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Antimicrobial and Anti-biofilm Effect of Samarium oxide Nanoparticles on *Pseudomonas aeruginosa* Isolated from Hospital Wards

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Abstract

The development of drug resistance, treatment ineffectiveness, and the occurrence of *Pseudomonas aeruginosa* infections are primarily attributed to biofilm formation and quorum sensing (QS)-dependent virulence factors. This study aimed to synthesize samarium oxide nanoparticles (Sm_2O_3NPs) using curcumin and evaluate their antimicrobial effects against *P. aeruginosa* isolated from different hospital wards. Based on TEM, XRD, and EDX analysis, the synthesized nanoparticles exhibited a cubic structure with an average size of 32.61 nm and a pure crystalline phase. The Sm_2O_3NPs exhibited notable antibacterial efficacy against *P. aeruginosa* strains. Based on the CV staining assay, sub-MIC of Sm_2O_3NPs inhibited the biofilm formation of *P. aeruginosa* strains by 16-69%. Additionally, the levels of pyocyanin in the NPs-treatment groups decreased by 29-58% compared to the control group. This research confirms the significant antibacterial potential of Sm_2O_3NPs, implying their potential utilization as an effective antibacterial agent against *P. aeruginosa* strains isolated from various hospital wards, after further characterization.

Key words: Nanoparticles; Samarium; *Pseudomonas aeruginosa*; Hospital environment; Biofilm

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Introduction

Pseudomonas aeruginosa is an opportunistic pathogen responsible for various infections, including bacteremia, burn wound infections, and cystic fibrosis. These infections are particularly prevalent among individuals with compromised immune systems or underlying conditions such as diabetes (Gellatly et al. 2013). The production of various virulence factors such as pyocyanin, proteases, elastase, and hemolysin, also biofilm-forming ability have led to the inclusion of *P. aeruginosa* in the World Health Organization's (WHO) priority list for the development of new antibiotics (Tacconelli et al. 2018).

Biofilms consist of a community of bacterial cells that are surrounded by a self-generated polymer matrix comprising exopolysaccharides (Pel, Psl, alginate), as well as extracellular DNA (eDNA). This organized bacterial community leads to evasion of the host's immune system and renders conventional antibiotic treatment ineffective. Pel and Psl polysaccharides participate as structural scaffolds during the initial phases of biofilm formation. Alginate plays a crucial role in maintaining the integrity of the biofilm matrix and enhancing the stability of *Pseudomonas* in the lungs. eDNA serves as a nutrient source for bacteria and facilitates cell-to-cell adhesion. Moreover, it provides protection to bacterial cells by binding to antibiotics (Rasamiravaka et al. 2015).

Nanoparticles (NPs) possess distinctive characteristics including a high surface area, small dimensions, minimal toxicity, and high stability (Khan et al. 2019a). various research studies have demonstrated the antimicrobial effects of diverse metal and metal oxide nanoparticles produced using different plant extracts (Khan et al. 2016). The main consequences of exposure to antimicrobial nanoparticles include the generation of reactive oxygen species (ROS), impairment of bacterial cell wall and extracellular polymeric substances (EPSs), DNA damage, inhibition of protein synthesis, and ultimately leading to bacterial cell death (Velsankar et al. 2020). Samarium, an important element of the lanthanide series, holds significant importance in various health and medical domains, such as drug delivery, medical imaging, and etc (Dasari et al. 2016). However, few studies have examined the antimicrobial activity of Sm_2O_3NPs. This research aims to investigate the impact of Sm_2O_3NPs on the production of virulence factors and biofilm formation in *P. aeruginosa* strains isolated from the hospital environment.

Materials and methods

P. aeruginosa strains

A total number of five strains of *P. aeruginosa* were isolated from different hospital wards and identified by Gram-stain, catalase, and oxidase test, citrate utilization, sugar fermentation in TSI medium, the ability to grow at 42 °C.

Synthesis and characterization of Sm_2O_3NPs

To produce Sm_2O_3NPs, 0.1 M samarium nitrate solution ($Sm(NO_3)_3 \cdot 6H_2O$) was gradually added to 0.2 M curcumin solution while stirring continuously for 1 hour. The resulting mixture was then centrifuged at 4000 rpm for 30 minutes. The obtained pellet was washed with water and ethanol and dried in an oven at 80 °C for 1 h. Finally, the pellet was calcined in a muffle furnace at 700 °C for 4 h.

The size and morphology of the NPs were analyzed using transmission electron microscopy (TEM). The crystalline phase of the synthesized NPs was investigated using X-ray diffractometer (XRD), and elemental analysis was conducted using energy-dispersive X-ray spectroscopy (EDS).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of Sm_2O_3NPs against the *P. aeruginosa* strains was determined using the broth microdilution method in 96-well plates, as previously described (Zahmatkesh et al. 2022). In brief, different concentrations of Sm_2O_3NPs (12.5-400 µg/mL) were prepared in the microtiter well plate, and 100 µL of fresh bacterial culture with a population of 1.5×10^6 CFU/mL was transferred to each well. The plates were then incubated at 37 °C for 24 h, and the turbidity of each well was assessed. The MIC was determined as the lowest concentration of Sm_2O_3NPs that inhibited bacterial growth.

To determine the Minimum bactericidal activi-

ty (MBC), 100µL of suspension from the wells without bacterial growth was cultured in the Muller Hinton Agar medium. The plates were then incubated at 37 °C for 24 h, and the bacterial growth was assessed and which resulted in no bacterial growth, was considered the MBC value.

Biofilm formation assay

The antibiofilm effect of the Sm_2O_3NPs against the *P. aeruginosa* strains was assessed using the crystal violet staining assay (Das and Dash 2014). In brief, bacterial cells were grown in the presence of ½ MIC of the NPs in 96-well plates and incubated at 37 °C for 2 days under static conditions. After incubation, the wells were washed with phosphate-buffered saline (PBS) and stained with 200 µL of 0.1% crystal violet solution for 15 minutes at room temperature. Following staining, the wells were washed three times with PBS and allowed to dry. To release the dye, 200 µL of 30% acetic acid was added, and the absorbance was measured at 570 nm to quantify the biofilm formation level. The biofilm inhibitory potential of the NPs was determined using the following formula:

Biofilm inhibition (%) = [(untreated well OD − Treated well OD)/untreated well OD] × 100.

Pyocyanin assay

The impact of Sm_2O_3NPs on pyocyanin production by *P. aeruginosa* was examined using a previously described method (Prithiviraj et al., 2005). Initially, an overnight culture of *P. aeruginosa* was diluted in LB medium at a 1:100 ratio. Subsequently, 250 µL of the diluted bacterial suspension was inoculated into 25 mL of LB medium with or without NPs and incubated in a shaker incubator at 37 °C for 48 hours. Following the incubation period, pyocyanin was extracted from the cell-free supernatant of both the NPs-treated and untreated *P. aeruginosa* cultures. The extraction process involved the addition of 2 mL of chloroform, followed by re-extraction into 1 mL of 0.1 N HCl, resulting in a pink-colored solution. The concentration of pyocyanin (in µg/mL) was determined by multiplying the optical density at 520 nm by the conversion factor of 17.072.

Results

Characterization of Sm_2O_3NPs

TEM imaging demonstrated that the Sm_2O_3NPs had a cubic shape with a size range of 17–51 nm (Fig. 1a, b). XRD analysis (Fig. 1c) confirmed the crystalline structure of the synthesized Sm_2O_3NPs, which matched the JCPDS card no. 01-088-2866. EDS analysis (Fig. 1d) confirmed the presence of samarium and oxygen atoms in the elemental composition of the Sm_2O_3NPs.

MIC and MBC

The microdilution method was employed to determine the MIC values of Sm_2O_3NPs against five strains of *P. aeruginosa*. As shown in Table 1, the MIC values of Sm_2O_3NPs against the studied bacteria ranged from 25 to 100 µg/mL. The PA01 and PA04 strains exhibited the lowest MIC, while the PA05 strain had the highest MIC of 100 µg/mL. Furthermore, the synthesized NPs did not exhibit bactericidal activity against the PA03 and PA05 strains.

Biofilm formation assay

Crystal violet staining was employed to measure the extent of biofilm formation in *P. aeruginosa* strains after treatment with Sm_2O_3NPs. Our findings indicated that the utilization of Sm_2O_3NPs led to a reduction in biofilm formation of *P. aeruginosa* strains ranging from 18 to 69% compared to the control. Strain PA04 exhibited the most significant reduction in biofilm formation, whereas strain PA05 showed a negligible decrease. The biofilm formation of various *P. aeruginosa* strains in the presence of Sm_2O_3NPs is depicted in Fig. 2.

Pyocyanin assay

In this study, we investigated the effect of ½ MIC of Sm_2O_3NPs on the production of pyocyanin pigment by *P. aeruginosa* strains. The results showed that the inhibition percentage of pyocyanin production ranged from 29 to 58. The strain PA03 exhibited the lowest percentage of inhibition, while strain PA02 showed the highest percentage of inhibition. The concentration of pyocyanin produced by each strain is illustrated in Fig. 3.

Discussion

Biofilm formation is a process controlled by the quorum sensing (QS) system and plays a significant role in the antibiotic resistance and

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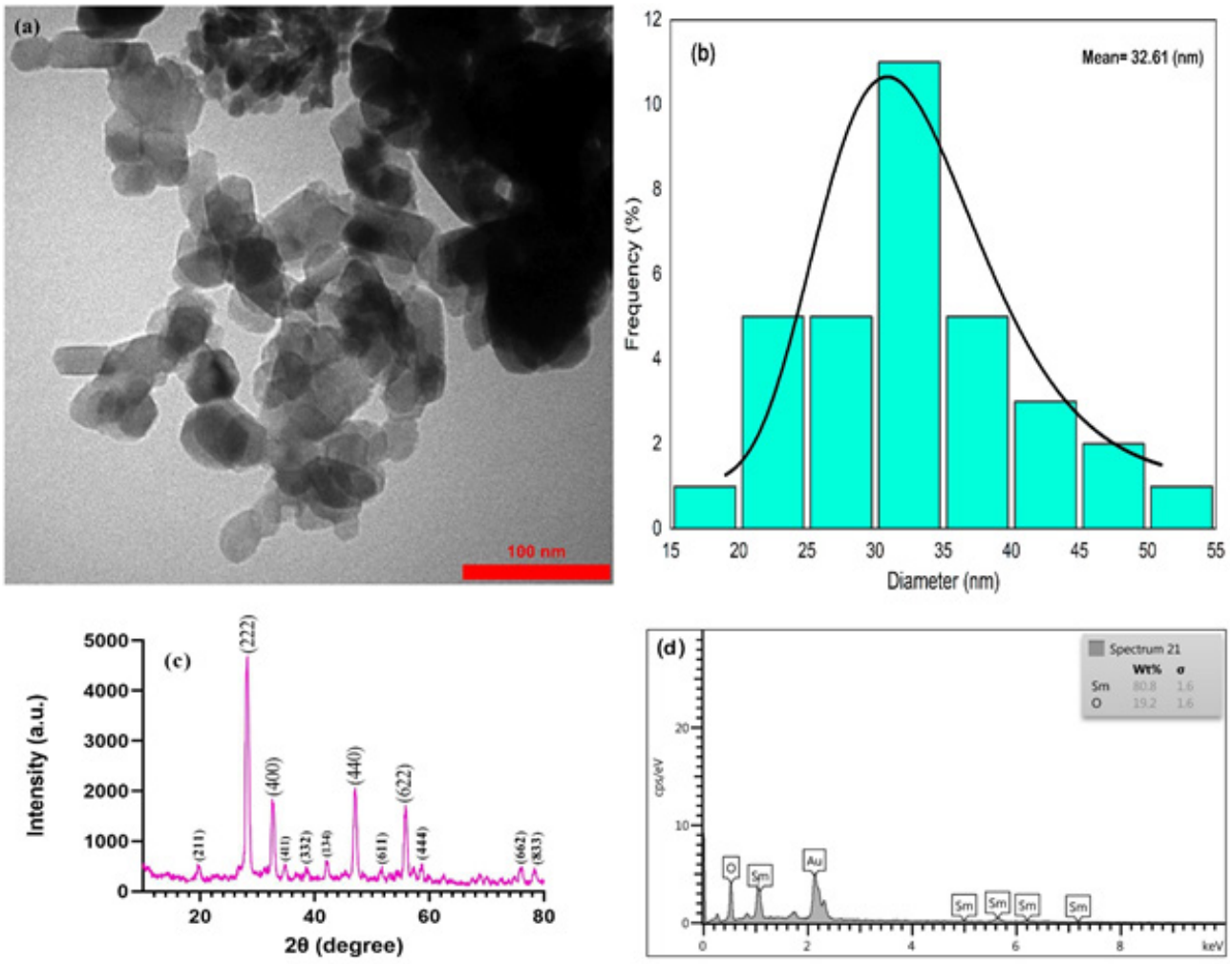

Fig. 1 (a) TEM images, (b) Particles size distribution histogram, (c) XRD pattern, and (d) EDX spectrum of Sm_2O_3NPs.

Bacteria	MIC (µg/mL)	MBC (µg/mL)
PA01	25	50
PA02	50	100
PA03	50	-
PA04	25	50
PA05	100	-

Table 1 MIC and MBC of Sm_2O_3 NPs against *P. aeruginosa* strains

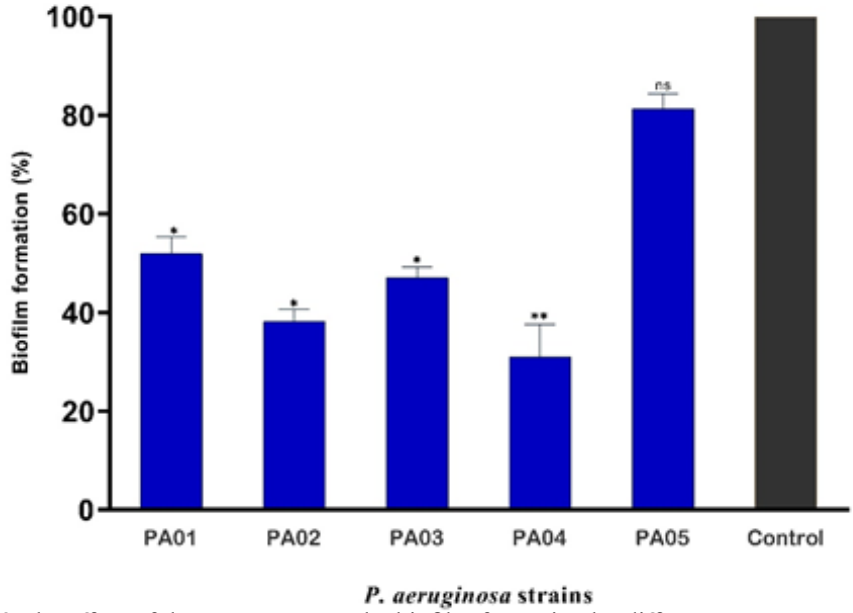

Fig. 2 The effect of the Sm_2O_3NPs on the biofilm formation by different *P. aeruginosa* strains.

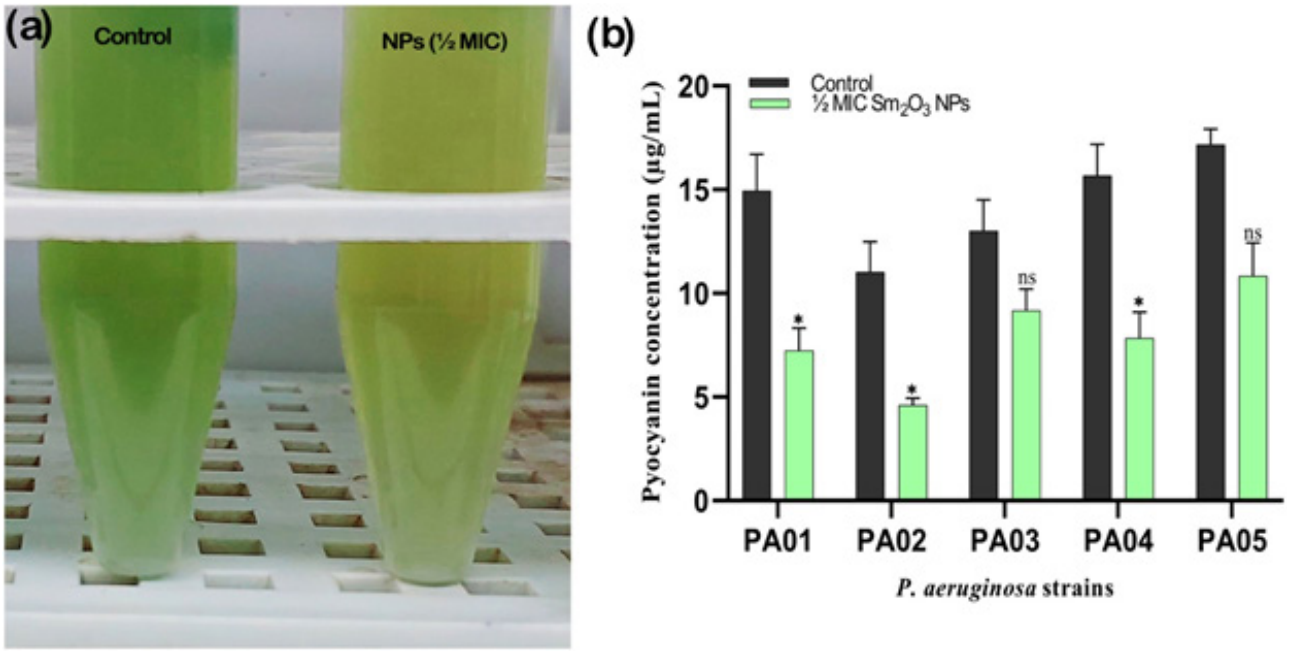

Fig. 3 (a) Visual representation of pyocyanin pigmentation produced by Sm_2O_3NPs treatment and a control group of *P. aeruginosa*, and (b) Pyocyanin concentration (µg/mL) of *P. aeruginosa* strains upon treatment with Sm_2O_3NPs vs control group.

pathogenicity of *P. aeruginosa* (Jurado-Martín et al. 2021). The antibiofilm activity of Sm_2O_3NPs may be attributed to their ability to penetrate the extracellular matrix through water channels involved in nutrient transport (Ansari et al. 2013). Additionally, the negative charge of bacterial extracellular polymeric substances (EPSs) allows for the adsorption of cations released from Sm_2O_3NPs and their subsequent penetration of the bacterial membrane. This process leads to membrane impairment, ultimately preventing biofilm formation by causing damage to bacterial cells (Ibáñez de Aldecoa et al. 2017). Sm_2O_3NPs may act against *P. aeruginosa* biofilms by generating reactive oxygen species (ROS) within the extracellular matrix. This ROS production is linked to the degradation of biofilm components, particularly extracellular DNA (eDNA)

(Shkodenko et al. 2020). This effect is similar to the antibiofilm activity observed by Khan et al. (2019b) using Fucoidan-Stabilized Gold Nanoparticles (F-AuNPs) in *P. aeruginosa*, where the researchers proposed that ROS generated by the nanoparticles hindered biofilm formation.

Pyocyanin, a virulence factor associated with antibiotic resistance in *P. aeruginosa*, serves as a protective barrier by impeding the penetration of antibiotics through the bacterial cell wall and cytoplasmic membrane. As a member of the phenazine group, pyocyanin generates reactive oxygen species (ROS) that directly damage DNA. Additionally, pyocyanin acts as a cellular signal in eukaryotic cells, influencing gene expression and altering cellular responses. Elevated concentrations of pyocyanin in the lungs of individuals with chronic cystic fibrosis infections can impair epithelial cell function and dampen the immune response (Das et al. 2013). Notably, the treatment of *P. aeruginosa* strains with Sm_2O_3NPs resulted in a significant reduction in pyocyanin production compared to the control group. This decrease in pyocyanin production may be attributed to the interference of Sm_2O_3NPs with the two quorum sensing pathways (rhl and pqs) responsible for regulating pyocyanin production (Chang et al. 2014).

The potential mechanism behind this reduction in virulence factor production could be attributed to the quorum-quenching effect of nanoparticles. These findings align with a prior study conducted by Ali et al. (2020), which observed significant inhibition of pyocyanin and protease production in *P. aeruginosa* isolates treated with ZnONPs. The authors suggested that the presence of nanoparticles inside the bacterial cells disrupts pathogenic mechanisms and the synthesis of virulence factors.

Conclusion

While previous studies have primarily focused on the development of samarium-doped nanoparticles for medical applications like orthopedics and dentistry, our work stands out as we synthesized pure Sm_2O_3NPs on a laboratory scale and specifically investigated their impact on the virulence factors of pathogenic *P. aeruginosa*. Given the significant antibacterial potential demonstrated by these synthesized nanoparticles, our research serves as a crucial stepping stone towards conducting future studies on these nanoparticles and the development of a novel class of antibacterial agents.

CONFLICT OF INTEREST: No conflict of interest declared.

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