Current Botany 2025, 16: 239-243
doi: 10.25081/cb.2025.v16.9573
https://updatepublishing.com/journal/index.php/cb

Research Article

ISSN: 2220-4822

Antibacterial efficacy of zinc oxide nanoparticles on *Proteus mirabilis*: MIC, MBC, and comparative analysis with conventional antibiotics

Vaishnavi K. Pidadi*, Vaishali U. Thool

Department of Microbiology, IHLR&SS, Sardar Patel Mahavidyalaya, Chandrapur-442402, Maharashtra, India

ABSTRACT

Catheter-associated urinary tract infections (CAUTI) attributable to colonisation and infection by *Proteus mirabilis* are a pressing issue owing to their ability to form crystalline biofilms that obstruct urinary catheters. In this study, we evaluated the antibiofilm activity of zinc oxide nanoparticles (ZnONPs) against *P. mirabilis* and compared it to conventional antibiotics. ZnONPs were characterised by energy-dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) to assess their morphology, size and surface characteristics, key determinants of their antimicrobial efficacy. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ZnONPs were 125 µg/mL and 175 µg/mL, respectively, with up to 99.7% bacterial growth inhibited. The results show that ZnONPs possess concentration-dependent antibacterial activity, often exceeding the effectiveness of standard antibiotics. The superior activity of ZnONPs is associated with their high surface area-to-volume ratio, which enhances their interactions with bacterial membranes. These findings demonstrate that ZnONPs are a promising alternative for treating *P. mirabilis* biofilms in CAUTIs, particularly in antibiotic-resistant cases. The potential clinical applications of this research include the development of ZnONP-based treatments for CAUTIs and the design of novel strategies to combat antibiotic resistance, highlighting the necessity for further research on their synergistic potential and clinical applications.

KEYWORDS: ZnONP, Antibiotic resistance, MIC, MBC, CAUTI

Received: May 03, 2025
Revised: September 10, 2025
Accepted: September 17, 2025
Published: October 07, 2025

***Corresponding author:**
Vaishnavi K. Pidadi
E-mail: vaishnavip48@gmail.com

INTRODUCTION

Urinary tract infections (UTI) present a significant risk to hospitalized patients. These infections are the most prevalent healthcare-associated infections affecting the bladder, ureters, and kidneys (Bhani *et al.*, 2017). A significant proportion of infections acquired during hospitalisation (approximately 75%) are linked to the use of a urinary catheter; it is concerning that between 15% and 25% of hospitalised patients undergo catheterisation at some point during their hospital stay, as catheters are among the most commonly used medical devices (Clarke, 2019). The prevalence of pathogens such as *Escherichia coli*, *Proteus*, *Enterococcus*, and others in CAUTIs, our study focuses on *Proteus mirabilis* due to its unique ability to form crystalline biofilms that obstruct urinary catheters, which are typically acquired from external sources through manipulation of the catheter and drainage device (Mortazavi-Tabatabaei *et al.*, 2019). Catheter-associated urinary tract infections are among the most common nosocomial infections. CAUTIS are associated with numerous adverse outcomes, such as septicemia, catheter encrustation, bladder stones, pyelonephritis, and endotoxic shock. They can be caused by both Gram-negative and Gram-positive bacteria and yeast (Rubi *et al.*, 2022).

P. mirabilis was chosen for this study because it is a primary contributor to catheter-associated urinary tract infections caused using catheters. *P. mirabilis* forms crystalline biofilms that can encrust and block urinary catheters, posing a significant challenge in managing these infections (Rakhi & Gupta, 2023). Bacteria within biofilms exhibit increased tolerance, and biofilms demonstrate a significantly distinct response to antimicrobial agents and host immune defenses compared to their planktonic counterparts, highlighting the urgent need for targeted therapeutic strategies. *P. mirabilis* is known to form dense, crystalline biofilms on the surface of urinary catheters. The formation of a *P. mirabilis* biofilm begins with the initial adherence of bacterial cells to the catheter surface, followed by the production of an extracellular polymeric matrix that encases the cells. The production of urease by *P. mirabilis* contributes to an increase in the surrounding environment's pH

by catalyzing the breakdown of urea into ammonia and carbon dioxide through hydrolysis. This pH increase promotes the precipitation of minerals such as struvite and apatite within the biofilm matrix, resulting in a characteristic crystalline structure (Durgadevi *et al.*, 2020; Yuan *et al.*, 2021).

Encrustation and blockage of urinary catheters by *P. mirabilis* biofilms can lead to serious complications, including urinary retention, reflux of infected urine, and the development of pyelonephritis (Wasfi *et al.*, 2020). Treating CAUTIs attributed to *P. mirabilis* has become more difficult due to the rise of antibiotic-resistant variants. Conventional antibiotic treatments frequently do not successfully address these infections because biofilm formation shields the bacteria. Therefore, it is urgent and crucial to investigate alternative antimicrobial approaches to tackle *P. mirabilis* biofilms and avoid catheter encrustation.

With the urgent need for alternative antimicrobial strategies, our attention turns to metallic nanoparticles, including silver and zinc, known for their promising antimicrobial properties. Zinc nanoparticles have displayed this activity, targeting a broad range of pathogenic bacteria, including *P. mirabilis*. The proposed mechanism underlying the antimicrobial effects of zinc nanoparticles involves disruption of bacterial cell membranes, generation of reactive oxygen species, and inhibition of cellular enzymes (Gharpure & Ankamwar, 2020). Furthermore, zinc nanoparticles have been observed to disrupt the formation of bacterial biofilms, suggesting their potential to address the challenges posed by *P. mirabilis* biofilms of catheter-associated urinary tract infections (Iribarnegaray *et al.*, 2019). The main objective of this study was to characterise zinc nanoparticles, evaluate their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against biofilm-forming strains of *P. mirabilis* isolated from patients with catheter-associated urinary tract infections (CAUTI), and analyse the zinc nanoparticles using various analytical techniques. The promising potential of zinc nanoparticles suggests a new approach to combating CAUTI

MATERIALS AND METHODS

Sample Collection and Bacterial Analysis

120 urine samples were collected from catheterised patients in the Chandrapur District of Maharashtra. The samples were obtained from various clinical wards and Intensive Care Units. Before sample collection, informed consent was secured from the patients admitted with diverse medical conditions. The urine samples were analysed to isolate and identify *Proteus mirabilis*, conduct antibiotic sensitivity testing, and evaluate the antibacterial activity of *P. mirabilis* against zinc nanoparticles. The zinc nanoparticles used in this study were purchased from Sai Biosystem and characterised using various analytical techniques, including energy-dispersive X-ray spectroscopy and scanning electron microscopy. The size, shape, and surface properties of the ZnO nanoparticles were analysed to assess their potential as antimicrobial agents.

Antibiotic Susceptibility Testing

The antibiotic resistance profile of *P. mirabilis* was evaluated using the Kirby-Bauer disk diffusion method. A standardized bacterial suspension was spread onto agar plates. Antibiotic discs containing Ciprofloxacin, Gentamicin, Cefoperazone, Tetracycline, Cefotaxime, and Ampicillin were placed on the surface (Bär *et al.*, 2009). Post-incubation, the zones of Inhibition were determined in millimetres. The zone diameters were interpreted by following CLSI guidelines, categorising the bacteria as sensitive, intermediate, or resistant.

Antibacterial Activity Assay

Zinc Oxide nanoparticles (ZnONPs) were assessed for their antibacterial activity by measuring the zone of Inhibition at different concentrations and volumes. The study involved two experimental setups: a control group to observe the natural growth of bacterial colonies and another group with varying concentrations of ZnONPs. The agar well diffusion method was used to evaluate the antibacterial effectiveness of ZnONPs at concentrations of 1 mg/mL and 5 mg/mL, utilizing different volumes. The resulting zones of Inhibition were measured in millimetres (Hasan *et al.*, 2022). Statistical analyses were conducted to determine the significance of the findings. Data were expressed as mean ± standard deviation and analysed using one-way ANOVA to assess whether the antibacterial activity of ZnONPs varied significantly across the different concentrations and volumes.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration Assessment

To determine the minimum inhibitory concentration (MIC) of ZnONPs we are using the broth microdilution method. Serial dilutions of ZnONPs were prepared in Mueller-Hinton broth, with final concentrations ranging from 10 to 200 μg/mL. A standardised bacterial inoculum was added to each well, and the plates were incubated overnight at 37 °C. The minimum inhibitory concentration was recorded as the lowest concentration of ZnONPs that inhibited visible bacterial growth. To determine the minimum bactericidal concentration (MBC), samples from wells that showed no visible growth in the minimum inhibitory concentration (MIC) assay were plated onto fresh agar plates and incubated for 24 hours. The MBC was defined as the lowest concentration of zinc oxide nanoparticles (ZnONP) that resulted in no bacterial growth on the agar plates. The percentage of bacterial growth inhibition was calculated using the formula:

$$\% \text{ Inhibition} = 100 \times (\text{mean colony count/Control})$$

This calculation was then used to evaluate both the minimum inhibitory concentration and the minimum bactericidal concentration.

RESULTS

P. mirabilis 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.

Antibiotic Sensitivity Assay

The antibiotic sensitivity assay demonstrated that the ZnO nanoparticles exhibited comparable or superior antibacterial activity against *P. mirabilis* compared to several common antibiotics. The Antibiotic Sensitivity Assay shows that the zone of Inhibition varied for the different antibiotics, ranging from 9.5±3.1 mm to 14.2±2.8 mm (Figure 2). Interestingly, the antibacterial efficacy of the ZnONP surpassed that of some of the tested antibiotics, indicating their potential as an effective alternative antimicrobial agent (Table 1).

Antibacterial Activity of ZnO Nanoparticles

The elemental analysis using EDAX APEX revealed that the zinc oxide nanoparticle sample consists of 79.4% zinc and 20.6% oxygen by weight (Figure 3). Additionally, the SEM analysis showed that the ZnONPs have an average size of 49.6 nm (Figure 4). The results demonstrate an evident concentration-dependent antibacterial activity of the ZnONPs. Higher concentrations of ZnONPs exhibited more significant inhibitory effects against *P. mirabilis*, with the 100 µL of 5 mg/mL concentration producing the largest zone of Inhibition, ranging from 12.5±2.07 mm to 17.38±2.99 mm. These findings suggest that ZnONPs possess potent antibacterial properties, and their efficacy increases with concentration. One-way analysis of variance showed a statistically notable difference in the antibacterial activity of ZnONPs across the different volumes tested at both 1 mg/mL and 5 mg/mL concentrations. This suggests that the inhibitory effects of the ZnONPs were affected by the volume of the nanoparticle solution used, with larger volumes exhibiting greater antibacterial effectiveness. The low p-values support the statistical significance of these findings, suggesting a strong concentration-dependent relationship between the ZnONPs' antibacterial properties against *P. mirabilis* (Table 2).

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration assessment

The results of the minimum inhibitory concentration and minimum bactericidal concentration assays provide valuable insights into the antibacterial efficacy of the zinc oxide nanoparticles against *P. mirabilis*. The MIC was determined to be 125 µg/mL (Figure 1), indicating that this concentration of ZnONPs could effectively inhibit the visible growth of the bacterial cells. This suggests that ZnONPs could disrupt the proliferation and viability of *P. mirabilis* cells at this concentration.

Furthermore, the MBC was found to be 175 µg/mL, with this concentration achieving 99.7% inhibition of bacterial growth. This demonstrates the potent bactericidal activity of the ZnONPs, as they could completely eradicate the bacterial population at this higher concentration. The high percentage of Inhibition underscores the remarkable antimicrobial efficacy of the ZnONPs against this problematic biofilm-forming pathogen. These findings highlight the significant potential of ZnONPs as a promising antimicrobial agent for managing *P. mirabilis* infections, especially in cases where conventional antibiotics may be less effective (Table 3).

The Minimum inhibitory Concentration (MIC) was determined as 125 µg/mL. The Minimum Bactericidal Concentration (MBC) was determined as 175 µg/mL, achieving 99.7% inhibition.

Table 1: Antibiotic sensitivity assay against *Proteus mirabilis*

Antibiotic	Zone of Inhibition (mm)
Ciprofloxacin (CIP) 5 mcg	10.5±2.5
Gentamicin (GEN) 10 mcg	12.0±3.0
Cefoperazone (CPZ) 75 mcg	14.2±2.8
Tetracycline (TE) 30 mcg	9.5±3.1
Cefotaxime (CTX) 30 mcg	12.4±2.6
Ampicillin (AMP) 10 mcg	11.8±3.5

Table 2: Antibacterial activity and one-way ANOVA results of ZnO nanoparticles

Dilution	25 µL (mm)	50 µL (mm)	100 µL (mm)	F-value	p-value
1 mg/mL	12.5±2.07	15.15±2.54	17.0±2.47	11.04	0.0002
5 mg/mL	12.6±1.96	14.62±2.66	17.38±2.99	10.5	0.0003

Table 3: MIC & MBC

Concentration	Mean Colony Count	% Inhibition
Control	283	0.00%
50 µg/mL	170	39.90%
60 µg/mL	261.8	7.50%
75 µg/mL	123.8	56.30%
100 µg/mL	201.6	28.80%
125 µg/mL	64	77.40%
150 µg/mL	1	99.60%
175 µg/mL	0.9	99.70%

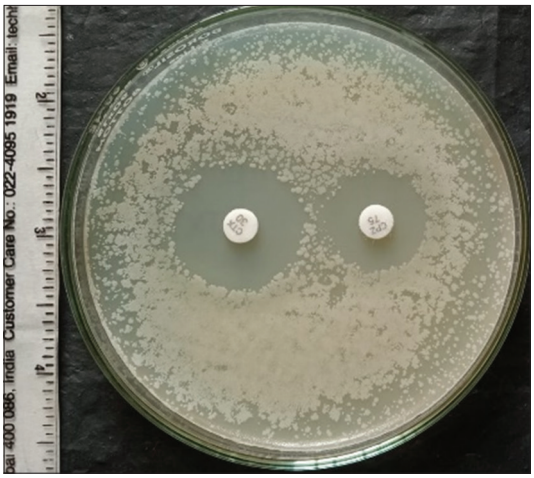

Figure 1: Antibiotic susceptibility testing of PMU46 using disc diffusion assay

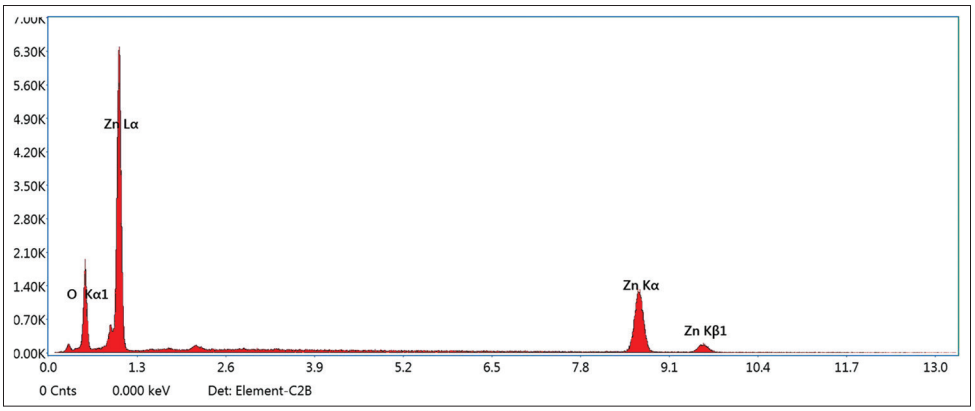

Figure 2: Representative EDX spectrum of ZnONP

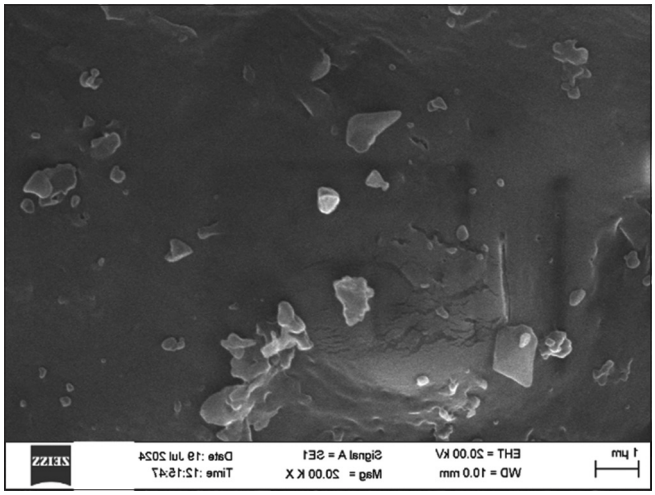

Figure 3: Representative scanning electron microscopy image of ZnONP

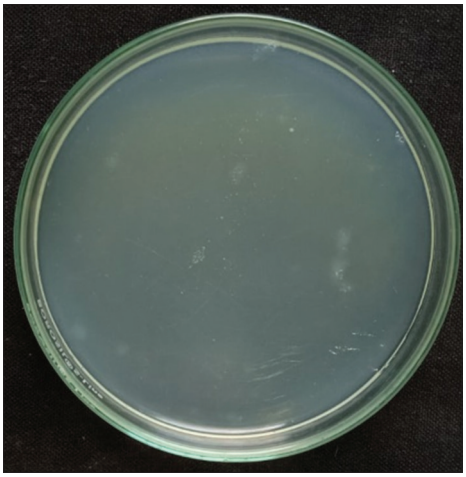

Figure 4: Antibacterial efficacy of ZnO (125 µg/mL): MIC analysis on PMU11

DISCUSSION

Zinc oxide nanoparticles represent a promising approach for combating bacterial infections, particularly those caused by biofilm-forming pathogens such as *P. mirabilis* (Mahdi *et al.*, 2024). Our findings demonstrate that ZnO nanoparticles exhibit significant antimicrobial activity, primarily through disruption of bacterial cell membranes. This mechanism aligns with existing literature, suggesting the potential of ZnO nanoparticles as a novel therapeutic approach. They can disrupt bacterial cell membranes and produce reactive oxygen species (ROS), damaging bacterial DNA and proteins (Wasfi *et al.*, 2020). The concentration-dependent antibacterial activity observed in this study aligns with previous research indicating that the inhibitory efficacy of ZnO nanoparticles is significantly influenced by both size and concentration (Palanikumar *et al.*, 2014). The ability of ZnO NPs to inhibit bacterial growth and biofilm formation suggests that they could serve as an alternative to traditional antibiotics, especially in cases of antibiotic resistance (Wasfi *et al.*, 2020). The superior activity of ZnO NPs can be attributed to their high surface area-to-volume ratio, which facilitates more effective contact with bacterial membranes and disrupts vital cellular functions (Palanikumar *et al.*, 2014). The MIC and MBC values revealed the capacity of ZnO nanoparticles to impede proliferation and induce the demise of *P. mirabilis* at relatively low concentrations, underscoring their potential as a compelling therapeutic intervention. The antibiotic sensitivity assay results showed that ZnO nanoparticles are more effective than several common antibiotics, demonstrating their potential as a superior alternative. Compared to a previous study reporting a higher MIC (Mahdi *et al.*, 2024), differences in nanoparticle synthesis methods, size, shape, bacterial strain, and experimental methodology may account for the variations in the observed MIC values (Padmavathy & Vijayaraghavan, 2008). Discussing the size and characteristics of the ZnO nanoparticles used and other influential factors would strengthen the analysis.

Furthermore, the synergistic potential of ZnO nanoparticles with other antimicrobial agents or nanoparticles, such as magnesium oxide, could enhance their efficacy and broaden their applications in combating bacterial infections (Wasfi *et al.*, 2020). Furthermore, the synergistic potential of ZnO nanoparticles with other antimicrobial agents or nanoparticles, such as magnesium oxide, could enhance their efficacy and broaden their applications in combating bacterial infections (Gharpure & Ankamwar, 2020). Combining zinc oxide

nanoparticles (ZnONPs) with other antimicrobial methods, such as antibiotics or metal oxide nanoparticles, may produce more substantial effects against *P. mirabilis*. Future studies should examine how well ZnO nanoparticles work in living organisms, check their safety and compatibility, and find better ways to deliver them to improve the treatment of *P. mirabilis* infections.

Comparative analyses with other published studies on the antimicrobial effects of ZnO nanoparticles against *P. mirabilis* could provide valuable insights into the consistency and reproducibility of these findings, potentially guiding the development of more effective nanoparticle-based therapeutics (Iribarnegaray *et al.*, 2019; Saleh *et al.*, 2019). The ability of *P. mirabilis* to form crystalline biofilms significantly complicates the treatment of infections, rendering biofilm-embedded cells considerably more resistant to antibacterial agents and the host immune response (Wasfi *et al.*, 2020). These biofilms, frequently observed on indwelling medical devices such as urinary catheters, lead to catheter-associated tract infections, emphasizing the vital need for innovative strategies to combat these recalcitrant microbial communities

CONCLUSION

This study highlights the potential of ZnONP as an effective antimicrobial strategy against *P. mirabilis*, a bacterium that can form problematic biofilms and cause complicated urinary tract infections (Wasfi *et al.*, 2020). The findings support using ZnO nanoparticles as a promising alternative to traditional antibiotics for combating *P. mirabilis* infections, offering a new therapeutic approach (Gharpure & Ankamwar, 2020). Future research should evaluate the in vivo efficacy and safety of ZnO nanoparticles and their potential for combination therapies with existing antibiotics or other antimicrobial agents. Additionally, studies should examine the chronic impact of ZnO nanoparticles on microbial communities and the possibility of resistance development. This research establishes a foundation for developing innovative strategies to address bio-film-associated infections caused by *P. mirabilis*, potentially enhancing patient outcomes and decreasing the impact of antibiotic resistance.

REFERENCES

Bär, W., Bäde-Schumann, U., Krebs, A., & Cromme, L. (2009). Rapid method for detection of minimal bactericidal concentration of antibiotics. *Journal of Microbiological Methods, 77*(1), 85-89. https://doi.org/10.1016/j.mimet.2009.01.010

Bhani, D., Bachhiwal, R., Sharma, R., & Maheshwari, R. K. (2017). Microbial profile and antimicrobial susceptibility pattern of uropathogens isolated from catheter associated urinary tract infection (CAUTI). *International Journal of Current Microbiology and Applied Sciences, 6*(8), 2446-2453. https://doi.org/10.20546/ijcmas.2017.608.289

Clarke, K. (2019). Catheter-associated urinary tract infections: Diagnosis, treatment, and prevention. *Women Health Care and Issues, 2*(1), 1. https://doi.org/10.31579/2642-9756/008

Durgadevi, R., Kaleeshwari, R., Swetha, T. K., Alexpandi, R., Pandian, S. K., & Ravi, A. V. (2020). Attenuation of *Proteus mirabilis* colonization and swarming motility on indwelling urinary catheter by antibiofilm impregnation: An *in vitro* study. *Colloids and Surfaces B: Biointerfaces, 194*, 111207. https://doi.org/10.1016/j.colsurfb.2020.111207

Gharpure, S., & Ankamwar, B. (2020). Synthesis and antimicrobial properties of zinc oxide nanoparticles. *Journal of Nanoscience and Nanotechnology, 20*(10), 5977-5996. https://doi.org/10.1166/jnn.2020.18707

Hasan, M., Zafar, A., Imran, M., Iqbal, K. J., Tariq, T., Iqbal, J., Shaheen, A., Hussain, R., Anjum, S. I., & Shu, X. (2022). Crest to trough cellular drifting of green-synthesized zinc oxide and silver nanoparticles. *ACS Omega, 7*(39), 34770-34778. https://doi.org/10.1021/acsomega.2c02178

Iribarnegaray, V., Navarro, N., Robino, L., Zunino, P., Morales, J., & Scavone, P. (2019). Magnesium-doped zinc oxide nanoparticles alter biofilm formation of *Proteus mirabilis*. *Nanomedicine, 14*(12), 1551-1564. https://doi.org/10.2217/nnm-2018-0420

Mahdi, S. M., Ahmed, M. E., & Abbas, A. F. (2024). Effect of Enterococcin–zinc oxide nanoparticles on gene expression of *rsbA* swarming genes in *Proteus mirabilis* isolated from catheter urine. *Biomedical and Pharmacology Journal, 17*(2), 1249-1264. https://doi.org/10.13005/bpj/2939

Mortazavi-Tabatabaei, S. A. R., Ghaderkhani, J., Nazari, A., Sayehmiri, K., Sayehmiri, F., & Pakzad, I. (2019). Pattern of antibacterial resistance in urinary tract infections: A systematic review and meta-analysis. *International Journal of Preventive Medicine, 10*, 169. https://doi.org/10.4103/ijpvm.IJPVM_419_17

Padmavathy, N., & Vijayaraghavan, R. (2008). Enhanced bioactivity of ZnO nanoparticles-An antimicrobial study. *Science and Technology of Advanced Materials, 9*(3), 035004. https://doi.org/10.1088/1468-6996/9/3/035004

Palanikumar, L., Ramasamy, S. N., & Balachandran, C. (2014). Size-dependent antimicrobial response of zinc oxide nanoparticles. *IET Nanobiotechnology, 8*(2), 111-117. https://doi.org/10.1049/iet-nbt.2012.0008

Rakhi, & Gupta, A. (2023). Alternative medicine for urinary tract infections (UTI) against *E. coli* and other bacterial population. *International Journal of Herbal Medicine, 11*(2), 44. https://doi.org/10.22271/flora.2023.v11.i2a.859

Rubi, H., Mudey, G., & Kunjalwar, R. (2022). Catheter-associated urinary tract infection (CAUTI). *Cureus, 14*(10), e30385. https://doi.org/10.7759/cureus.30385

Saleh, T. H., Hashim, S. T., Malik, S. N., & AL-Rubaii, B. A. L. (2019). Down-regulation of *fliL* gene expression by Ag nanoparticles and TiO_2 nanoparticles in pragmatic clinical isolates of *Proteus mirabilis* and *Proteus vulgaris* from urinary tract infection. *Nanobiotechnology, 11*(4), 321-332. https://www.sciopen.com/article/10.5101/nbe.v11i4.p321-332

Wasfi, R., Hamed, S. M., Amer, M. A., & Fahmy, L. I. (2020). *Proteus mirabilis* biofilm: Development and therapeutic strategies. *Frontiers in Cellular and Infection Microbiology, 10*, 414. https://doi.org/10.3389/fcimb.2020.00414

Yuan, F., Huang, Z., Yang, T., Wang, G., Li, P., Yang, B., & Li, J. (2021). Pathogenesis of *Proteus mirabilis* in catheter-associated urinary tract infections. *Urologia Internationalis, 105*(5-6), 354-361. https://doi.org/10.1159/000514097

Author Query???

AQ1: KIndly note the figure citations are not in chronology order. Please check and arrange them in order