and Gram-positive bacteria. Continued research in this area is essential for advancing our understanding of nanoparticle-based antibacterial materials and their suitability for clinical use.

Materials and method

Antibiotic and Nanomaterial Sensitivity Test

The antibiotic sensitivity of the selected isolate was assessed using the disc diffusion method (wild type). A sterile cotton swab was immersed in the inoculum (freshly cultured, 18 hr.) and swabbed across the entire surface of brain heart infusion agar plates three times to achieve uniform distribution. Antibiotic disks were then placed on the plates with sterile forceps after allowing excess moisture to evaporate at room temperature for 10 minutes. The plates were incubated inverted at 37°C for 18 hours, and the widths of inhibition zones were measured to determine antibiotic sensitivity according to the National Committee for Clinical Laboratory Standards guidelines (Ferraro et al., 2009).

To evaluate bacterial susceptibility to nanoparticles, the disc diffusion method was adapted, where nanoparticle-coated discs were placed on agar plates inoculated with bacteria. The zone of inhibition was measured after incubation. Furthermore, the minimum inhibitory concentration (MIC) of nanoparticles was determined using broth microdilution assays. Serial dilutions of nanoparticles were prepared in liquid growth medium containing bacteria, and growth inhibition was assessed after incubation (Malarkodi et al., 2013).

Curing of Plasmid with Nanoparticles

Curing experiments and plasmid isolation were conducted on the isolated bacteria using nanoparticles (TiO2) as a curing agent. Bacterial cells were grown to mid-log phase in brain heart infusion broth. Tubes containing fresh brain heart broth with varying concentrations of titanium nanoparticles (1% to 10%) were inoculated with the growth culture inoculum. All tubes were then incubated for 24-48 hours at 37°C. The effect of titanium nanoparticles on bacterial growth was assessed by comparing the growth density of different tubes to the control (Fidel Martinez-Gutierrez et al., 2013).

The procedure for isolating plasmids from bacterial cultures treated with titanium nanoparticles involved several steps: bacterial culture was spread onto brain heart infusion agar plates and incubated overnight. Colonies from the master plate were then transferred to multiple antibiotic-containing agar plates to assess antibiotic resistance. Plasmids were isolated by preparing a lysate from bacterial culture, followed by column purification and elution of plasmid DNA. The isolated plasmids were analyzed using agarose gel electrophoresis to determine their pattern and number (Robert et al., 2018). This protocol ensures the successful isolation of plasmids from bacterial cultures treated with titanium nanoparticles, facilitating further analysis of plasmid content and antibiotic resistance patterns.

Results And Discussion

Isolation and identification of Staphylococcus aureus:

Fifteen clinical samples, including wound and burn swabs, were collected during research at Al-Kadymia Hospital in Baghdad. Standard cultural protocols were followed, and samples with positive growth underwent further analysis for primary species identification. Gram staining and routine biochemical assays were employed for this purpose.

Staphylococcus aureus was detected in seven isolates based on their gram-positive nature, characterized by thicker and denser peptidoglycan layers in their cell walls. Microscopic examination revealed their characteristic appearance as cocci arranged in clusters resembling grape-like structures.

Mannitol Salt Agar served as a selective medium for Staphylococcus aureus, promoting its growth while inhibiting other bacterial species. Clinical isolates of Staphylococcus aureus demonstrated mannitol fermentation, leading to acid production and a yellow color change in the medium (Figure 1).

Ten clinical samples, including stool specimens from patients with diarrhea and urinary tract infections, were collected during the same research. Bacterial isolates were identified using conventional cultural procedures based on morphological, biochemical, and physiological characteristics. Isolates exhibiting positive growth underwent further examination for primary species identification through Gram staining and routine biochemical assays. Among the isolates, six were identified as Escherichia coli due to their gram-negative nature and rod-shaped morphology observed under microscopic examination. MacConkey agar served as a selective medium for Escherichia coli. The clinical isolates were found to ferment lactose, as depicted in Figure 2.

These approaches enable accurate identification of pathogenic bacteria and provide valuable insights into their metabolic capabilities, aiding in the diagnosis and management of bacterial infections. Selective media and biochemical assays play a crucial role in clinical microbiology by facilitating the precise identification of pathogenic organisms.

The standard disk diffusion method was utilized to assess the susceptibility of E. coli and S. aureus to various antibiotics, aiming to confirm their sensitivity and resistance profiles for selecting markers for the plasmid curing experiment.

Table 1 showed the multiresistance patterns of clinical isolates to antibiotics. Cefixime exhibited 100% sensitivity, potentially suitable for growth suppression treatment. However, 83% of isolates showed resistance to tetracycline and erythromycin, 67% to chloramphenicol, 50% to novobiocin, and 33% to neomycin and kanamycin. Conversely, Table 2 showed that 100% of isolates were sensitive to neomycin, indicating its potential utility in treating intestinal bacterial growth suppression. However, all isolates demonstrated resistance to chloramphenicol, with 71% resistance to tetracycline and kanamycin, 57% to novobiocin and erythromycin, and 43% to cefixime.

Table 2 showed the antibiotic resistance patterns among E. coli and S. aureus isolates, showing variability based on the isolate or antibiotic type. Notably, 75% of isolates exhibited resistance to tetracycline, erythromycin, and chloramphenicol. The susceptibility of Staphylococcus aureus isolates, previously characterized in our research, to four different antibiotics with distinct mechanisms of action was tested. Results indicated multidrug resistance among the examined pathogenic isolates to the four antibiotics under consideration, as illustrated in Figure 3.

Based on these results, E3 for E. coli and S5 for S. aureus were selected due to their multiresistance to antibiotics, as indicated by previous studies (Priyanka et al., 2014; Lowrence et al., 2015). These

Figure 1. Mannitol fermentation by *Staphylococcus aureu*s grown on Mannitol Salt Agar Medium Isolation and identification of *Escherichia coli*.

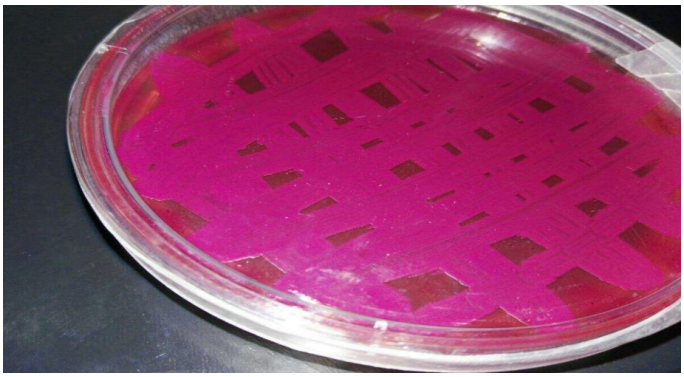

Figure 2. MacConkey agar by *Escherichia coli* aureus grown on MacConkey agar media.

Table 1. Antibiotic susceptibility of *E.coli* isolate.

Antibiotics	**Code**	**E1**	**E2**	**E3**	**E4**	**E5**	**E6**
Tetracyclin	TE	R	S	R	R	R	R
Cefixme	CFM	S	S	S	S	S	S
Erythromycin	E	R	S	R	R	R	R
Neomycin	N	S	S	R	S	R	S
Kanamycin	K	R	S	R	S	S	S
Chloramphenicol	C	R	S	S	R	R	R
Novobiocin	K	S	S	R	R	R	S

Table 2. Antibiotic susceptibility of *S. aureus* isolate.

Antibiotics	**Code**	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**
Tetracyclin	TE	R	S	R	R	R	R	S
Cefixme	CFM	S	S	S	R	R	S	R
Erythromycin	E	R	S	R	R	R	S	S
Neomycin	N	S	S	S	S	S	S	S
Kanamycin	K	R	S	R	S	R	R	R
Chloramphenicol	C	R	R	R	R	R	R	R
Novobiocin	K	S	S	R	R	R	R	S
R: Resistance, S: Sensitive								

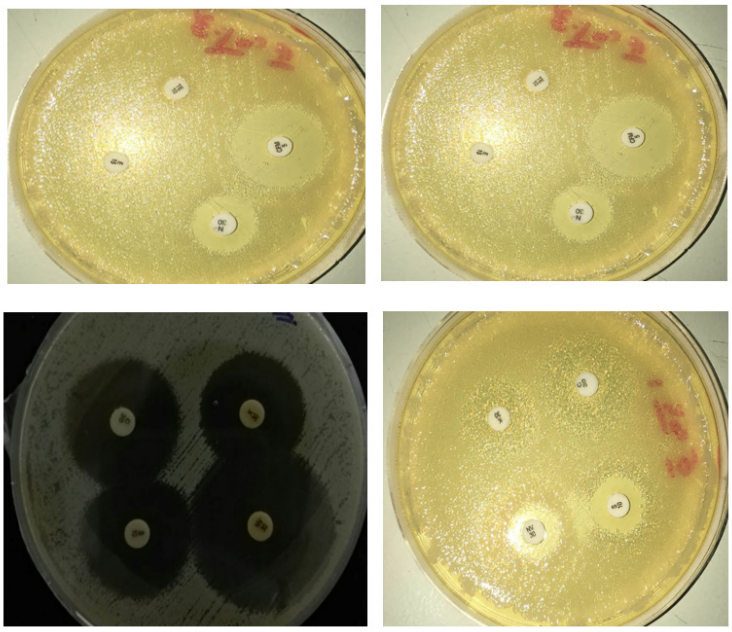

Figure 3. Antibiotic susceptibility pattern of *Staph aureus* and *E. coli* bacteria on Mueller Hinton agar media.

Table 3. Effect of SDS and nano TiO2 and mixer of both nano Tio2 and SDS on the growth of *E. coli* (E3) and *S.aureus* (S5).

Concentration%	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
Bacterial growth (E3) (SDS)	+++	+++	++	++	++	+	+	±	-	-
Bacterial growth (S5)(SDS)	+++	+++	++	++	+	+	±	-	-	-
Bacterial growth (E3) (TiO_2)	+++	+++	++	++	+	±	-	-	-	-
Bacterial growth (S5)(TiO_2)	+++	+++	++	++	+	±	-	-	-	-
Bacterial growth (E3) (SDS and TiO_2)	+++	+++	++	++	+	±	-	-	-	-
Bacterial growth (S5)(SDS and TiO_2)	+++	+++	++	++	+	±	-	-	-	-
(+++): very good growth, (++) : Good growth, (+) : Moderate growth, (±) : Slightly growth, (-) : No growth										

Table 4. Concentration and purity of DNA extracted from *E. coli* and *S. aureus*.

Extracted DNA from:	DNA concentration ng\µl	Purity of DNA (260 /280)
E. coli (E3) DNA with SDS	104.8	1.6
E. coli with TiO_2	205	1.7
E. coli with TiO2and SDS	198	1.8
S. aureus (S5) DNA with SDS	98	1.6
S.aureus with TiO_2	183	1.7
S.aureus with TiO2and SDS	180	1.8

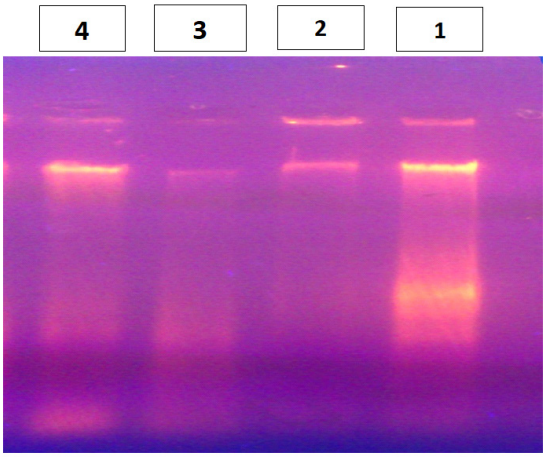

Figure 4. Gel electrophoresis of plasmids content of E. Coli before and after treatment with SDS and nano TiO_2 And the mixer on agarose gel (0.7%) in TBE buffer at 5V/cm. (1: Plasmid content of *E. Coli* (E3), 2: Plasmid content of cured strain with TiO_2 (E3) 3: Plasmid content of cured strain with SDS (E3) 4: Plasmid content of cured strain with TiO_2 and SDS (E

findings guided the selection of appropriate isolates for subsequent plasmid curing experiments.

Multidrug resistance in bacteria can be attributed to several factors. The impermeability of the outer membrane acts as a barrier, limiting the entry of antibiotics into bacterial cells. This reduced permeability contributes to the ineffectiveness of antibiotic treatments against these bacteria. Additionally, mutations within the bacterial genome, stemming from the overuse and misuse of antibiotics, can exacerbate resistance. Furthermore, plasmids carrying resistance genes play a significant role in disseminating multidrug resistance among bacterial strains (Sengan et al., 2016). These factors collectively contribute to the challenge of combating multidrug-resistant bacteria in clinical settings.

Plasmid Isolation:

To determine the plasmid profile of E. coli and S. aureus isolates, genomic DNA was extracted using a Qiagen kit for genomic DNA following cultivation in Luria-Bertani broth. Extraction procedures were carried out according to the manufacturer's instructions. Subsequently, a plasmid profile was obtained by analyzing the presence of DNA before curing. Results revealed that two isolates contained small plasmid DNA bands. It is possible that the bacterial isolates tested in this study may harbor other undetected plasmids, potentially due to their larger size (Gloria et al., 2017; Debashish et al., 2018).

The relationship between plasmid curing by SDS and Nanoparticles:

Plasmid curing experiments were conducted to determine if genes responsible for antibacterial resistance and virulence factors were located on plasmids in bacterial isolates. E. coli and S. aureus isolates (E3 and S5) were chosen for their multidrug resistance to antibiotics (Linlin et al., 2017; Dutta et al., 2012). The standard disk diffusion method was employed to ascertain the susceptibility of these isolates to different antibiotics, aiding in the selection of appropriate markers for the curing experiment (Steven et al., 2012; Jun Feng et al., 2013).

Results indicated that a concentration of 8% SDS was required for the growth of E. coli and S. aureus (E3), while a concentration of 6% nano TiO2 allowed for their growth. Additionally, a mixture of SDS and nano TiO2 (v/v) at a concentration of 0.6 enabled growth of both E. coli and S. aureus (E3, S5). Following these treatments, appropriate dilutions were prepared for each isolate and spread onto brain heart infusion agar. Subsequently, 100 colonies were selected and tested on selective media containing specific antibiotics to identify cured colonies that could not grow on these antibiotics. The obtained cured colonies were then tested against another specific antibiotic (Muhammad et al., 2017; Ningning et al., 2015).

DNA purity and quantity were assessed before and after using nanoparticles, with the DNA content ranging from 98 to 205 ng/l and the DNA purity from 1.6 to 1.8 (Dongshu et al., 2015). Table 4 demonstrated an improvement in DNA quality post-nanoparticle treatment, with the purity of extracted DNA increasing from 1.6 to 1.7-1.8 after extraction with nanoparticles. Moreover, DNA concentration significantly increased when recovered from both bacterial species using TiO2 nanoparticles.

To validate the results of the curing experiment, plasmid DNA was isolated from a cured strain of E. coli (E3) that had lost its resistance to certain antibiotics. Comparison with the plasmid content of the original isolate revealed that the cured strain (E3) had lost its plasmid DNA bands following treatment with nano TiO2 as a curing agent. This finding supported the results of the curing experiments, indicating that genes responsible for antibiotic resistance, such as tetracycline, were located on plasmids. Furthermore, all isolates exhibited small plasmid DNA bands after treatment with nanoparticles, indicating successful plasmid curing. This study marked the first application of nanoparticles, particularly TiO2, as a curing agent for bacterial plasmids. The findings not only underscore the effectiveness of nanoparticles in plasmid elimination but also highlight their potential to enhance the purity and quantity of extracted DNA. The use of SDS as a curing agent also yielded promising results, consistent with previous research findings.

However, this study revealed the significant potential of nanoparticles, specifically TiO2, in curing bacterial plasmids and improving DNA extraction protocols. Further research involving different types of nanoparticles and bacterial species/strains is recommended to validate and expand upon these findings (Daoud et al., 2015; V. Berzin & M. K., 2013).

Conclusion

In conclusion, this study demonstrated the significant potential of nano-TiO2 in enhancing DNA extraction efficiency and curing bacterial plasmids, thereby contributing to advancements in molecular biology and the fight against antimicrobial resistance. The observed enhancement in DNA extraction can be attributed to the ability of TiO2 nanoparticles to disrupt cell membranes and interact with extraction proteins effectively. Furthermore, the successful elimination of plasmids from bacterial isolates suggests a novel approach to combating antimicrobial resistance.

However, while this research highlighted the promising applications of nano-TiO2, further studies might be warranted to comprehensively understand the effects of different nanoparticles on plasmid curing across a wider range of bacterial species and strains. Such investigations will not only help validate the findings of this study but also expand our knowledge of nanoparticle-based interventions in molecular biology and antimicrobial resistance mechanisms.

Overall, this study underscored the importance of exploring innovative approaches, such as nano-TiO2, in addressing challenges related to DNA extraction and antimicrobial resistance. By continuing to investigate and refine these techniques, researchers can potentially develop more effective strategies for diagnosing and treating bacterial infections while mitigating the risk of antibiotic resistance.

Limitations of the study include potential variations in bacterial response to nano-TiO2 treatment among different strains and environmental factors impacting nanoparticle interactions, potentially yielding different results. The generalizability of findings to diverse bacterial species or environmental conditions may be limited. Future studies should explore nano-TiO2 efficacy across a broader range of bacterial strains and optimal conditions. The study focused solely on the beneficial effects of nano-TiO2 in DNA extraction and plasmid curing, neglecting nanoparticle toxicity. Future research should investigate nano-TiO2 toxicity on bacterial and human cells and assess its long-term stability. Mechanisms underlying nano-TiO2 effects on plasmid curing and DNA extraction, such as cell membrane lysis and protein interactions, remain unclear and warrant further exploration. Future directions should include global risk evaluation, optimization of nanomaterial properties, elucidation of molecular mechanisms, and assessment of nano-TiO2 applications under realistic conditions. Addressing these limitations can maximize nano-TiO2 potential in molecular biology and combatting antibiotic resistance, offering innovative solutions in microbiology and biotechnology.

Author contributions

N.A.H. conducted study design, analyzed data, S.K.A.H. wrote and drafted the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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