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Polyvinylpyrrolidone-loaded Zn and ZnO nanoparticles influence *Escherichia coli* isolated from urinary tract infection patients

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ABSTRACT

The biofilm formed by *Escherichia coli* contributes to pathogenesis through the intercellular contact within the biofilm, thereby facilitating bacterial proliferation and infection. This underscores the need for alternative antimicrobial agents with enhanced efficacy compared to traditional antibiotics. In this study, urinary tract infection (UTI)-derived *E. coli* isolates were assessed for susceptibility to zinc (Zn) and zinc oxide (ZnO) nanoparticles stabilised with the use of polyvinylpyrrolidone. Thirty isolates were treated with Zn and ZnO nanoparticles, and conventional polymerase chain reaction (PCR) was employed in order to detect the presence of virulence genes (*phoA*, *VTL*, and *LT1*). The phoA gene was detected in the majority of isolates, whereas the VTL and LT1 genes were not observed. Bacterial counts were higher in untreated controls than in nanoparticle-treated samples, with this difference being particularly pronounced in *phoA*-positive isolates. These findings suggest that Zn and ZnO nanoparticles hold therapeutic potential against UTI-causing *E. coli* strains.

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1. Introduction

The global prevalence of urinary tract infections (UTIs) caused by *Escherichia coli* is largely attributed to increasing antibiotic resistance[1]. Zinc oxide (ZnO) nanoparticles and zinc (Zn) have demonstrated promising antibacterial properties. ZnO is particularly effective due to its ability to generate reactive oxygen species (ROS) and disrupt bacterial

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membranes. Polyvinylpyrrolidone (PVP) serves as a stabilizing agent for these nanoparticles, enhancing dispersibility and modulating release profiles; features that make the Zn/ZnO-PVP nanocomposite suitable for treating persistent infections[2]. Pathogenic *E. coli* strains associated with UTIs require the alkaline phosphatase-encoding alkaline phosphatase (phoA) gene for survival[3]. This study investigates the bactericidal efficacy of the Zn/ZnO-PVP nanocomposite against UTI-associated *E. coli*, and examines the *phoA* expression as a potential biomarker for alternative therapeutic approaches.

2. Methodology

2.1. Sample collection

Thirty urine specimens were collected from patients with symptomatic UTIs attending the Hillah Teaching Hospital (Iraq). Diagnostic criteria included dysuria and urinary frequency. Samples were processed in order to isolate *E. coli*, which were compared with control strains not associated with UTIs. Clean-catch midstream samples were used for microbiological analysis. The urine microbiome was evaluated in order to confirm the *E. coli* presence and assess antibiotic resistance.

2.2. Primer preparation

Primers targeting the phoA, verotoxin (VT1), and heat-labile toxin (LT1) genes were designed and synthesized by an industrial oligonucleotide supplier following standard protocols. Bioinformatics validation confirmed primer specificity to *E. coli* genomic sequences. Upon receipt, primers were reconstituted to 100 μM in nuclease-free water and were subsequently diluted to 10 μM for polymerase chain reaction (PCR) assays. Sequence alignments demonstrated exclusive (to *E. coli*) target matches[4,5].

2.3. PCR assay

Multiplex PCR was performed in order to amplify the phoA, VT1, and LT1 genes from *E. coli* isolates. Each 50-μL reaction contained 10 μL of PCR buffer, 1 μL of each primer (forward and reverse: *phoA*, 5’-GTGACAAAAGCCCGGACACCATAAATGCCT-3’, 5’-TACACTGTCATTACGTTGCGGATTTGGCGT-3’; *VT1*, 5’-GAACGAAATAATTTATATGTG-3’, 5’-CCTGATGATGGCAATTCAGTA-3’; *LT1*, 5’-TTACGGCGTTACTATCCTCTCTA-3’, 5’-GGTCTCGGTCAGATATGTGATTC-3’), 0.5 μL of DNA polymerase, 1–5 μL of template DNA, and nuclease-free water. Thermal cycling involved an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation (94°C, 1 min), annealing (56°C, 1 min), and extension (72°C, 1 min), with a final extension at 72°C for 10 min. Agarose gel electrophoresis confirmed amplification of the target genes (*phoA*, *VT1*, and *LT1*)[6].

2.4. Nanoparticle preparation

Nanoparticles were synthesized *via* laser ablation in liquids using 99%+ purity Zn targets sourced from Sigma-Aldrich (Germany). A 1% PVP solution in deionised water was prepared as a stabilizing medium. Prior to ablation, Zn targets were cleansed with ethanol and deionised water. Ablation was performed using a switched Nd:YAG laser at 1064 nm, 5-nsec pulse width, 6-Hz repetition rate, and 160 mJ per pulse[7].

2.5. Transmission electron microscopy

For the precise morphological characterization of core/shell Zn/ZnO nanoparticles, transmission electron microscopy (TEM) was conducted using the Zeiss EM10C system operated at 100 kV. This upgraded method was selected over previous field emission scanning electron microscopy (FE-SEM) for improved dimensional accuracy.

3. Results and Discussion

Out of 30 *E. coli* isolates, 19 were found to be *phoA*-positive; VT1 and LT1 genes were not detected (Figure 1). The undertaken UV-Vis spectral analysis revealed distinct absorption edges at 400 nm and 300 nm for Zn and ZnO nanoparticles, respectively, consistent with electronic band gap characteristics. The PVP polymer exhibited a peak at 279 nm, which is indicative of func-

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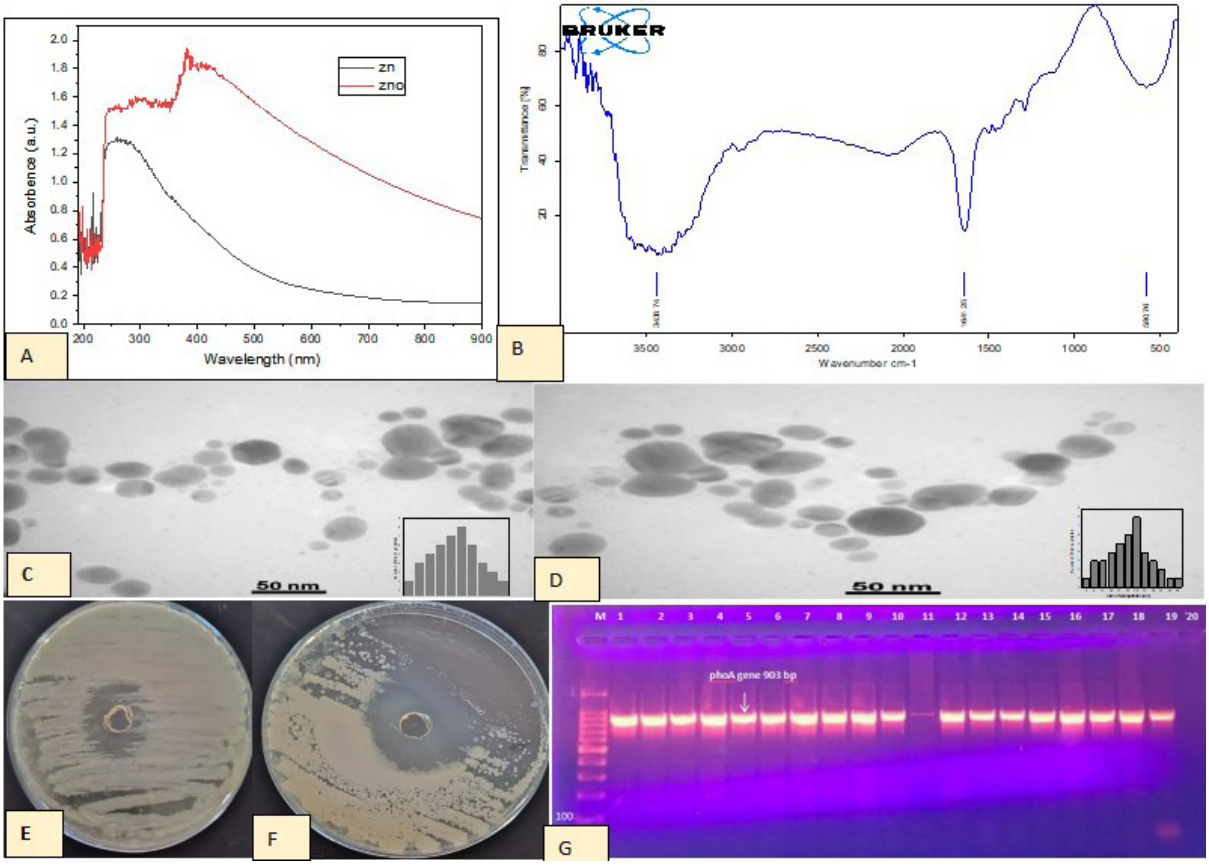

Figure 1. *(A): UV-Vis spectra of zinc (Zn) and zinc oxide (ZnO) nanoparticles stabilized with a polyvinylpyrrolidone (PVP) polymer. (B): Fourier transform infrared spectroscopy (FTIR) spectra indicating absorption peaks at 3436, 1641, and 580 cm^{-1}. (C): Transmission electron microscopy (TEM) micrograph of Zn nanoparticles. (D): TEM micrograph of ZnO nanoparticles. (E–F): Zones of inhibition and minimum inhibitory concentrations (MIC) of ZnO (15 mm) and Zn (30 mm) nanoparticles against E. coli on Mueller-Hinton agar. (G): Gel electrophoresis of the 903 bp phoA amplicon from urinary tract infection-derived E. coli; 1.5% agarose gel run at 75 V for 45 min; lanes 1–19 show positive amplification; M denotes the molecular marker.*

tional groups. Fourier-transform infrared spectroscopy (FTIR) confirmed the chemical identity of the synthesized nanoparticles: absorption bands at 3400 cm^{-1} corresponded to O–H stretching (hydroxyl groups), at 1630 cm^{-1} to C=O stretching (carbonyl or amide groups), and at 590 cm^{-1} to metal–oxygen bonds of Zn/ZnO (Figure 1).

TEM imaging illustrated uniform spherical morphology in Zn and ZnO nanoparticles (Figure 1), with PVP stabilization limiting particle sizes to 30 nm and 28 nm, respectively. This controlled nanoparticle architecture enhances their biomedical utility. Given that biofilms contribute significantly to *E. coli* resistance and survival, chemical agents and nanoparticles such as those synthesized in this study are critical in disrupting biofilm formation[8].

In the undertaken direct bacterial inhibition assays, Zn nanoparticles exhibited superior antibacterial activity compared to ZnO nanoparticles. Mechanistically, nanoparticle-induced ROS production and Zn^{2+} ion release underpin bactericidal effects. The phoA gene product (alkaline phosphatase) facilitates phosphorus mineralization, but also contributes to virulence *via* the hydrolysis of phosphate monoesters[9].

4. Conclusion

The herein assessed Zn nanoparticles effectively inhibited *E. coli* proliferation, indicating their potential as safe, non-antibiotic alternatives in UTI management. This is particularly relevant in the context of antimicrobial resistance driven by genetic mutations.

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Conflicts of interest

None exist.

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