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Vancomycin-coated cerium oxide nanoparticles: synthesis, characterization and in-vitro antibacterial evaluation

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Abstract

Antibacterial nano-platforms have been established in response to the growing incidence of bacterial resistance to traditional antibiotics guaranteeing an appealing approach to tackle this resistance. The objective of this research is to improve vancomycin's antibacterial activity as a potent nano-platform against a variety of resistant bacteria. Cerium oxide nanoparticles (CeO_2NPs) and cerium oxide nanoparticles functionalized with vancomycin (CeO_2-VanNPs) were synthesized using the hydroxide-mediated method. Both CeO_2NPs and CeO_2-VanNPs were properly synthesized and exhibited particle sizes of 2.5 ± 0.23 and 5 ± 0.4 nm, along with hydrodynamic size of 31.01 ±2.4 and 151.5 ±3.2 nm, respectively. The zeta potentials for CeO_2NPs and CeO_2-VanNPs were measured to show +28.8 and +10.7 mV, respectively indicating the stability of the synthesized nanoparticles. The in-vitro antibacterial activity of vancomycin, CeO_2-NPs and CeO_2-VanNPs against clinically isolated gram-negative (Escherichia coli) and gram-positive (Staphylococcus aureus) bacteria was investigated. The combination of CeO_2-NPs and vancomycin in a single nanostructure showed a synergistic effect in inhibiting bacterial growth, where Staphylococcus aureus and E. coli displayed larger inhibition zones of 39 ±21 mm and 29 ±24 mm, respectively as compared to vancomycin only. This enhanced antibacterial activity indicates the potential of CeO_2-VanNPs as promising alternative antibiotics for combating vancomycin-resistant bacteria.

Keywords: Vancomycin, antibacterial activity, nanomaterial, cerium nanoparticles and synergistic antibacterial

1. Introduction

Staphylococcus aureus is a gram-positive bacterium that causes a wide variety of clinical diseases [1]. It has been recognized as a prominent human opportunistic pathogen since its discovery in the 1880s, and it can cause a variety of diseases, from minor skin infections to severe bacteremia and necrotizing pneumonia [2]. The emergence of multi-drug-resistant strains, like MRSA (Methicillin-Resistant Staphylococcus aureus), makes treatment difficult [3]. Additionally, Escherichia coli, commonly known as E. coli, is a type of bacteria that is found in the intestines of humans and animals [4]. While most strains of E. coli are harmless, some can cause serious diseases. Numerous strains of E. coli are known to cause a wide range of symptoms, from mild, self-limiting gastroenteritis to renal failure and septic shock [5]. E. coli is more likely to resist host defenses and develop resistance to common antibiotics due to its virulence [6].

Many dangerous bacterial infections have been successfully treated with antibiotics. However, recent overuse has led to significant drug resistance in bacteria, including resistance to multi-

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ple drugs [7]. Gram-positive bacteria were treated with vancomycin (glycopeptide antibiotic) for the first time in clinical settings in 1958 [8]. Vancomycin's bactericidal action was long believed to be immune to resistance because it binds to the bacterial cell membrane instead of a protein target like other antibiotics [9]. Vancomycin's efficacy in treating infections is challenged by two emerging resistance mechanisms, each involving a complex multi-enzyme pathway, and now prevalent in pathogenic species. According to preliminary studies, S. aureus strains, particularly those resistant to methicillin, can withstand vancomycin [10, 11]. Gram-negative bacteria (E. coli) are normally resistant to vancomycin which can not significantly penetrate the outer membrane [12]. The looking for alternative emerging strategies to overcome this resistance without causing additional side effects was inspired by the growing resistance to vancomycin.

Advances in nanoscience and nanotechnologies across several scientific fields have made life easier these days. Nanoparticles like cerium, silver, cellulose, titanium, aluminum, iron, manganese, gold, or their mixtures with antibiotics are presently being researched [13, 14]. Nanoparticles have shown promise as a potential alternative to traditional antibiotics. Their small size allows them to penetrate bacterial cell walls more effectively, leading to improved antibacterial activity. Additionally, nanoparticles can be engineered to target specific pathogens, reducing the risk of antibiotic resistance. Cerium nanoparticles (CeO2-NPs) show great promise in a variety of applications, particularly in the biomedical field. It has been discovered that CeO_2NPs' intrinsic capacity to display varying oxidation states significantly reduces the growth of a wide range of bacterial strains [15]. CeO_2-NPs exhibited antibacterial activity against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, suggesting their potential use in combating bacterial infections [16, 17]. The main goal of this study was to combine vancomycin and CeO_2NPs in a single basic structure to increase the antibacterial efficacy of the two compounds. This would demonstrate an efficient synergistic action to eradicate the antibiotic-resistant bacterial strains linked to both Gram-positive and Gram-negative bacteria.

2. Materials and methods

2.1. Chemicals

Vancomycin hydrochloride ($C_{66}H_{75}C_{l2}N_9O_{24}$• HCl, M.Wt.1449.25, 99.9%), cerium nitrate hexahydrate ($Ce(NO_3)_3 \cdot 6\ H_2O$, M.Wt. 434.22, 99.0%), sodium hydroxide (NaOH, M.Wt.40.0, >98%), Mueller-Hinton broth and Tryptic Soy Broth (TSA) were purchased from Sigma-Aldrich Company (Darmstadt, Germany). To avoid photochemical reactions, deionized water was used to prepare all of the solutions. The glassware was thoroughly cleaned with a fresh HNO_3/HCl (3:1 v/v) solution, thoroughly rinsed with double-distilled water, and dried before being used.

2.2. Isolation and identification of bacteria

Clinically isolated gram-negative (Escherichia coli) and gram-positive (Staphylococcus aureus) bacterial strains were isolated from radioactive lab on a Petri dish having Tryptic Soy agr (TSA) medium and left to incubate for 24 hours at 37 °C in an incubator. A parallel agar plate was used to further cultivate the pure colonies that were visible. Subsequently, they were biochemically identified based on biochemical reactions and the microorganism's use of nutrients using the automation system VITEK 2 (serial number VK2C6280) [18, 19].

2.3. Synthesis of CeO2-NPs

The hydroxide-mediated approach was used for the synthesis of CeO_2-NPs [20]. In two different 250 mL beakers, a 0.1 M solution of cerium nitrate hexahydrate and a 0.3 M solution of sodium hydroxide were made using deionized water. The cerium hexahydrate solution was stirred continuously while sodium hydroxide was added dropwise until precipitation formed. Following that, the precipitation will undergo a 15 minute centrifugation at 8000 rpm. The precipitate was rinsed three times with water and once more with ethanol. At 200 °C, the precipitate dried in an oven. The dried precipitate was finally crushed into fine particles using a mortar and pestle.

2.4. Synthesis of CeO2-VanNPs

The hydroxide-assisted method was also used to synthesize CeO_2-VanNPs with minor adjustments. In a 250 mL beaker, a 0.1 M solution of cerium nitrate hexahydrate and 0.03 M solution of vancomycin were stirred together for 2 h. Next, dropwise additions of 0.3 M sodium hydroxide were made until precipitation developed. Then, the mixture was centrifuged for 15 min at 8000 rpm. The precipitate was exposed to three cycles of washing with water and one with ethanol. Then, it was oven-dried at 60 °C before being ground into fine particles.

2.5. Nanoparticles characterization

FEI CM20 microscope images obtained at a 200 kV voltage were utilized to measure the particle size using the transmission electron microscope (TEM) (Ted Pella, Redding, CA, USA). The average hydrodynamic particle diameter in solution was determined using dynamic light scattering (DLS) equipment (Malvern Zetasizer Nano ZS 90, ATA Scientific Pty. Ltd., Australia) with a 90-degree scattering optic. A photocorrelation spectrometer (PCS) was used to determine the zeta potential. The Perkin Elmer Spectrum 100 Spectrometer was utilized to acquire the FT-IR spectra, ensuring the production of CeO2-VanNPs and CeO2-NPs.

2.6. Antibacterial activity

The antibacterial activity of vancomycin, CeO_2-NPs and CeO_2-VanNPs was assessed in-vitro against clinically isolated gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacterial strains using a modified agar-well disc diffusion method [21]. prepared suspension of 0.5 ml activated bacteria contained 1.5×10^5 CFU/ml, which were then spread onto agar plates [22]. A range of 5 mm filter papers impregnated with the tested antibacterial agents (vancomycin (1.4mg/ml), CeO_2-NPs (1.4mg/ml) and CeO_2-VanNPs(1.4mg/ml)) were gently placed over the inoculated agar plates and incubated at 37°C. After a 24 hour incubation period, the antibacterial activities of the prepared samples were determined by measuring the diameter of the clear media surrounding the filter paper, which is also referred to as the zone of inhibition [23]. To determine the mean value of the zone of inhibition, antibacterial activity was tested three times.

3. Result and discussion

3.1. Isolation and identification of bacteria

Gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacterial strains were identified using the automated VITEK 2 system (Fig. 1). Biochemical reactions are the foundation for identification in the automated VITEK 2 system [24]. These reactions are determined by the microorganism's use of nutrients and biochemical reactions. To pass the test, a certain amount of growth must be obtained within a predefined growth period of 18–70 h [25]. The system detects alterations in metabolism and bacterial growth in the microwells of thin plastic cards using fluorescence-based technology [18].

3.2. Synthesis and characterization of CeO_2-NPs

Cerium oxide nanoparticles (CeO_2-NPs) were synthesized using a simple and economical hydroxide-mediated method, using cerium nitrate hexahydrate as a precursor [26]. After dissolving the cerium nitrate hexahydrate in deionized water to facilitate the change from the Ce^{3+} to the Ce^{4+} state, the mixture was combined with sodium hydroxide to create yellowish-white nanoparticles [27]. CeO_2-NPs were assessed for particles size using TEM. CeO_2-NPs showed a small particle size of 2.5 ± 0.2 nm (Fig. 2A). CeO_2-NPs showed a hydrodynamic particle size of 31.01 ± 2.4 nm (Fig. 2B). The hydrodynamic size of CeO_2-NPs, measured by DLS, was greater than the size observed by TEM. This variation might be due to the wet and expanded hydrodynamic diameter of the CeO_2-NPs suspension, whereas TEM displays a dry and contracted configuration of CeO_2-NPs [28]. The zeta potential was measured using PCS, revealing a positive +28.8 mV for CeO_2-NPs, indicating a stable suspension (Fig. 2C) [29]. FT-IR spectroscopy was utilized to confirm the formation of CeO_2-NPs within the 4000–400 cm^{-1} wavenumber range, as depicted in Figure 2D. The FTIR analysis revealed the presence of CeO_2 in the sample; the strong

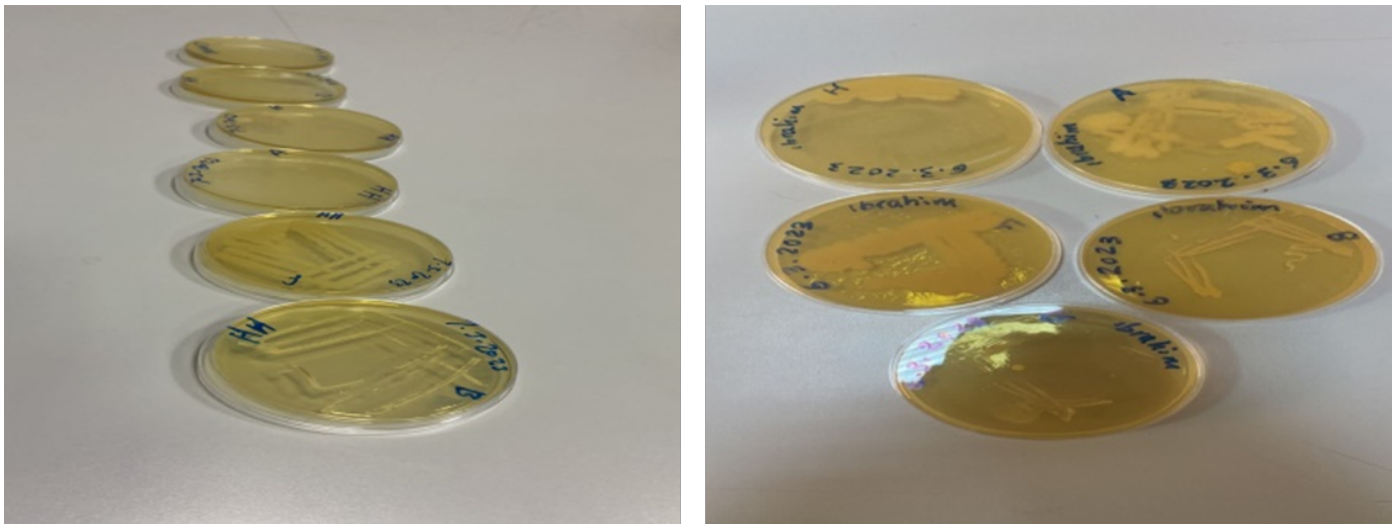

Figure 1: Photosof agar plates demonstrating bacterial isolates purified colonies

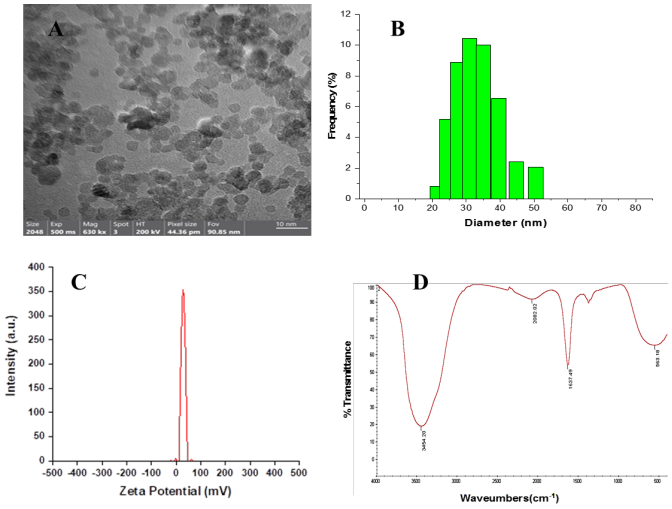

Figure 2: Physicochemical characterization of CeO_2-NPs (A) TEM image, (B) Hydrodynamic size, (C) The zeta potential and (D) FTIR spectra

band at 563 cm^{-1} was conclusively linked to the CeO_2 nanoparticles [30]. The peaks at 3454 cm^{-1} and 1637 cm^{-1} correspond to O-H stretching and bending modes in water molecules [31]. The FTIR study revealed that the trace quantity of nitrate was excluded during the washing step, since there was no peak for the N-O stretch.

3.3. Synthesis and characterization of CeO2-VanNPs

The hydroxide-mediated method was successfully used to synthesize brownish-yellow cerium oxide nanoparticles coated with vancomycin (CeO_2-VanNPs) [27]. CeO_2-VanNPs' particle size was determined through TEM analysis, revealing a diameter of 5 ± 0.4 nm, as illustrated in Figure 3A. CeO_2-VanNPs were reported by DLS to have a hydrodynamic particle size of 151.5 ± 3.2 nm (Fig. 3B). PCS was utilized for zeta potential determination, revealing a positive potential of +10.7 mV for CeO_2-VanNPs (Fig. 3C), indicating the attainment of a stable suspension. The zeta potential of CeO_2-NPs was +28.8, while CeO_2-VanNPs was +10.7, suggesting vancomycin accumulation on cerium oxide nanoparticles and the existence of numerous hydroxyl groups [32]. This led to charge equalization, reducing the reading to +10.7. FT-IR spectroscopy was used to verify the proper functionalization of CeO_2-VanNPs in the 4000–400 cm^{-1} wavenumber region, shown in Figure 3D. FT-IR spectroscopy of CeO_2-VanNPs showed intense absorption peaks around 482, 669 and 849 cm^{-1}. these peaks were following the bands of CeO_2-NPs with oxygen from the hydroxyl groups of vancomycin [33]. The broad band at 3423 cm^{-1} was due to O-H stretching, while the band at 2924 cm^{-1} was corresponding to C-H stretching. The stretching vibration of C=O and the bending vibration of O-H of vancomycin were noticed at 1630 and 1462 cm^{-1}, respectively. Herein, the FT-IR spectra demonstrate the presence of van der Waals force associations between CeO_2-NPs and vancomycin [34].

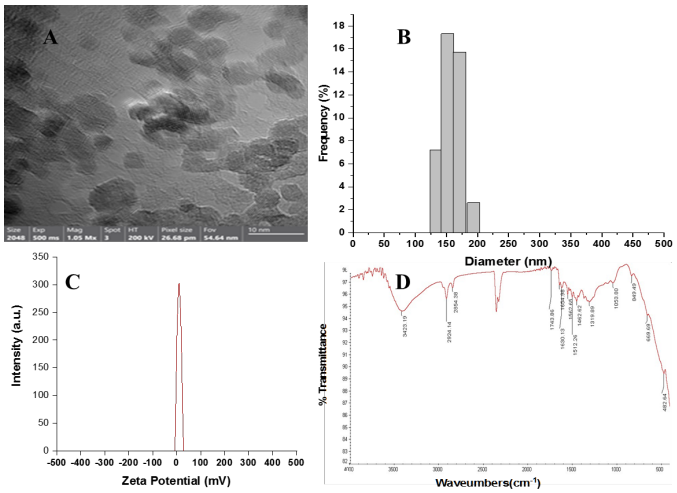

Figure 3: Physicochemicalcharacterization of CeO_2-VanNPs (A) TEM image, (B) Hydrodynamic size, (C) The zeta potential and (D) FTIR spectra

3.4. Antibacterial activity

The antibacterial activity of vancomycin, CeO_2-NPs and CeO_2-VanNPs against the isolated bacterial strains was assessed, as shown in Fig. 4. The results showed that Staphylococcus aureus had inhibition zones measuring 30 ± 2 mm, indicating that it was sensitive to free vancomycin. Nevertheless, E. coli displayed resistance to free vancomycin, with an inhibition zone of 10 ± 0.2 which is consistent with other studies (Table 1) [35]. Furtherly, Staphylococcus aureus was more sensitive to the newly prepared CeO_2-VanNPs than free vancomycin as indicated by its inhibition zones of 39 ± 21 mm. The most interesting was showing that the vancomycin-resistant bacteria, E. coli, showed a higher degree of sensitivity to CeO_2-VanNPs with an inhibition zone of 29 ± 24 mm. The synergistic interaction between vancomycin and cerium oxide nanoparticles might be responsible for CeO_2-VanNPs' increased antibacterial activity when compared to free vancomycin. This combination probably increases the antibiotic's ability to pass through the bacterial cell wall, increasing its effectiveness against strains that are both sensitive and resistant [36–38]. According to the findings, CeO_2-VanNPs are a viable option for the development of innovative antimicrobial treatments because they can potentially overcome antibiotic resistance in specific bacterial strains.

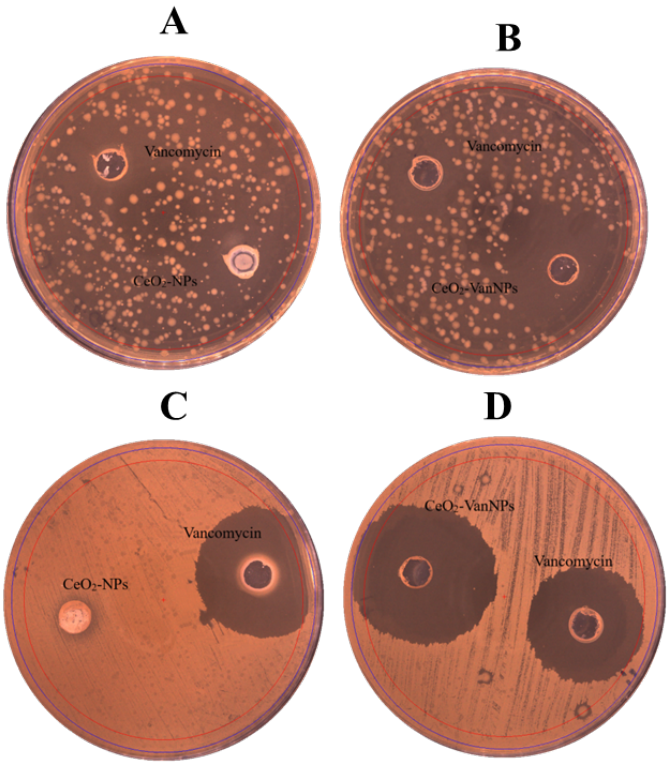

Figure 4: Photos of agar plates demonstrating vancomycin, CeO2-NPs and CeO2-VanNPs antibacterial effectiveness in terms of zone of inhibition against (A, B) Escherichia coli, (C, D) Staphylococcus aureus

Table 1: Vancomycin, CeO_2-NPs and CeO_2-VanNPs antibacterial effectiveness in terms of zone of inhibition against the clinically isolated Gram-negative Escherichia coli and Gram-positive strains Staphylococcus aureus.

Antibiotic Bacteria	zone of inhibition		
	van-comycin	CeO_2-NPs	CeO_2-VanNPs
Staphylococcus aureus	30 ± 2	5 ± 0	39 ± 21
Escherichia coli	10 ± 0.2	5 ± 0	29 ± 24

4. Conclusions

The study demonstrated the potential of employing the hydroxide-mediated technique for synthesizing CeO_2-NPs with a size of 2.5 ± 0.2 nm, as well as CeO_2-VanNPs with a size of 5 ± 0.4 nm. CeO_2-VanNPs demonstrated superior antibacterial capabilities in comparison to vancomycin when it came to strains of Staphylococcus aureus and Escherichia coli that were clinically isolated. Com-

bining cerium nanoparticles with the antibiotic vancomycin effectively treated vancomycin resistance in gram-negative bacteria like Escherichia coli. While this combined strategy shows greater effectiveness against Gram-positive bacteria, particularly Staphylococcus aureus. Generally, CeO_2-VanNPs might provide a dual-action strategy for bacterial infection treatment, especially targeting those resistant to conventional antibiotics, by simultaneously attacking and exterminating bacteria.

Conflict of Interest

No conflicts of interest were disclosed by the writers.

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