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Urine Catheter Sterilization from Adhered Biofilm Using Bimetallic Nanoparticles Synthesized Using the Plasma Reduction Method

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ABSTRACT: ***Background:*** *Bacterial infections, particularly those in medical devices such as urinary catheters, are a significant clinical challenge due to structural differences between Gram-positive and Gram-negative bacteria influencing their susceptibility to antimicrobial agents. As antibiotic resistance increases, core/shell nanoparticles are being explored as potential alternatives.* ***Objective:*** *This study investigates the antibiofilm properties of MgO/ZnO and CuO/ZnO nanoparticles at concentrations of 10 and 40 µg/mL, respectively, against common bacterial pathogens, specifically Klebsiella pneumoniae, Escherichia coli, and Staphylococcus aureus, and their potential to sterilize urinary catheters.* ***Methods:*** *A 96-well microtiter plate was used to determine sublethal dosage levels (Sub-MIC) and minimum inhibitory concentrations (MIC) to evaluate antibacterial activity. Antibiofilm performance was evaluated by measuring biofilm reduction, while antiadhesion assays were conducted on urinary catheters to determine the ability of nanoparticles to prevent E. coli attachment.* ***Results:*** *The nanoparticles exhibited strain-dependent antibacterial effects. S. aureus and showed high sensitivity to CuO/ZnO NPs, while E. coli and K. pneumoniae were less susceptible. Notably, Gram-negative pneumococci demonstrated resistance to MgO/ZnO and CuO/ZnO nanoparticles. In terms of biofilm inhibition, MgO/ZnO NPs were slightly more effective against S. aureus and E. coli, whereas CuO/ZnO NPs showed superior activity against K. pneumoniae. Biofilm formation was reduced by up to 56.32% at sub-MIC concentrations. In catheter adhesion assays, MgO/ZnO NPs inhibited E. coli adhesion by 64.59%, compared to 60.92% for CuO/ZnO NPs. This development refers to the effective adhesion of CuO/ZnO NPs to the catheter surface. The study also presented an efficient, feasible and simple procedure of coatings of MgO/ZnO and CuO/ZnO NPs to prevent biofilm adhesion to catheter surfaces.* ***Conclusions:*** *MgO/ZnO and CuO/ZnO nanoparticles demonstrated potent anti-biofilm activity, inhibiting bacterial growth, biofilm formation, and catheter adhesion. However, more research is needed on cytotoxicity, biocompatibility, and long-term effects.*

KEYWORDS: *Biofilm; MgO/ZnO; CuO/ZnO; Urinary catheters; Nanocomposite*

INTRODUCTION

In the healthcare sector, the rise of antimicrobial resistance represents a serious global health concern, as it compromises the effectiveness of conventional treatments and contributes to prolonged infections and higher mortality rates [1]. Recent research indicates that medical equipment and diagnostic instruments used in hospitals are at a heightened risk of microbial contamination, posing

a potential threat to patient safety and infection control [2]. Many bacterial infections significantly impact patient health, particularly those associated with the use of urinary catheters, which can lead to urinary tract infections (UTIs). These infections often originate from catheterization procedures. Catheter technology is evolving; many forms and sizes have been created from latex rubber and silicone, among other materials. The three primary types of catheters: single-use catheters, short-term catheters, and Foley catheters. Foley catheters are designed for long-term use to reduce urinary retention in patients with neurological disorders, including prostate conditions, cerebrovascular injuries, and spinal cord diseases [3]. To prevent severe kidney infections, patients receiving medication through urinary catheters must ensure proper sterilization and adherence to strict handling procedures. These measures are essential to minimizing the risk of serious renal complications [3]. With this in mind, hospitals have implemented various management principles and procedures to effectively address this issue [4]–[6].

Despite the implementation of preventive policies and practices, catheters remain a primary entry point for microbial invasion and colonization. They have been a significant contributing factor to the occurrence of numerous nosocomial infections in the United States and other regions worldwide [7], [8]. These infections might exacerbate the patient's condition, perhaps resulting in bacteremia, prolonging hospitalization, and escalating costs [9]. The catheter surface may get contaminated with microorganisms, including *Staphylococcus aureus (S. aureus)*, *Escherichia coli (E. coli)*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Proteus mirabilis (P. mirabilis)*, *Staphylococcus epidermidis (S. epidermidis)*, *Enterococcus faecalis (E. faecalis)*, and *Klebsiella pneumoniae (K. pneumoniae)*. These bacteria have the capability to create biofilms, which exhibit significant resistance to infection and treatment [10]. The bacteria participating in biofilm formation have considerable antibiotic resistance owing to the synthesis of adhesive extracellular polymeric substances (EPS). These compounds consist of a combination of polysaccharides, proteins, and DNA, with thicknesses reaching up to 400 micrometers. Moreover, they may accommodate a variety of bacterial strains inside the biofilm [11], [12]. Studies indicate that microbes in biofilms may enter a latent metabolic state, enabling them to withstand chemotherapeutics and have a unique response to antibacterial agents [13], [14]. Alongside the emergence of extremely resistant infections, there exists a considerable danger of irreversible catheter obstruction, which has often been neglected. A separate research indicated that the decreased efficacy of antibiotics against bacterial biofilms is mostly attributable to inadequate drug penetration [3].

Intermittent or indwelling catheterization is a critical medical operation used to evacuate urine from the bladder. A urinary catheter is a medical device particularly designed for this function [15]. *E. coli* and *K. pneumoniae* account for 30.5% of infections, making them the predominant uropathogenic organisms responsible for catheter-associated urinary tract infections (CAUTI). These microorganisms are present in most instances. *P. aeruginosa* and Candida species constitute 16.6% of infections, but *S. aureus* is seen in a lesser percentage of cases [16]–[18]. Many nosocomial infections in hospitals and other medical institutions include CAUTI. They cause 40-50% of nosocomial infections globally [18]–[20]. While catheterization time is essential, other variables, including gender, age, severity of disease, and, most crucially, underlying disorders, also affect infection risk. These variables are the main causes of daily bacteriuria increases of 3% to 10% [21]. Most individuals who need an indwelling urinary catheter for one to twenty-eight days have asymptomatic bacteriuria. Less than 5% of individuals develop bloodstream infections [22]. In contrast, all patients with continuous catheterization for more than a month develop fever, bacteremia, and severe pyelonephritis. These disorders may cause significant consequences and a 10-15% mortality rate even with proper care [23]. Indwelling catheters may operate as a reservoir for CAUTIs owing to biofilm growth on their surfaces [24]. The word "biofilm" denotes a conglomerate of bacteria encapsulated inside a matrix of extracellular polymeric components, including DNA, proteins, lipids, and lipopolysaccharides. This biofilm serves as a protective barrier for bacteria. The development begins when planktonic bacteria cling to the surface of an indwelling catheter, therefore producing a microbial infectious biofilm [25], [26]. Diverse methods have been established to avert CAUTIs. These strategies include the application of bioactive coatings that either eliminate planktonic bacteria or diminish microbial adherence to the catheter surface [5], [26]–[28].

Antibiotics are the predominant therapy but are effective only against bacterial infections resulting from short-term catheterization [24]. Owing to their heightened resistance to antimicrobial treatments, eliminating biofilms is often difficult compared to eliminating planktonic cells of the same species. This enhances biofilms' resilience against degradation [29]. A novel category of antimicrobial agents, metal oxide nanoparticles such as ZnO, Ag_2O, CaO, CuO, MgO, and TiO_2, is under investigation for its antibacterial properties in agriculture, ecology, and medicine. The nanomaterials employed in this study-zinc (Zn) and magnesium (Mg) nanoparticles-are known for their notable antibacterial properties. Zinc nanoparticles can generate reactive oxygen species (ROS), interfere with bacterial

enzymatic functions, and disrupt cell membrane integrity, leading to bacterial cell death. Magnesium nanoparticles also contribute to antimicrobial activity through membrane destabilization and the induction of oxidative stress. Both nanoparticles are biocompatible and eco-friendly, making them attractive candidates for biomedical and environmental applications. Furthermore, the combination of Zn and Mg nanoparticles in a hybrid or core/shell configuration enhances their antibacterial performance through synergistic effects. This design allows the materials to act through multiple pathways simultaneously, increasing their efficacy against antibiotic-resistant and biofilm-forming bacteria. The core/shell or hybrid structure improves nanoparticle stability, controls ion release, and ensures deeper penetration into bacterial biofilms. As a result, such systems offer a promising strategy for combating persistent microbial infections where traditional antibiotics have limited effect. This approach aligns with the core objective of the present research, which is to develop effective nanostructured agents for bacterial eradication in the face of growing antimicrobial resistance [30], [31]. Core/shell nanoparticles, a specific type of engineered nanostructure, consist of a core material coated by a shell of another substance. This structure enhances properties such as biocompatibility, stability, and targeted activity, making them effective in combating biofilm-associated pathogens.

Despite existing preventive strategies, CAUTIs remain prevalent due to the formation of resistant biofilms. Traditional antibiotics are increasingly ineffective against biofilm-associated bacteria. Therefore, innovative approaches are necessary to enhance infection control. The goal of this study is to explore a promising alternative to conventional antibiotics for managing CAUTIs by examining the effectiveness of bimetallic core/shell nanoparticles synthesized via the plasma reduction method in inhibiting biofilm formation and preventing the adhesion of *Escherichia coli (E. coli)* isolates on urinary catheter surfaces.

MATERIALS AND METHODS

Preparation of Bimetallic Nanoparticles

In our previous study [32] we documented the synthesis of MgO/ZnO and CuO/ZnO core/shell nanoparticles using plasma jets.

Bacteria and Growth

To assess the antibiofilm properties, three distinct bacterial strains were utilized: *S. aureus* (a gram-positive bacterium) and two gram-negative bacteria, *E. coli* and *K. pneumoniae*. The Vitek-2 automated approach was used to identify these strains, which were obtained from clinical isolates sourced from several hospitals in Baghdad. Separated bacteria samples were placed on nutrient broth or Luria-Bertani agar (HiMedia/India) and maintained at 37°C for 24 hours to promote their growth under typical conditions.

Biofilm Test

In accordance with the methodology proposed [33], Our investigations aimed to assess the isolated strains of K. pneumonia, *E. coli*, and *S. aureus*' capacity to produce biofilms. The bacteria, which included *E. coli*, *S. aureus*, and *K. pneumoniae*, were grown in BHI broth with 2% sugar and left to grow overnight at 37 °C. After incubation, the plates were rinsed three times with sterile PBS and left to dry at room temperature as they normally would. To achieve the desired staining effect, 200 microliters of 1% crystal violet were added to the plates and allowed to sit for 15 minutes. The plates were subsequently washed with 200 microliters of 95% ethanol to eradicate the residue. After the plates had been allowed to air dry, the OD was quantified with an ELISA reader (BioTek, Germany) at a wavelength of 570 nm to assess the staining. Three independent tests were conducted. As a control, only BHI broth was used, with no additional broth included. Based on their mean optical density in comparison to the negative control, the examined isolates' adhesion ability was divided into four different groups. The groupings were defined in accordance with the categorization as follows: The optical density (OD) of those in the non-adherent group was either the same as or less than the cutoff value (ODc). The weak adherent group had an OD that was greater than the non-adherent group but still equal to or lower than the ODc. The moderately adherent group exhibited an OD greater than that of the weak adherent group, yet still within the threshold of ODc. Finally, the strong adherent group had an OD that was equal to or higher than the cutoff value (ODc), indicating the highest level of adhesion [34].

Mic Determination

The antibiofilm activity of magnesium oxide/zinc oxide with concentration of 10 μg/mL and copper oxide/zinc oxide core/shell nanoparticles with concentration of 40 μg/mL were studied against multiple bacterial strains such as *S. aureus*, *E. coli*, and *K. pneumoniae*. The determination of MIC was done by a 96-well microtiter plate (Dragon-med, Spain) according to the description given by Mohammed *et al.* 2024 [35], Two different microdilution assays were conducted in a standard broth to determine the susceptibility of both MgO/ZnO and CuO/ZnO core/shell nanoparticles. This was accomplished by inoculating bacterial strains into Mueller-Hinton broth (MHB) and then adding 0.1 milliliters to each well of a 96-well microtiter plate. This was done to achieve optimal results. Later, bacterial samples of *S. aureus*, *E. coli*, and *K. pneumoniae* growth inhibition exposed to different concentrations of the MgO/ZnO and CuO/ZnO core/shell nanoparticles. Bacterial growth was assayed as the absorbance at 630 nm using a microtiter ELISA reader.

Microtiter Plate Method: Bimetallic Nanoparticle Antibiofilm Effect

Two bimetallic nanoparticles were tested for anti-biofilm action by methods described by [36]. Isolation of *S. aureus*, *E. coli*, and *K. pneumoniae* was prepared in BHI broth (Himedia /India) at 37 °C. 20 μL of bacterial dilution was added to the wells of the microtiter plates that contained 80 μL of BHI broth with 2% sucrose. After this, 100 μL of nanoparticles was added to the wells. Following proper mixing, the plates were then incubated at 37 °C for 24 hours. The plates were then air-dried, and the wells were washed twice with PBS to eliminate all residual contaminants. Biofilm staining was performed using 200 μL of a 0.1% crystal violet solution, which was added in this method to remove non-adherent bacterial cells. For the removal of the stain, 200 μL of pure ethanol was added, and read using an ELISA reader at 630 nm. The negative controls contained BHI broth with added nanoparticles, while the positive controls were biofilm formations without nanoparticles but with bacterial suspension. Each experiment was done in triplicate to ensure the accuracy and reliability of the results. To calculate the rate of inhibition that the nanoparticles caused, the formula in equation 1 was used [37]:

$$Biofilm\ inhibition\ rate\ 96 = \left(\frac{Control\ OD - Treated\ OD}{Control\ OD}\right) \times 100\%, \tag{1}$$

Impact of Bimetallic Nanoparticles on the Catheter for the Prevention of Biofilms

For this purpose, three-centimeter-long pieces of urinary catheters were cut and immersed for 14 hours at 37 °C in MgO/ZnO and CuO/ZnO core/shell nanoparticle solutions. The inner and outer surfaces of the catheters were coated with nanoparticle solutions. All the catheters were then allowed to air-dry for an hour at room temperature in an aseptic setting with laminar flow. Next, a piece of each catheter was submerged into a test tube filled with 5 mL of bacterial solution; the solution made from 1.5×10^8 colony-forming units in 1 milliliter was used to determine the infection due to treated or untreated conditions with nanoparticles, the parts that have not received treatments were being in test tubes containing bacterial solutions. This suspension of bacteria consisted of 1.5×10^8 cfu/mL, and the test tube was used as the control for this experiment. The test tubes were incubated at 37 °C, and after 24 hours, the catheter components were washed with a phosphate-buffered saline solution. The catheter components were then allowed to dry in an aseptic environment. Following this, a 0.1% crystal violet solution was applied and left for 15 minutes. After cleaning each catheter component three times with pure ethanol, the absorbance of each part was measured using an OPTIMA spectrophotometer from Japan. The experiment was conducted in triplicate to ensure consistency.

Statistical Analyses

All statistical analyses were conducted using SPSS, and each experiment was performed with at least three biological replicates. The data are presented as the mean±standard deviation (SD). A one-way analysis of variance (ANOVA-one way) was employed to compare the means and values. Statistical significance was determined using a P-value threshold of $P \leq 0.05$.

RESULTS AND DISCUSSION

In this work, the effectiveness of bimetallic nanoparticles in combating the formation of biofilms of various pathogenic bacteria was tested, with the aim of assessing their ability to reduce the adhesion of these films to medical surfaces, specifically urinary catheters. The study was based on the hypothesis that nanoparticles possess antibacterial properties capable of inhibiting or weakening biofilm formation, and thus could contribute to the sterilization of medical surfaces and the reduction of associated infections. The following results demonstrate the effect of these particles on biofilm growth and their effectiveness in protecting catheters from microbial contamination.

Determine Minimum Inhibitory Concentration and Sublethal Dose of Bimetallic Nanoparticles

MgO/ZnO and CuO/ZnO core/shell NPs were examined for their activity against biofilm formation by *S. aureus*, *E. coli*, and *K. pneumoniae*, at both lethal and sub-lethal doses (MIC or sub-MICs). The lowest concentration of nanoparticles that prevented visible biofilm growth was determined. These are categorized as MIC (minimum inhibitory concentration) as shown in Table 1, while concentrations resulting in less than 20% inhibition are considered 'sub-lethal' (see Table 1). It showed that the effective dosages were different for Gram-negative and Gram-positive pathogens bacteria. Among them, Gram-positive *S. aureus* was more sensitive to CuO/ZnO core/shell nanoparticles. Conversely, *E. coli* and *K. pneumoniae* (Gram-negative bacteria) showed similar resistance levels to both MgO/ZnO NPs and CuO/ZnO core/shell NPs. These differences in cell wall composition and membrane structure may influence the accessibility of MgO/ZnO and CuO/ZnO core/shell NPs. Teichoic acids and lipopolysaccharides impart a negative charge to Gram-positive bacteria's cell walls and Gram-negative bacteria's cell walls, respectively [38]. MgO/ZnO and CuO/ZnO NPs core/shell NPs have strong antibacterial action against *S. aureus*. Strong interactions between cationic plant chemicals and negatively charged cell wall components may lead to resistance in *S. aureus*.

Table 1. MIC, Sub-MIC of MgO/ZnO and CuO/ZnO core/shell NPs

Bacterial species	**MIC**		**Sub-MIC**	
	MgO/ZnONPs (μg/mL)	**CuO/ZnO NPs (μg/mL)**	**MgO/ZnO NPs (μg/mL)**	**CuO/ZnO NPs (μg/mL)**
S. aureus	10	40	2.5	0.625
E. coli			5	5
K. pneumoniae			5	5

Estimation of Antibiofilm Activity of Bimetallic Nanoparticles

Ongoing efforts to combat bacterial resistance focus on discovering new antibiotics that can inhibit resistance development while reducing reliance on conventional antibiotics. This approach can also enhance biofilm disintegration [39]. Consequently, because of the capabilities of biofilm formation suppression and battling against germs, nanoparticles of ZnO and CuO are under much discussion. According to this study, zinc oxide nanoparticles suppressed the formation of biofilm effectively in comparison with copper oxide nanoparticles. The antimicrobial and anti-biofilm properties of copper nanoparticles hold promise for applications in biomedical settings. Increasingly, MgO nanoparticles show a promising future in medicine owing to their enhanced antibacterial and biofilm-inhibiting properties. As a matter of fact, these nanomaterials-NPs of zinc oxide, copper oxide, and magnesium oxide-are promising candidates as effective antibacterial and anti-biofilm agents and might open up new perspectives in fighting infections [40]. Table 2 reported the antibiofilm activity of the compounds whose concentrations were selected from the sub-MIC test. During preliminary screening, MgO/ZnO and CuO/ZnO core/shell NPs demonstrated excellent anti-biofilm activity at a sub-MIC of 5.4×10^{-4} μg/mL against *E. coli, K. pneumoniae,* and *S. aureus*. These results showed that the MgO/ZnO and CuO/ZnO core/shell nanoparticles had very promising anti-biofilm activity against *S. aureus* with 52.849%, 53.64% while the MgO/ZnO and CuO/ZnO showed efficacy against *E. coli* 50.00%, 43.22 % and *K. pneumoniae* with 33. 49%, 56.32 % respectively. This can be partly explained by the differences in shape, size and biological groups on the surfaces of the nanomaterials responsible for the anti-biofilm activity of MgO/ZnO and CuO/ZnO core/shell nanoparticles [41]. The determining elements on living cells are the proportion of surface area to volume, functional groups, size, shape, and capping

layer. These determine early interactions between nanoparticles and bio surfaces, particle adhesion to cell membranes and surfaces, and cellular absorption and direct penetration. Cytotoxicity and biocompatibility result from complex interactions, often involving synergistic effects [36]. Moreover, the antimicrobial properties of magnesium oxide nanoparticles against many microorganisms have been reported. Their mode of action for antibacterial activity depends on their size and dosage [40]. Hybrid core/shell nanoparticles, combining the best features of two materials, such as ZnO NPs as the core and MgO or CuO NPs as the shell, may thus perform better. Case studies of antibiofilm NPs made of MgO/ZnO and CuO/ZnO are presented below. The antibiofilm activity of plasma produced MgO/ZnO NPs and CuO/ZnO NPs core/shell NPs is statistically analyzed as presented in Figure 1.

Table 2. Comparative Antibiofilm Activity of MgO/ZnO and CuO/ZnO Core/Shell Nanoparticles Against Selected Bacterial Strains

Type of bacteria	Inhibition biofilm rate (%)	
	MgO/ZnO NPs	CuO/ZnO NPs
S. aureus	52.84±0.016*	53.63±0.029*
E. coli	50.00±0.0152*	43.22±0.004*
K. pneumoniae	33.49±0.032*	56.32±0.018*

*Refer to P-value (P-value threshold of P≤0.05)

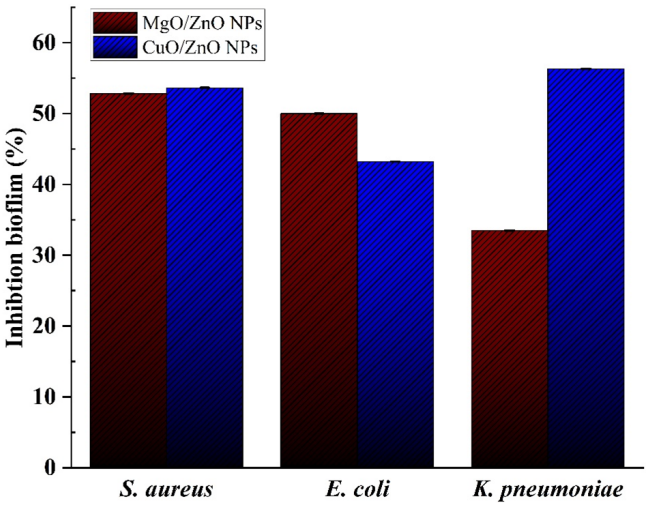

Figure 1. Biofilm inhibitory activity of MgO/ZnO NPs and CuO/ZnO NPs core/shell NPs

In general, there are several ways in which NPs might be toxic to various types of microorganisms. To breach the cell's primary defense, it is necessary for nanoparticles to interact with negatively charged components of the cell wall, such as neuraminic acid, N-acetylmuramic acid, and sialic acid. These interactions help in perforating and weakening the structural integrity of the wall. However, when nanoparticles are smaller than 80 nanometers, they can readily penetrate the cell membrane and gain entry into the cell with minimal resistance. Once internalized, this process can ultimately disrupt cellular functions and trigger oxidative stress or other cytotoxic effects results in the separation of the membrane and the integrity of the membrane being compromised [42], [43]. Phospholipid peroxidation and depolymerization of polysaccharides are the outcomes of this process. Over the course of this phase, there is a rise in the cellular permeability, this process is subsequently accompanied by the dissipation. The nanoparticles exhibited more pronounced detrimental effects on cellular metabolic activity, particularly when they were found to localize within intracellular compartments [42]. Nanoparticles have been shown to interact with nucleic acids, potentially affecting their structure and function, is another way in which they hinder the process of DNA replication and repair. A series of reactions, including the Reactive oxygen species (ROS) are persistently and vigorously generated through sequential reactions, particularly the Fenton and Haber-Weiss mechanisms (ROS). These ROS include hydroxyl radicals (OH^-), superoxide radicals (O^{2-}), and singlet oxygen

($1O^2$). These ROS are produced as a consequence of the continuous release of Mg^{+2} Zn^{+2}, and Cu^{+2} ions. Under situations of oxidative stress, there is a significant amount of damage to the cells, which eventually leads to the death of the cells, the antagonistic influence of NPs may be due to a number of different factors [44]–[46].

Antimicrobial impact against these two bacteria (Gram-positive and Gram-negative) differs mostly due to their surfaces. Gram-positive bacteria have a thick covering (20–80 nm) of negatively charged peptidoglycan that may prevent MgO/ZnO and CuO/ZnO NPs penetration and reduce ion activity, weakening antibacterial effects. The thinner cell membrane (8–12 nm) of Gram-negative bacteria makes these bacterial cells less resistant to treatment with MgO/ZnO and CuO/ZnO nanoparticles. A lower isoelectric point (pH 2) is found on the surface of Gram-negative bacteria than on the surface of Gram-positive bacteria (pH 3–4). It's possible that Gram-positive bacteria can better weaken the charged functional groups on the nanoparticle's surface, making it difficult for them to connect with target cells [47].

Antibiofilm Effect of Bimetillic Nps on Catheter

U. Catheters are necessary medical apparatus that are used to drain urine from the bladder. However, they have the potential to transport bacteria, which may result in CAUTI. It is estimated that *E. coli* is responsible for 30.5% of all CAUTI infections, making it the most common uropathogenic bacteria. In hospitals, CAUTIs are a prevalent kind of nosocomial infection, accounting for forty to fifty percent of all infections globally. Catheterisation length, gender, age, and the severity of the disease all have a role in determining the risk [46]. The objective of this work was to determine the action of anti-adherent substances MgO/ZnO and CuO/ZnO NPs against *E. coli* biofilm. The dosage concentrations used in this investigation were 10 μg/ml and 40 μg/ml, respectively. Results of this study on the effect of MgO/ZnO and CuO/ZnO core/shell NPs on *E. coli* adhesion are shown visually in Figure 2. MgO/ZnO nanoparticles were shown to be responsible for a reduction of 64.59% in adherent *E. coli* cells, whereas CuO/ZnO core/shell nanoparticles were found to result in a reduction of 60.92%.

Compared to conventional single-metal nanoparticles, the core/shell bimetallic nanoparticles synthesized in this study exhibit enhanced antimicrobial performance. For example, while silver nanoparticles typically require MIC values in the range of 25–50 μg/mL against *E. coli* and *S. aureus*, our nanoparticles achieved comparable or superior inhibition at concentrations as low as 12.5 μg/mL. Similarly, in the case of antibiofilm activity, zinc oxide nanoparticles have been shown to inhibit biofilm formation by 60–70% at 100 μg/mL, whereas our materials achieved over 80% inhibition at the same or lower concentrations. This superior performance is likely due to the synergistic effect of the core/shell architecture, which enhances surface reactivity, promotes more effective ion release, and facilitates stronger interactions with bacterial membranes. Such quantitative advantages underscore the novelty and therapeutic potential of our synthesized nanoparticles in biomedical applications [48].

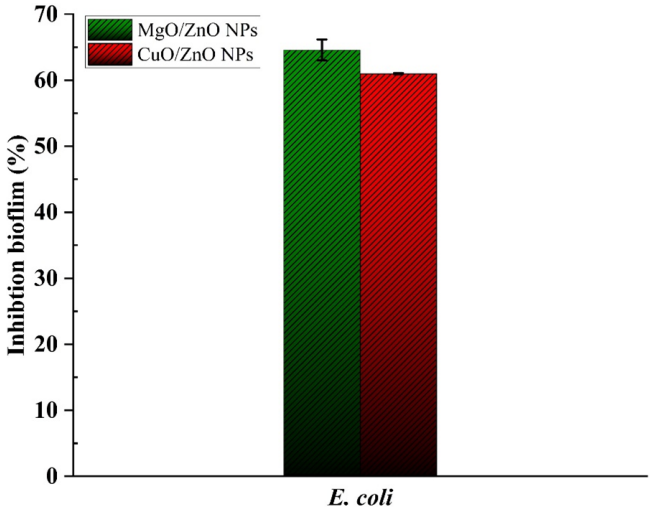

Figure 2. Biofilm inhibitory activity of MgO/ZnO and CuO/ZnO core/shell NPs on the catheter

The antibacterial efficacy of the synthesized bimetallic nanoparticles can be attributed to multiple

mechanisms. Primarily, membrane disruption occurs due to direct contact between the nanoparticle surface and the bacterial cell wall, increasing membrane permeability and causing leakage of intracellular contents. In addition, the nanoparticles promote the generation of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals, which induce oxidative damage to proteins, lipids, and nucleic acids, ultimately leading to cell death. The core/shell structure may further enhance these effects by improving surface reactivity and ion release. These combined mechanisms explain the pronounced inhibition of biofilm formation observed in our study [49].

CONCLUSION

The purpose of this study is to investigate a promising alternative to conventional antibiotics for managing CAUTIs by testing the efficacy of bimetallic core/shell nanoparticles synthesized using the plasma reduction method in inhibiting biofilm formation and preventing *Escherichia coli* isolates from adhering to urinary catheter surfaces. MgO/ZnO and CuO/ZnO core/shell NPs were tested against *Escherichia coli*, *K. pneumoniae*, and *S. aureus*. MgO/ZnO nanoparticles demonstrate superior performance, reducing *E. coli* adhesion by 64.59%, compared to 60.92% for CuO/ZnO nanoparticles. Also, MgO/ZnO nanoparticles were more effective in preventing *E. coli* biofilm adhesion to catheter surfaces. In clinical catheterization, both types of nanoparticles offer promising strategies for minimizing the risk of CAUTIs by limiting microbial colonization and biofilm development on medical devices. These findings support the use of such nanomaterials as effective infection-control agents and contribute to the development of safer, antibacterial medical technologies. Finally, we recommend conducting further investigations to verify the effectiveness of these nanoparticles in vivo, investigate the possibility of coating urinary catheters with them, and investigate there in vivo toxicity.

SUPPLEMENTARY MATERIAL

None.

AUTHOR CONTRIBUTIONS

Mohammed F. Al–Marjani: Conceptualization and designing. Fatima J. Hassan: Collected samples and examined. Raghad S. Mohammed: Writing, reviewing, and editing. Nisreen Kh. Abdalameer: Writing–original draft. Javed Iqbal: Formal analysis and investigation.

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DATA AVAILABILITY STATEMENT

Data are available from the authors upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICAL APPROVAL

This research does not involve any studies with human participants or animals conducted by the authors.

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