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ANTIBACTERIAL EFFICACY OF SILVER AND COPPER NANOPARTICLES AGAINST *E. COLI* IN CATHETER-ASSOCIATED URINARY TRACT INFECTIONS

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ABSTRACT

The rise of antibiotic-resistant *E. coli* in catheter-associated urinary tract infections necessitates the exploration of new treatment strategies. This study examined the antibacterial efficacy of silver and copper oxide nanoparticles against *E. coli* isolates obtained from CAUTIs. Antibiotic susceptibility testing demonstrated notable resistance to frequently prescribed antibiotics, underscoring the critical need for alternative treatment modalities. Silver nanoparticles, measuring approximately 6.66 nm, demonstrated potent antibacterial activity, producing inhibition zones ranging from 11 mm to 19 mm at concentrations of 1 mg/ml and 5 mg/ml, respectively. Conversely, copper oxide nanoparticles, with an average size of 39.64 nm, exhibited moderate antibacterial effects, especially at higher concentrations. The superior efficacy of AgNPs is attributed to their smaller size, larger surface area-to-volume ratio, and increased reactivity. Additionally, scanning electron microscopy and energy-dispersive X-ray spectroscopy confirmed the morphology and elemental composition of the nanoparticles. These findings indicate that AgNPs have significant potential as an alternative treatment for *E. coli*-induced CAUTIs with increasing antibiotic resistance. Nevertheless, further research is essential to refine nanoparticle formulations and investigate their genetic and molecular interactions, emphasizing the continuous effort required from the scientific community in this area.

INTRODUCTION

Urinary tract infections (UTIs) are a significant global health issue affecting approximately 150 million people annually. The incidence is notably higher in females, with a lifetime risk of 50-60%, compared to 13% in males. UPEC, or uropathogenic*E. coli*, is the primary causative agent of urinary tract infections, possessing a plethora of virulence factors that enable it to adhere to and invade the host bladder epithelium. (Terlizzi *et al.*, 2017) These factors include adhesins, toxins, and other secreted proteins that facilitate the ability of the bacterium to colonize the urinary tract and evade host defences. (Zhou *et al.*, 2023)

In cases of asymptomatic bacteriuria, UPEC establishes a quiescent intracellular reservoir within the bladder, leading to recurrent infections (Subashchandrabose& Mobley, 2015). Furthermore, UPEC has been shown to penetrate the bladder epithelium and access the bloodstream, potentially resulting in life-threatening sepsis. (Subashchandrabose& Mobley, 2015) The development of multidrug-resistant UPEC strains has emerged as a significant challenge in the treatment of UTIs, particularly those associated with indwelling catheters. The increasing prevalence of antimicrobial resistance among uropathogenshighlights the need for alternative therapeutic and preventative strategies to combat these infections. (Terlizzi *et al.*, 2017) (Clegg & Murphy, 2016) (Zhou *et al.*, 2023) (Subashchandrabose& Mobley, 2015)

Silver and copper were chosen due to their known antibacterial properties. Previous studies have shown that silver nanoparticles exhibit effective bactericidal effects against a variety of microorganisms, including the common urinary tract pathogen Escherichia coli (Khodashenas, 2015). Copper nanoparticles have also been investigated for their antimicrobial activity, but they appear to be less potent than silver nanoparticles. (Zia et al., 2018) Notably, the antimicrobial activity of nanoparticles has been observed to be strain-specific, underscoring the importance of evaluating the comparative efficacy of silver and copper nanoparticles against the specific E. coli strain associated with catheter-related urinary tract infections.

The pathogens most frequently associated with CAUTI are *E. coli*, *Proteus*, *Enterococcus*, *Pseudomonas*, *Enterobacter*, *Serratia*, and *Candida species*, which are typically acquired exogenously through manipulation of the catheter and drainage device [Soto SM. 2014]. The distinctive physicochemical properties of nanoparticles enable researchers to develop

innovative methods for targeting bacterial pathogens (Karaman *et al.*, 2017). Their diminutive size and high surface-to-volume ratio facilitate efficient interactions with bacterial cells, potentially resulting in disruption of cellular functions, membrane damage, or oxidative stress (Seil & Webster, 2012). Furthermore, the tunability of nanoparticle characteristics, such as size, shape, and surface chemistry, can facilitate the development of targeted antimicrobial strategies (Makabenta et al., 2020).

Silver and copper exhibit potent antimicrobial properties, making them promising candidates for combating catheter-associated urinary tract infections, particularly those caused by *E. coli*. The broad-spectrum antibacterial activity of Ag stems from the disruption of bacterial cell walls, interference with DNA replication, and inhibition of enzyme activity, which effectively targets a wide range of bacteria, including drug-resistant strains (Kannan *et al.*, 2021). Research has shown that Cu acts against bacteria by breaking cell membranes and altering essential cellular functions (Kannan *et al.*, 2021), making it an economical alternative to Ag. Including the exact amounts of Cu and Ag in this combination helps protect against bacteria more effectively than either metal alone, without harming cells. Researchers are interested in developing CAUTI solutions because silver and copper exhibit strong antimicrobial effects against drug-resistant bacteria.This study investigated the antibacterial properties of silver and copper nanoparticles as potential interventions for catheter-associated urinary tract infections caused by *E. coli*.

MATERIALS AND METHOD

Sample collection and bacterial analysis
A total of 120 urine samples were collected from patients catheterized in the Chandrapur District of Maharashtra. Essential demographic information, including sex, catheterization duration, and reason for catheterization, were recorded for each patient. Samples were obtained from various clinical wards and Intensive Care Units (ICUs). Informed consent was obtained from patients admitted to multiple medical conditions prior to sample collection. Urine samples were analyzed for the isolation and identification of Escherichia coli, antibiotic sensitivity testing, and evaluation of the antibacterial activity of *E. coli* against silver and copper nanoparticles. Descriptive statistical methods, including percentage distribution, were employed to analyze the trends and associations in the data

Antibiotic Resistance Profiling
Antibiotic susceptibility testing of *E. coli* was conducted using the Kirby Bauer disk diffusion method on Muller-Hinton agar. The antibiotics evaluated were Ciprofloxacin (CIP) 5µg, Gentamicin (GEN) 10µg, Cefoperazone (CPZ) 75µg, Tetracycline (TE) 30µg, Cefotaxime (CTX) 30µg and Ampicillin (AMP) 10µg. Antibiotic susceptibility testing is essential for determining the antibiotic resistance patterns of various antibiotics in *E coli*.

Nanomaterial-Based Antibacterial Assay
The nanoparticles were procured from Sai Biosystem, Nagpur. Subsequently, their size, morphology, and other properties were characterized using Scanning Electron Microscopy (SEM) and Energy-dispersive X-ray spectroscopy (EDX). The antimicrobial efficacy of silver and copper nanoparticles was evaluated using a well diffusion assay following CLSI guidelines. Standardized bacterial suspensions were uniformly inoculated onto Mueller-Hinton agar plates to ensure homogeneous bacterial growth. Wells measuring 6–8 mm in diameter were created in the agar medium. Silver and copper nanoparticle suspensions at concentrations of 1 mg and 5 mg, respectively, were introduced along with an appropriate control to ensure experimental validity. The plates were incubated at 37°C for 24 h to measure the antibacterial activity by comparing zone sizes.

RESULT AND DISCUSSION

Sample collection and bacterial analysis
This study included 120 patients hospitalized for catheterization with a mean age of 55.5 years. The sex distribution was 56.67% female and 43.33% male patients. Catheterization was predominantly necessitated by accidents (15.83%), surgery (12.5%), maternity-related issues (10%), and laparoscopy (8.33%). Additional indications included cardiac arrest (6.67%), paralysis (5.83%), ICU unit (ICU; 5%), hip fractures (5%), multiple sclerosis (5%), and chronic renal failure (4.17%) (Table 1). The most prevalent catheterization duration was 4-5 days (21.66%), followed by extended periods of one month or more (20.84%), three days (17.5%), one week (15.83%), two weeks (10%), and 6-15 days (14.17%) (Table 2).

E coli was successfully identified and isolated from urinary samples. Initial isolation was conducted on HiChrome UTI agar, followed by Gram staining to confirm the presence of gram-negative bacilli. Further confirmation involved a series of tests using the VITECH identification system.

The EDAX APEX analysis was conducted to confirm the presence of nanoparticles, revealing the presence of Copper Oxide (CuO) with 24.3% oxygen and 75.7% copper by weight (Figure 1A). The analysis also confirmed the presence of Ag (Silver), with 100% silver by weight and atomic percentage (Figure 1B). Both analyses were performed under 20 kV accelerating voltage, 2500x magnification, 37° takeoff angle, and a 50-second live time. According to the SEM analysis results, Nanoparticle Size Analysis demonstrated that the AgNPs in Figure 2A had 419 detected particles with an average size of 6.66 nm. CuO nanoparticles (Figure 2B) exhibited an average size of 39.64 nm; however, their total count was not recorded.

Antibiotic Resistance Profiling
The antimicrobial susceptibility of *E. coli* was evaluated using the disk diffusion method, and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Gentamicin and Ampicillin were classified as sensitive, with mean zone diameters of 16±2.75 mm and 19±5.22 mm, respectively. Cefoperazone was moderately sensitive (mean zone of 18±2.75 mm), whereas Ciprofloxacin, Tetracycline, and Cefotaxime were resistant (mean zones of 13±4.72 mm, 14±3.92 mm, and 16±2.08 mm, respectively). *E. coli* displays high levels of resistance to Ciprofloxacin and Tetracycline indicating that these bacteria rapidly develop resistance to many antibiotics. This resistance is attributed to the excessive and inappropriate use of antibiotics, which has contributed to an increase in multidrug-resistant *E. coli* strains in clinical settings. (Table 3)

Nanomaterial-Based Antibacterial Assay
The efficacy of silver and copper nanoparticles in combating various *E. coli* strains was assessed. Two stock concentrations, 1 mg/ml and 5 mg/ml, were diluted to 25, 50, and 100 µL for experimental purposes. At a concentration of 1 mg/ml, AgNPs demonstrated significant antibacterial activity against ECU-08, ECU-84, and ECU-63, with inhibition zones ranging from 11 to 19 mm, contingent upon the volume. CuO nanoparticles exhibited efficacy at higher volumes, particularly against ECU-08 and ECU-84. At 5 mg/ml, AgNPs displayed consistent broad-spectrum activity across diverse strains and volumes, generating inhibition zones of up to 16 mm. (Table 4).

CuO demonstrated moderate effectiveness at higher volumes for specific strains. These results highlight the notable antibacterial properties of AgNPs and suggest their potential as alternative antibacterial agents against *E. coli*.

Table 1. Reason for Catheterization	
Reason	Patients in %
Accident	15.83
Surgery	12.50
Maternity ward	10.00
Laparoscopy	08.33
Cardiac arrest	06.67
Paralysis	05.83
ICU surgery	05.00
Fracture in the hip joint	05.00
Multiple sclerosis	05.00
Chronic Renal Failure	04.17
Others (≤ 4 patients each)	18.34

Table 2. Duration of Catheterisation	
Duration	Patients (%)
Three days	17.50
One week	15.83
4-5 days	21.66
Two weeks	10.00
Prolong (≥1 month)	20.84
Other (6-15 days)	14.17

Table 3. Antibiotic sensitivity assay against *Escherichia coli*	
Antibiotic	zone of inhibition in mm
Ciprofloxacin (CIP) 5mcg	13±4.72
Gentamicin (GEN) 10mcg	16±2.75
Cefoperazone (CPZ) 75mcg	18±2.75
Tetracycline (TE) 30mcg	14±3.92
Cefotaxime (CTX) 30mcg	16±2.08
Ampicillin (AMP) 10mcg	19±5.22

Table 4. Antibacterial Activity (Zone of Inhibition in mm) of silver and copper nanoparticle				
Metal	Dilution	25µl	50µl	100µl
Silver	1 mg/ml	13.2 ± 0.4	14.5 ± 0.6	15.3 ± 0.7
Silver	5 mg/ml	13.4 ± 0.5	15.0 ± 0.6	15.6 ± 0.8
Copper	1 mg/ml	0.00 ± 0.00	11.5 ± 0.3	13.7 ± 0.5
Copper	5 mg/ml	0.00 ± 0.00	12.1 ± 0.4	14.1 ± 0.5

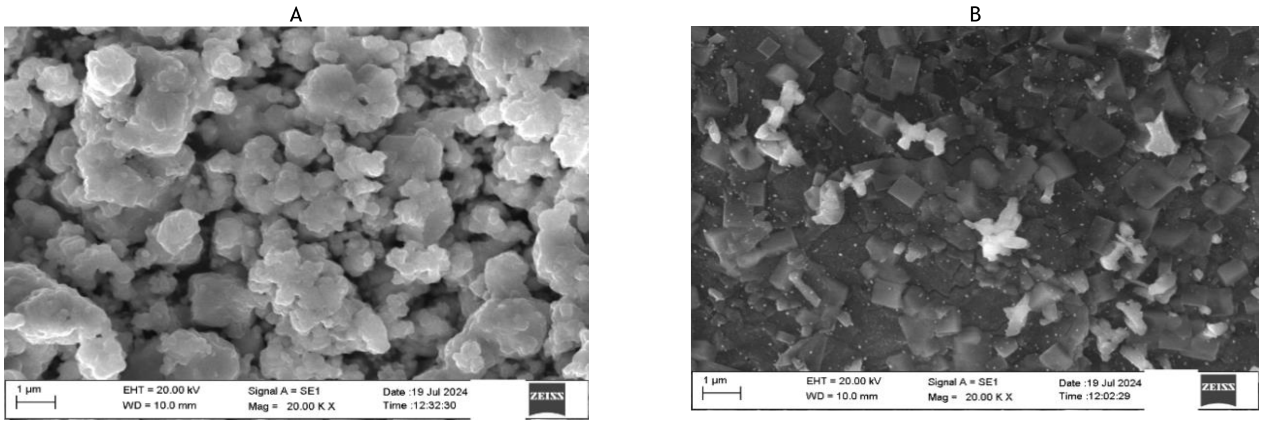

Fig. 1 Representative scanning electron microscopy image of (A) CuONPs and (B) AgNPs

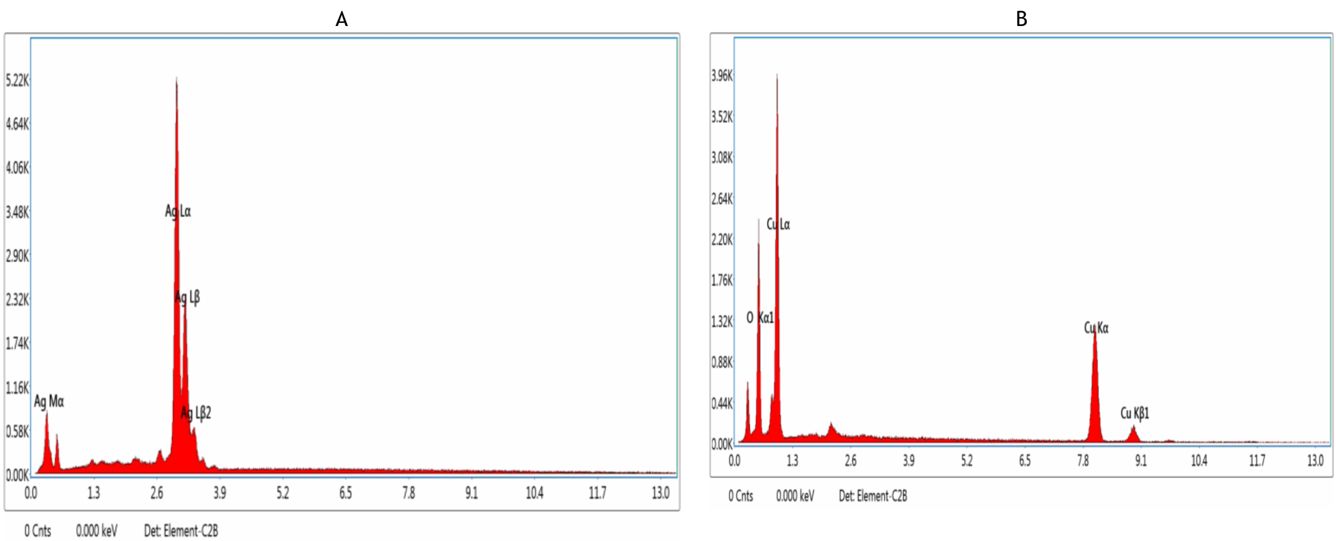

Fig. 2 Representative EDX spectrum of (A) Silver and (B) Copper oxide nanoparticle

The antimicrobial activity of metal nanoparticles has been widely explored, and studies have suggested that their small size and high surface-area-to-volume ratio are key factors contributing to their potent antibacterial effects (Krishnan *et al.*, 2015) (Thirugnanasambandan& Alagar, 2011) (Godymchuk*et al.*, 2015). These findings are consistent with our findings. We observed that silver nanoparticles (AgNPs), with an average size of 6.66 nm, displayed significant antibacterial activity at concentrations of 1 mg/ml and 5 mg/ml, producing inhibition zones ranging from 11 to 19 mm. AgNPs have demonstrated strong bactericidal, fungicidal, and antiviral properties, making them a promising alternative to traditional antimicrobial agents. (Madla-Cruz *et al.*, 2020)

Copper nanoparticles have demonstrated significant inhibitory effects against Gram-positive and Gram-negative bacteria, including Escherichia coli. (Godymchuk et al., 2015) (Thirugnanasambandan& Alagar, 2011) Copper oxide nanoparticles (CuONPs), with a larger average size of 39.64 nm, exhibited moderate antibacterial activity, primarily at elevated concentrations. In a comparative analysis of AgNPs and CuONPs, AgNPs demonstrated superior performance to copper, with nanoparticle size being one of the contributing factors. These findings are consistent with (Seil and Webster's 2012) finding that AgNPs outperform CuONPs in antibacterial tests because of their enhanced production of reactive oxygen species and more efficacious action. (Goda *et al.* 2022) reported that green synthesis of AgNPs using pomegranate rind exhibited optimal antibacterial efficacy. The resulting nanoparticles ranged in size from 15 to 25 nm

The exact mechanisms by which metal nanoparticles exert their antimicrobial properties are not fully understood, but several potential pathways have been proposed. One mechanism is the ability of nanoparticles to disrupt the cell membrane, leading to the leakage of cellular contents and, ultimately, cell death. Another potential mechanism is the generation of reactive oxygen species during the surface oxidation of nanoparticles, which can cause oxidative damage to cellular components. (Madla-Cruz et al., 2020) Metal nanoparticles may also interfere with vital cellular processes, such as nutrient uptake, enzyme function, and DNA replication. (Godymchuk*et al.*, 2015; Madla-Cruz *et al.*, 2020; Thirugnanasambandan& Alagar, 2011) these mechanisms underscore the potential of silver and copper nanoparticle a effective agents against antibiotic resistance bacterial strains

The significant resistance of E. coli strains to commonly used antibiotics, such as ciprofloxacin and tetracycline, observed in this study mirrors the growing concern over multidrug-resistant bacteria in clinical settings(Arsene *et al.*, 2021). This escalating resistance underscores the urgent need for alternative treatments, such as nanoparticle therapy, which can bypass traditional resistance mechanisms. Notably, the ability of AgNPs to inhibit biofilm formation on catheters presents a strategic advantage in managing catheter-associated urinary tract infections (CAUTIs) because biofilms can significantly contribute to antibiotic resistance.

This study did not focus on identifying the genes responsible for antibiotic resistance in *E. coli*, thus emphasizing the need for future research. highlighted the necessity of analyzing the genetic composition of *E. coli*, as a deeper understanding of its genomic structure is essential for scientific progress. Additionally, Soto SM demonstrated that horizontal gene transfer played a key role in the dissemination of antibiotic resistance (Soto, 2014). These findings provide valuable insights that could aid the development of nanoparticle-based strategies to combat resistant bacterial strains more effectively. Silver nanoparticles show powerful potential as a new medicine for *E. coli* infections; however, copper nanoparticles need further development before they can be used for medical treatment.

CONCLUSION

This study assessed the antibacterial efficacy of silver and copper oxide nanoparticles against *Escherichia coli* isolates obtained from patients diagnosed with catheter-associated urinary tract infections. Antibiotic susceptibility testing revealed notable resistance of the isolates to commonly prescribed antibiotics, underscoring the critical need for alternative therapeutic strategies. AgNPs with an approximate size of 6.66 nm, demonstrated potent antibacterial activity, evidenced by inhibition zones ranging from 11 mm to 19 mm at concentrations of 1 mg/ml and 5 mg/ml, respectively. Conversely, CuONPs with an average size of 39.64 nm, exhibited comparatively moderate antibacterial effects, particularly at higher concentrations. The enhanced efficacy of AgNPs is attributed to their smaller size, larger surface-area-to-volume ratio, and increased reactivity. These findings suggest that AgNPs hold significant promise as an alternative treatment for *E. coli*-induced CAUTIs, particularly in the context of escalating antibiotic resistance. However, further research is warranted to optimize nanoparticle formulations and elucidate the underlying genetic and molecular mechanisms of their interaction with bacterial cells.

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