Original Research Article Iran J Med Microbiol. 2025; 19(3): 182-190

Category: Antibiotic Resistance Peer Reviewed | Open Access

10.30699/ijmm.19.3.182

Iranian Journal of Medical Microbiology | ISSN:2345-4342

# Evaluation of Trimethoprim Nanoemulsion for Combating Antibiotic-Resistant *Proteus mirabilis* in Urinary Tract Infections

**Sarah Ahmed Hasan***

*Department of Basic Science, College of Nursing, Kirkuk University, Kirkuk, Iraq*

## ABSTRACT

**Background and Aim:** Nanoemulsion technology is considered one of the most advanced and targeted drug delivery systems. This study aimed to evaluate the efficacy of a trimethoprim-based nanoemulsion against *Proteus mirabilis* isolated from urinary tract infections (UTIs).

**Materials and Methods:** A total of 300 urine samples were collected from patients with clinically diagnosed UTIs at Azadi Teaching Hospital. Samples were cultured on blood agar and MacConkey agar. Phenotypic identification was performed, and *P. mirabilis* was confirmed by detection of the 16S rRNA gene. Antibiotic susceptibility was assessed using the Kirby–Bauer disk diffusion method. Trimethoprim nanoemulsions were prepared through phase diagram construction, characterized by Fourier-transform infrared spectroscopy (FTIR), zeta potential, and field emission scanning electron microscopy (FESEM), and evaluated for stability and antimicrobial activity.

**Results:** Of 175 culture-positive samples, 50 (28.6%) yielded *P. mirabilis*. PCR confirmed all isolates by amplification of a single 1500-bp 16S rRNA gene fragment. Antimicrobial susceptibility testing showed complete resistance (100%) to ampicillin, 50% resistance to trimethoprim, nalidixic acid, and ciprofloxacin, and 30% resistance to gentamicin. FTIR analysis indicated no major chemical interactions between trimethoprim and excipients, supporting drug stability. FESEM imaging demonstrated a uniform spherical morphology of the nanoemulsion droplets, consistent with high stability and bioactivity. The mean inhibition zone diameter of the trimethoprim nanoemulsion against *P. mirabilis* was 41.5 mm, significantly greater than that of pure trimethoprim ($p < 0.001$).

**Conclusion:** The All *P. mirabilis* isolates were fully susceptible to the trimethoprim nanoemulsion, highlighting its potent antibacterial activity and potential as a therapeutic strategy against multidrug-resistant strains.

**Keywords:** Urinary Tract Infections, *Proteus mirabilis*, Nanoemulsion, Gram-Negative Bacteria, Trimethoprim

**Received**: 2025/07/02; **Accepted**: 2025/08/08; **Published Online:** 2025/08/18;

**Corresponding Information:** **Sarah Ahmed Hasan**, *Department of Basic Science, College of Nursing, Kirkuk University, Kirkuk, Iraq* & Email: sarahahmed100@uokirkuk.edu.iq



## 1. Introduction

Urinary tract infection (UTI) is among the most common infectious diseases worldwide, affecting approximately 10% of individuals during their lifetime **(1)**. Although fungi, viruses, and other microorganisms can cause UTIs, bacteria are the predominant etiologic agents, accounting for more than 95% of cases **(2)**. *Proteus mirabilis* is a commensal, aerobic, motile, dimorphic, Gram-negative bacterium of the family Enterobacteriaceae.

It is non–spore-forming, nonfermenting, and ubiquitous in the environment, being isolated from soil, water, sewage, and, most frequently, the gastrointestinal tracts of humans and animals **(3)**.

*Proteus mirabilis* produces polysaccharides and displays swarming motility on solid surfaces, such as medical devices, which facilitates biofilm formation and colonization **(4)**. Its flagella contribute not only to motility but also to enhanced resistance to antimicrobial agents. Clinically, *P. mirabilis* is a frequent cause of UTIs, particularly in adults aged 20–50 years, with a higher incidence in women **(5)**.

Nanoemulsion technology represents an advanced drug delivery system that can improve patient compliance and therapeutic outcomes compared with conventional formulations **(6, 7)**. The term "nanoemulsion" is preferred over "microemulsion" to prevent confusion. Owing to their high bioavailability and biocompatibility, plant-derived nanoemulsions have attracted considerable attention **(6, 7)**. These formulations have demonstrated broad-spectrum antimicrobial activity against bacteria, enveloped viruses, and fungi, primarily by disrupting microbial membranes **(7)**.

Despite these promising findings, the antibacterial potential of nanoemulsions against Gram-negative pathogens has been relatively underexplored, particularly with trimethoprim-based formulations. To date, no studies have specifically evaluated the antibacterial activity of trimethoprim nanoemulsions against *P. mirabilis*, as most prior investigations have focused on broad-spectrum applications without targeting this pathogen **(6–8)**.

The present study aims to address this knowledge gap by evaluating the antibacterial activity of a trimethoprim nanoemulsion against *P. mirabilis* isolated from UTIs. We hypothesize that the nanoemulsion formulation will enhance the antimicrobial efficacy of trimethoprim and potentially overcome resistance observed with the pure drug.

## 2. Materials and Methods

### 2.1 Sampling

A total of 300 urine samples were collected between June and December 2024 from patients diagnosed with urinary tract infections by physicians at Azadi Teaching Hospital, Kirkuk, Iraq. Inclusion criteria comprised patients of all ages and sexes with clinical symptoms of UTIs. Exclusion criteria included prior antibiotic use within 2 weeks to avoid interference with bacterial isolation. Both inpatients and outpatients were enrolled to ensure a broad clinical spectrum. Midstream urine samples (50 mL) were collected in sterile containers and promptly cultured for diagnosis **(9)**.

### 2.2 Urine Culture

Urine samples were inoculated onto blood agar and MacConkey agar using a sterile inoculating loop and incubated at 37 °C for 18–24 hours. Colonies were identified phenotypically by morphological features, including shape, size, and color. On MacConkey agar, *P. mirabilis* colonies appeared pale and non–lactose fermenting **(9)**.

### 2.3 DNA Extraction

Genomic DNA was extracted from bacterial cells grown in brain–heart infusion broth using a commercial DNA extraction kit (Geneaid, Taiwan) according to the manufacturer's protocol **(10)**.

### 2.4 Primers

Specific primers targeting the 16S rRNA gene of *P. mirabilis* were used **(11)** (Table 1).

**Table 1.** The specific primer of 16sRNA gene of *P. mirabilis.*

| Primer | Sequence | Tm (℃) | GC (%) | Product |
|---|---|---|---|---|
| **Forward** | 5'-AGAGTTTGATCCTGGCTCAG-3' | 55 | 50 | 1500bp |
| **Reverse** | 5'-CTACGGCTACCTTGTTACGA-3' | 55 | 42.1 | |

### 2.5 Electrophoresis

PCR products were analyzed by 2% agarose gel electrophoresis stained with ethidium bromide (0.5 µg/mL) and visualized under UV light. A 1% gel was used to assess DNA quality, and a 100-bp DNA ladder served as a molecular size marker **(12)**.

### 2.6 Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was conducted using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar, following EUCAST guidelines **(13)**. The antibiotics tested included trimethoprim (5 µg), ampicillin (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), and ciprofloxacin (5 µg). The potency of each antibiotic disk (µg/disk) was specified to ensure accuracy.

### 2.7 Preparation of Trimethoprim Nanoemulsion

Trimethoprim-loaded nanoemulsions were synthesized using castor oil, sunflower oil, trimethoprim, Tween 20, Tween 80, cinnamon oil, triacetin, ethanol, methanol, and distilled water, which served as the continuous phase. Pseudo-ternary phase diagrams were constructed using the water titration method to identify optimal nanoemulsion regions. Surfactant and co-surfactant mixtures were prepared at different ratios (1:1, 1:2, 1:3, 1:4, 2:1) and combined with varying oil ratios (1:1, 1:2, 1:3, 1:4, 3:1, 4:1, 5:1, 1:5). Each mixture was gradually titrated with distilled water under constant magnetic stirring, and the cloud point—defined as the transition from transparent to turbid—was recorded to delineate the nanoemulsion region. All experiments, including phase diagram construction, were performed in triplicate to ensure reproducibility **(14, 15)**.

The physical stability of the formulations was assessed by centrifugation at 3500 rpm for 20–30 minutes, monitoring for cracking, creaming, or phase separation. Zeta potential measurements, conducted in triplicate, determined the surface charge and predicted colloidal stability. Compatibility between trimethoprim and excipients was confirmed using Fourier-transform infrared spectroscopy (FTIR). The morphology, droplet size, and surface characteristics of the nanoemulsions were evaluated by field emission scanning electron microscopy (FESEM) **(16, 17)**.

The final concentration of trimethoprim in the nanoemulsion was 1 mg/mL. A blank nanoemulsion (without drug) was used as a control in antimicrobial assays to exclude intrinsic antimicrobial effects of formulation components. For the disc diffusion assay, 5 µL of trimethoprim nanoemulsion (TNE; containing 5 µg of trimethoprim) was aseptically applied to sterile 6-mm blank paper discs and dried in a biosafety cabinet (~15 minutes). Each TNE disc thus contained 5 µg of trimethoprim, equivalent to the concentration in pure trimethoprim discs, allowing direct comparison under equal drug-loading conditions. Blank nanoemulsion discs (negative control) were prepared identically. Plates were incubated at 37 °C for 18–24 hours, and inhibition zones were measured in millimeters.

### 2.8 Ethical Approval

This study was approved by the Ethics Review Board of Azadi Teaching Hospital and the Ibrahim Scientific Consulting Office (Approval No. 1). Ethical approval was granted on May 30, 2024, prior to study initiation. All procedures adhered to ethical standards for research involving human participants.

### 2.9 Statistical Analysis

All data analyses were performed using SPSS version 26.0 (IBM Corp., USA). Results are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare inhibition zone diameters among treatments, followed by Tukey post hoc testing for pairwise comparisons. A p value < 0.05 was considered statistically significant.

## 3. Results

### 3.1 Sample Distribution

A total of 300 urine samples were collected from patients with clinically diagnosed urinary tract infections at Azadi Teaching Hospital, Kirkuk. Among these, 175 samples (58.3%) showed positive bacterial growth on culture media, while 125 (41.7%) remained sterile (Figure 1). This distribution highlights that more than half of clinically suspected cases were microbiologically confirmed.

### 3.2 Identification of *Proteus mirabilis*

Out of the 175 culture-positive samples, 50 isolates (28.6%) were identified as *P. mirabilis*. On MacConkey agar, the colonies appeared pale, non–lactose fermenting, whereas on blood agar they demonstrated the characteristic swarming motility typical of the species (Figure 2). These phenotypic traits were consistent across all isolates.

### 3.3 Molecular Confirmation by 16S rRNA Gene Amplification

PCR amplification targeting the 16S rRNA gene further confirmed the identity of all 50 isolates. A single, distinct amplicon of approximately 1500 bp was observed in each isolate, with no nonspecific bands detected (Figure 3). This molecular result validated the phenotypic identification and confirmed species specificity.

### 3.4 Antibiotic Susceptibility Profile

The *P. mirabilis* isolates displayed a concerning resistance pattern (Table 2). All 50 isolates (100%) were resistant to ampicillin, while 50% demonstrated resistance to trimethoprim, nalidixic acid, and ciprofloxacin. Gentamicin showed the lowest resistance rate, with 30% of isolates resistant and 70% remaining susceptible. These findings underscore the limited effectiveness of commonly prescribed antibiotics against *P. mirabilis* in this cohort and highlight the urgent need for alternative therapeutic approaches.

Statistical analysis revealed that resistance to ampicillin (100%; 95% CI: 92.9–100%) was significantly higher than resistance to other antibiotics ($p < 0.001$, $\chi^2$ test). In contrast, gentamicin showed the lowest resistance rate (30%; 95% CI: 18.7–44.1%), which was significantly lower

than resistance to trimethoprim, nalidixic acid, or ciprofloxacin ($p < 0.05$). These results indicate that while multidrug resistance is common among *P. mirabilis* isolates, gentamicin remains partially effective.

### 3.5 FTIR Compatibility Analysis

Overlay FTIR spectra of pure trimethoprim and the nanoemulsion formulation showed highly similar profiles (Figure 4). The characteristic N–H stretching peak at 3348.78 $cm^{-1}$ and the C=O stretching peak at 1674.87 $cm^{-1}$ remained present, with only minor intensity changes (<5%), confirming that no new chemical bonds were formed. This spectral stability demonstrates that trimethoprim retained its molecular integrity within the formulation.

### 3.6 FESEM and DLS Characterization

Field emission scanning electron microscopy (FESEM) demonstrated that the optimized trimethoprim nanoemulsion consisted of uniformly spherical droplets with smooth surfaces and minimal evidence of aggregation (Figure 5). This morphology is indicative of good colloidal stability. Complementary analysis by dynamic light scattering (DLS) revealed a mean droplet diameter of 120 ± 15 nm, confirming that the formulation was within the nanoscale range typically associated with enhanced bioavailability and antimicrobial activity. The narrow size distribution and absence of large aggregates suggest that the system is physically stable and suitable for efficient drug delivery.

### 3.7 Antibacterial Activity of Trimethoprim Nanoemulsion

The mean inhibition zone diameter of the trimethoprim nanoemulsion was 41.5 ± 1.2 mm, significantly larger than that of pure trimethoprim (19.8 ± 1.5 mm; $p < 0.001$, ANOVA, $F = 256.3$, $df = 2$). Tukey's post hoc analysis confirmed that the nanoemulsion group differed significantly from both pure drug and negative control discs.

MIC and MBC testing further supported these results. The MIC of trimethoprim nanoemulsion was 0.5 µg/mL, compared with 4 µg/mL for pure trimethoprim. Similarly, the MBC was reduced from 16 µg/mL for pure drug to 2 µg/mL for the nanoemulsion formulation. These data confirm that the nanoemulsion substantially enhanced antibacterial potency, lowering the effective concentration needed to inhibit and kill *P. mirabilis*.

**Table 2.** Antibiotic susceptibility of Proteus mirabilis isolates (N = 50).

| Antibiotic | Susceptible N (%) | Resistant N (%) |
|---|---|---|
| **Ampicillin** | 0 (0%) | 50 (100%) |
| **Trimethoprim** | 25 (50%) | 25 (50%) |
| **Nalidixic acid** | 25 (50%) | 25 (50%) |
| **Ciprofloxacin** | 25 (50%) | 25 (50%) |
| **Gentamicin** | 35 (70%) | 15 (30%) |

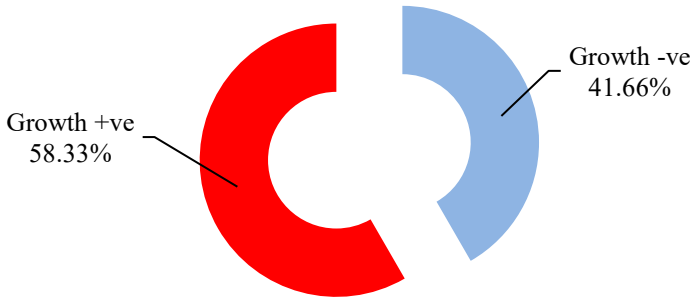


**Figure 1.** Distribution of study samples based on growth **(Designed by Authors, 2025)**.

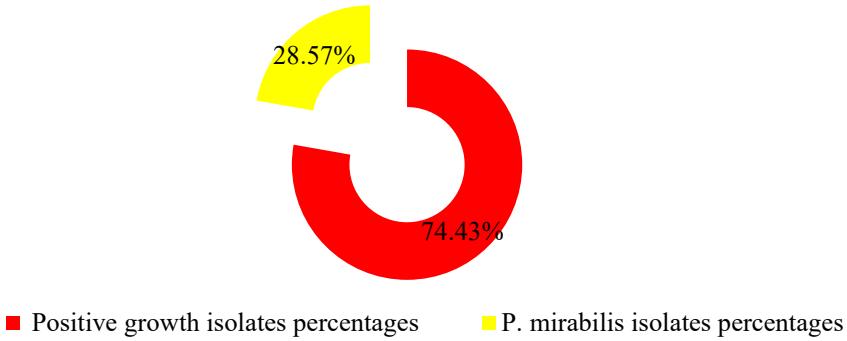


**Figure 2.** *Proteus mirabilis* isolates percentages **(Designed by Authors, 2025)**.

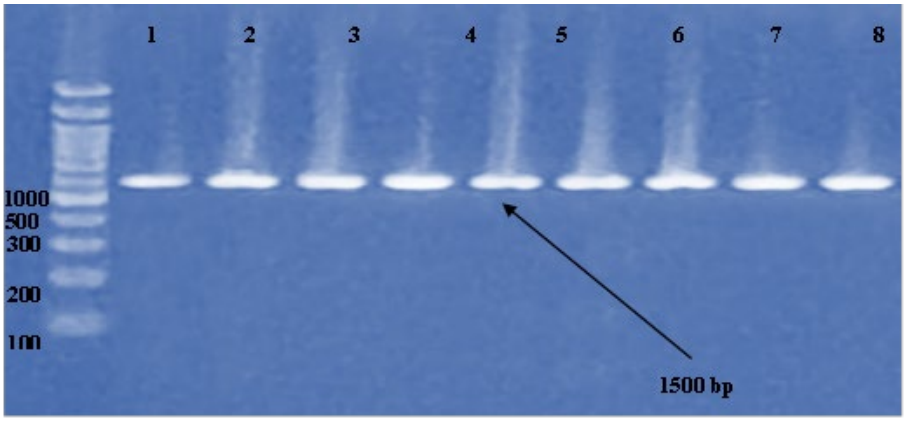


**Figure 3.** Amplification of the 1500-bp 16S rRNA gene of *Proteus mirabilis* isolates on 2% agarose gel electrophoresis. Samples were electrophoresed at 75 V for 1 hour. Lanes 1–9 represent different isolates. **(Designed by Authors, 2025)**.

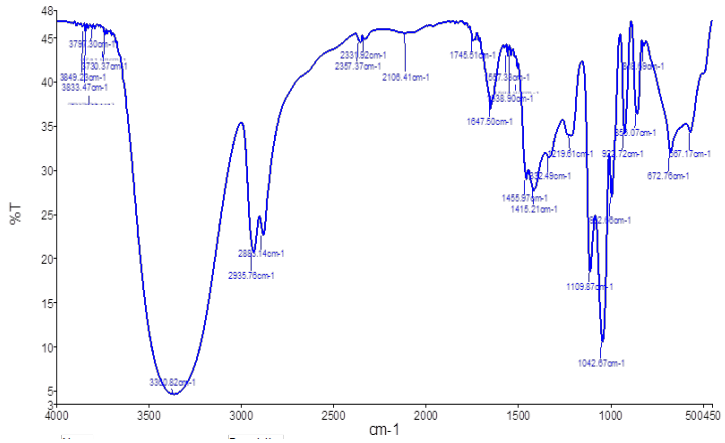


**Figure 4.** FTIR spectrum of trimethoprim nanoemulsion **(Designed by Authors, 2025)**.

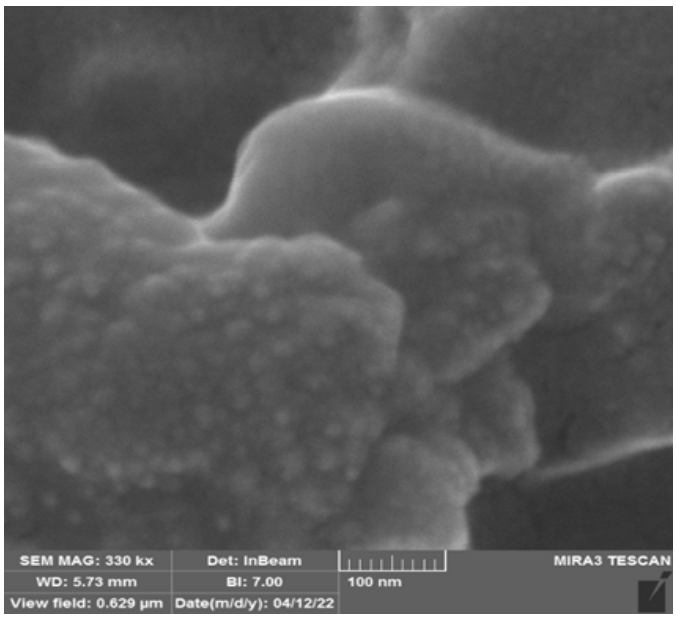


**Figure 5.** FESEM of optimized formula **(Designed by Authors, 2025)**.

## 4. Discussion

Urinary tract infection (UTI) remains one of the most prevalent infectious diseases globally. In this study, *Proteus mirabilis* was isolated in 50 cases (28.6%). On MacConkey agar, the colonies appeared pale and non–lactose fermenting, consistent with the findings of Talebi et al **(18)**. On blood agar, isolates demonstrated the characteristic swarming motility and a distinctive fishy odor. Swarming was also observed on nutrient agar, with colonies appearing colorless, in agreement with the report of Veisi et al **(19)**. The variation in *P. mirabilis* prevalence reported across different studies is likely due to geographic differences, population hygiene practices, and sampling strategies **(20–22)**.

Molecular identification using the 16S rRNA gene confirmed all *P. mirabilis* isolates in the present study. This supports the observations of Saleh et al **(23)**, who emphasized the reliability of this marker for species recognition in *Proteus* spp. from UTIs. In contrast, Abed and Shareef **(24)** reported closer similarity of local isolates to global reference strains deposited in GenBank, highlighting potential genetic diversity among strains from different regions.

The antibiotic susceptibility testing revealed a worrying resistance profile. All isolates were resistant to ampicillin (100%), consistent with earlier studies **(25)**. Ciprofloxacin exhibited moderate effectiveness (50% sensitivity), aligning with a 60% sensitivity rate reported elsewhere **(26)**. Resistance rates to trimethoprim (50%) and gentamicin (30%) were also comparable with previously published data **(27, 28)**. Similarly, nalidixic acid resistance (50%) was in line with findings from Najaf (47.5%) and Diyala (44%) **(25, 28)**. Such variations in antimicrobial resistance patterns likely reflect geographic and environmental factors, infection type, and hospital sanitation practices **(29–33)**.

A major contribution of this study was the demonstration of the potent antibacterial activity of trimethoprim nanoemulsion. The mean inhibition zone diameter (41.5 ± 1.2 mm) was significantly greater than that of trimethoprim alone ($p < 0.001$). This enhancement is likely due to the nanoscale droplet size, which increases surface area, promotes drug penetration across bacterial membranes, and may disrupt biofilm structures. Comparable synergistic effects of nanoemulsions with antibiotics and essential oils have been reported in prior studies **(8, 34)**.

Stability studies confirmed the suitability of the formulation. FTIR spectra revealed no significant interactions between trimethoprim and excipients, indicating chemical compatibility, while FESEM imaging showed spherical droplets with uniform distribution, consistent with reports on other antimicrobial nanoemulsion systems **(35, 36)**. Together, these findings highlight the potential of nanoformulation technology to enhance drug stability and therapeutic performance.

Despite these promising results, the study is limited to *in vitro* assays. Therefore, caution must be exercised when extrapolating these findings to clinical applications. Further investigations, including *in vivo* studies and clinical trials, are necessary to validate efficacy, assess toxicity and pharmacokinetics, and evaluate the risk of resistance development under clinical conditions.

## 5. Conclusion

This study demonstrated that trimethoprim nanoemulsion exhibits significantly greater antibacterial activity against *P. mirabilis* than pure trimethoprim under *in vitro* conditions. These results underscore the potential of nanoemulsion-based formulations as innovative therapeutic approaches to improve antibiotic efficacy and combat multidrug resistance. However, given the limitations of the current design, the findings should be interpreted cautiously. Comprehensive *in vivo* experiments and well-controlled clinical trials are required to establish safety, pharmacokinetics, pharmacodynamics, and therapeutic potential. If validated, trimethoprim nanoemulsion could represent a critical step toward addressing the global challenge of antimicrobial resistance.

## 6. Declarations

### 6.1 Acknowledgment

None.

### 6.2 Ethical Considerations

This study was approved by the Ethics Review Board of Azadi Teaching Hospital and the Ibrahim Scientific Consulting Office (Approval No. 1). Ethical approval was granted on May 30, 2024, prior to study initiation. All procedures were conducted in accordance with ethical standards for research involving human participants.

### 6.3 Authors' Contributions

All work was conducted solely by Sarah A. Hasan Ali.

### 6.4 Conflict of Interests

The author declares no conflict of interest.

### 6.5 Financial Support and Sponsorship

None.

### 6.6 Using Artificial Intelligence Tools (AI Tools)

No AI-assisted tools were used in the design, execution, or writing of this study.

## References


1. Yang A, Tian Y, Li X. Unveiling the hidden arsenal: new insights into Proteus mirabilis virulence in UTIs. Front Cell Infect Microbiol. 2024;14:1465460. [PMID] [PMCID] [DOI:10.3389/fcimb.2024.1465460]
2. Zhang S, Li Q, Wang M, Jia R, Chen S, Liu M, et al. Genomic analysis of Proteus mirabilis: Unraveling global epidemiology and antimicrobial resistance dissemination - emerging challenges for public health and biosecurity. Environ Int. 2025;199:109487. [DOI:10.1016/j.envint.2025.109487] [PMID]
3. Sujith S, Solomon AP, Rayappan JBB. Comprehensive insights into UTIs: from pathophysiology to precision diagnosis and management. Front Cell Infect Microbiol. 2024;14:1402941. [PMID] [PMCID] [DOI:10.3389/fcimb.2024.1402941]
4. Jamil RT, Foris LA, Snowden J. Proteus mirabilis Infections. StatPearls. Treasure Island (FL): StatPearls Publishing Copyright © 2022, StatPearls Publishing LLC.; 2022.
5. Nissanka MC, Dilhari A, Munasinghe JA, Priyadarshana G, Bandara K, Munaweera I, et al. Decoding Proteus mirabilis biofilms: expression of selected virulence genes and association with antibiotic resistance. BMC Microbiol. 2025;25(1):481. [PMID] [PMCID] [DOI:10.1186/s12866-025-04212-z]
6. Jacob S, Kather FS, Boddu SHS, Shah J, Nair AB. Innovations in Nanoemulsion Technology: Enhancing Drug Delivery for Oral, Parenteral, and Ophthalmic Applications. Pharmaceutics. 2024;16(10):1333. [PMID] [PMCID] [DOI:10.3390/pharmaceutics16101333]
7. Pandey P, Gulati N, Makhija M, Purohit D, Dureja H. Nanoemulsion: A Novel Drug Delivery Approach for Enhancement of Bioavailability. Recent Pat Nanotechnol. 2020; 14(4):276-93. [DOI:10.2174/1872210514666200604145755] [PMID]
8. Garcia CR, Malik MH, Biswas S, Tam VH, Rumbaugh KP, Li W, et al. Nanoemulsion delivery systems for enhanced efficacy of antimicrobials and essential oils. Biomater Sci. 2022;10(3):633-53. [DOI:10.1039/D1BM01537K] [PMID]
9. Pernille H, Lars B, Marjukka M, Volkert S, Anne H. Sampling of urine for diagnosing urinary

tract infection in general practice - First-void or mid-stream urine?. Scand J Prim Health Care. 2019;37(1):113-9. [PMID] [PMCID] [DOI:10.1080/02813432.2019.1568708]

10. Mirzaei A, Habibi M, Bouzari S, Asadi Karam MR. Characterization of Antibiotic-Susceptibility Patterns, Virulence Factor Profiles and Clonal Relatedness in Proteus mirabilis Isolates from Patients with Urinary Tract Infection in Iran. Infect Drug Resist. 2019;12:3967-79. [DOI:10.2147/IDR.S230303] [PMID] [PMCID]
11. Zhang W, Niu Z, Yin K, Liu P, Chen L. Quick identification and quantification of Proteus mirabilis by polymerase chain reaction (PCR) assays. Ann Microbiol. 2012;63(2):683-9. [DOI:10.1007/s13213-012-0520-x]
12. Aleman-Duarte MI, Aguilar-Uscanga BR, Garcia-Robles G, Ramirez-Salazar FJ, Benitez-Garcia I, Balcazar-Lopez E, et al. Improvement and Validation of a Genomic DNA Extraction Method for Human Breastmilk. Methods Protoc. 2023;6(2):34. [DOI:10.3390/mps6020034] [PMID] [PMCID]
13. Matuschek E, Copsey-Mawer S, Petersson S, Ahman J, Morris TE, Kahlmeter G. The European committee on antimicrobial susceptibility testing disc diffusion susceptibility testing method for frequently isolated anaerobic bacteria. Clin Microbiol Infect. 2023;29(6):795 e1- e7. [DOI:10.1016/j.cmi.2023.01.027] [PMID]
14. Cai D, Ye Y, Song J, Liu S, Zeng X, Zhu B, et al. A chitosan-modified tea tree oil self-nanoemulsion improves antibacterial effects in vivo and in vitro and promotes wound healing by influencing metabolic processes against multidrug-resistant bacterial infections. Int J Biol Macromol. 2024;281(Pt 3):136404. [PMID] [DOI:10.1016/j.ijbiomac.2024.136404]
15. Confessor MVA, Agreles MAA, Campos LAA, Silva Neto AF, Borges JC, Martins RM, et al. Olive oil nanoemulsion containing curcumin: antimicrobial agent against multidrug-resistant bacteria. Appl Microbiol Biotechnol. 2024;108(1):241. [PMID] [PMCID] [DOI:10.1007/s00253-024-13057-x]
16. Bunaciu AA, Hoang VD, Aboul-Enein HY. Fourier transform infrared spectroscopy used in drug excipients compatibility studies. Applied Spectroscopy Reviews. 2024;60(5): 385-403. [DOI:10.1080/05704928.2024.2438747]
17. Saleh AH. Potential effect of green zinc oxide nanoparticles in treatment of kidney lesions that induced by Burkholderia mallei in albino male rats. Biochem Cel Arch. 2019;19:2439-43.
18. Talebi A, Momtaz H, Tajbakhsh E. Frequency distribution of virulence factors and antibiotic resistance genes in uropathogenic Proteus species isolated from clinical samples. Lett Appl Microbiol. 2023;76(2):ovac043. [DOI:10.1093/lambio/ovac043] [PMID]
19. Veisi M, Hosseini-Nave H, Tadjrobehkar O. Biofilm formation ability and swarming motility are associated with some virulence genes in Proteus mirabilis. BMC Microbiol. 2025;25(1):388. [PMID] [PMCID] [DOI:10.1186/s12866-025-04090-5]
20. Fakhraddin Raheem T, Ahmed Hasan Ali S. Prevalence and Multi-Drug Resistance Patterns of Uropathogenic E.coli isolated from Women Patients in Kirkuk city, Iraq. Iran J Med Microbiol. 2022;16(6):609-14. [DOI:10.30699/ijmm.16.6.609]
21. Ahmed Hasan S, Mohammed Bakr M. Bacteriological and Molecular Detection of Klebsiella oxytoca and its Resistance to Antibiotics among Clinical Specimens from Kirkuk, Iraq. Arch Razi Inst. 2022;77(5):1521-5.
22. Ahmed Hasan S, Fakhraddin Raheem T, Mohammed Abdulla H. Phenotypic, Antibiotyping, and Molecular Detection of Klebsiella Pneumoniae Isolates from Clinical Specimens in Kirkuk, Iraq. Arch Razi Inst. 2021; 76(4):1061-7.
23. Saleh TH, Hashim ST, Malik SN, Al-Rubaii BAL. Down-Regulation of fliL Gene Expression by Ag Nanoparticles and TiO2 Nanoparticles in Pragmatic Clinical Isolates of Proteus mirabilis and Proteus vulgaris from Urinary Tract Infection. Nano Biomed Eng. 2019;11(4):321-32. [DOI:10.5101/nbe.v11i4.p321-332]
24. Abed MK, Shareef HK. Isolation and Molecular Identification of proteus mirabilis isolated from hospitals in the capital Baghdad. Indian J Forensic Med Toxicol. 2021;15(1):2216-23.
25. Abdul-hussein B, Hassan BA. Detection of MDR Proteus mirabilis Genes Isolates From Urinary Tract Infection. BioGecko. 2023;12: 2230-5807.
26. Kwiecinska-Pirog J, Skowron K, Zniszczol K, Gospodarek E. The assessment of Proteus mirabilis susceptibility to ceftazidime and ciprofloxacin and the impact of these

antibiotics at subinhibitory concentrations on Proteus mirabilis biofilms. Biomed Res Int. 2013;2013(1):930876. [DOI:10.1155/2013/930876] [PMID] [PMCID]

27. Alhusayni AA, Al-Khikani FHO, Aljaburi HK, Alkareawiu BAA, Abadi RM. Antibiotic susceptibility profile of bacterial uropathogens in Al-Shomali General Hospital, Babylon, Iraq. J Prev Diagn Treat Strateg Med. 2022;1(4):240-5. [DOI:10.4103/jpdtsm.jpdtsm_45_22]

28. Abed Gumar E, Salim Hamzah A, Fadhil Hamad W. Study of Some Resistance Genes in Clinical Proteus mirabilis. Arch Razi Inst. 2022;77(6): 2235-42.

29. Hasan SA, Raoof WM, Ahmed KK. Antibacterial activity of deer musk and Ziziphus spina-christi against carbapebem resis-tant gram negative bacteria isolated from patients with burns and wounds. Regul Mech Biosyst. 2024;15(2):267-78. [DOI:10.15421/022439]

30. Hasan S, Raoof W, Ahmed K. First report of co-harboring bleomycin resistance gene (bleMBL) and carbapenemase resistance gene (blaNDM-1) Klebsiella pneumoniae in Iraq with comparison study among the sensitivity test, the BD PHOENIX CPO derect test, and the RAPIDEC® CARBA NP test. Sib J Life Sci Agric. 2025;16(4):208-37. [DOI:10.12731/2658-6649-2024-16-4-1249]

31. Majeed ZH, Hasan SA, Ismail RM. Evaluate the benefit effects of Dodonaea viscosa in kidney of infected rats with Staphylococcus aureus. J Pharm Negat Results. 2022;13(3):291. [DOI:10.47750/pnr.2022.13.03.046]

32. Hasan SA, Abass KS. Prevalence of Gram Negative Bacteria Isolated from Patients with Burn Infection and their Antimicrobial Susceptibility Patterns in Kirkuk City, Iraq. Indian J Public Health Res Dev. 2019;10(8): 2197.[DOI:10.5958/0976-5506.2019.02184.3]

33. Hasan SA. Drug resistant bacteria and antimicrobial activity of medicinal plants. Int J Nutrology. 2025;18(3):e25312. [DOI:10.54448/ijn25312]

34. Lila ASA, Rajab AAH, Abdallah MH, Rizvi SMD, Moin A, Khafagy ES, et al. Biofilm Lifestyle in Recurrent Urinary Tract Infections. Life (Basel). 2023;13(1):148. [DOI:10.3390/life13010148] [PMID] [PMCID]

35. Chen H, Zhong Q. Physical and antimicrobial properties of self-emulsified nanoemulsions containing three synergistic essential oils. Int J Food Microbiol. 2022;365:109557. [PMID] [DOI:10.1016/j.ijfoodmicro.2022.109557]

36. Kowalczuk D. FTIR Characterization of the Development of Antimicrobial Catheter Coatings Loaded with Fluoroquinolones. Coatings. 2020;10(9):818. [DOI:10.3390/coatings10090818]