

Prevalence and Antibiotic Susceptibility of *Citrobacter* spp. in Patients with Urinary Tract Infections

Amina Shafique Sair ¹, Kinza Shafiq ¹, Nida Faryad ¹, Talha Mannan ¹

Abstract

Background

Citrobacter, a genus within the *Enterobacteriaceae* family, is an opportunistic pathogen increasingly recognized as a causative agent of both hospital-acquired and community-acquired urinary tract infections (UTIs), particularly among immunocompromised patients and neonates.

Objective

To determine the prevalence of *Citrobacter* species in patients presenting with UTIs and to evaluate the antibiotic susceptibility pattern of *Citrobacter* isolates.

Methods

Urine cultures collected at the Microbiology Laboratory, Shalamar Hospital, Lahore, over a two-year period (January 2021–December 2022) were retrospectively reviewed. Identification of isolates was carried out using standard laboratory protocols, and susceptibility testing was conducted by the Kirby–Bauer disk diffusion method following CLSI standards.

Results

Of 7074 urine samples, 1979 (27.9%) showed significant bacterial growth. *Citrobacter* spp. were isolated in 63 cases (3.1%), while non-*Citrobacter* organisms accounted for 1916 cases (96.9%). Among *Citrobacter* isolates, 35 (55.6%) were from females and 28 (44.4%) from males (female-to-male ratio: 1.25:1). The most effective antimicrobial agents against *Citrobacter* spp. were amikacin (90.5% sensitivity), meropenem (90%), and imipenem (89%).

Conclusion

Citrobacter spp. represent a clinically significant cause of UTIs and exhibit multidrug resistance, underscoring their role as important nosocomial pathogens. Strict infection-control practices and judicious use of urinary instrumentation are essential to reduce their spread.

Keywords: *Citrobacter* spp.; Antibiotic susceptibility; Urinary tract infection

Affiliations:

¹ Shalamar Medical & Dental College, Lahore, Pakistan

Email: tmannan87@gmail.com

Corresponding Author: Talha Mannan

Introduction

Urinary tract infection (UTI) is defined as the invasion and proliferation of microorganisms within the urinary tract, including the kidneys, ureters, bladder, and urethra. Virulence factors contributing to infection include pili, lipopolysaccharides, capsules, and toxins [1]. Common symptoms are frequency, dysuria, and flank or lower abdominal pain. UTIs are more prevalent in females due to their shorter urethra and anatomical proximity to the rectum [2].

Bacteria are responsible for more than 95% of UTIs, with Gram-negative species accounting for 80–85% and Gram-positive species for 15–20% [3].

While *Escherichia coli* remains the predominant uropathogen, *Citrobacter* isolates rank among the most frequent hospital-acquired pathogens, after *E. coli* and *Proteus* spp., contributing to approximately 9.4% of isolates [4].

Citrobacter are the aerobic as well as Gram-negative bacilli belonging to the family of *Enterobacteriaceae*. Extensively dispersed in soil, food, water and the (gastrointestinal) tracts (of humans and animals, these organisms can utilize citrate as their sole carbon source [5]. Clinically important species include *Citrobacter freundii*, *Citrobacter koseri*, and *Citrobacter amalonaticus* [6]. They have been implicated in both community-acquired and healthcare-associated UTIs [7].

Beyond UTIs, *Citrobacter* spp. can cause a spectrum of infections including bloodstream infections, pneumonia, wound infections, meningitis, endocarditis, and peritonitis [8]. A study from the All-India Institute of Medical Sciences (AIIMS), New Delhi, reported *Citrobacter* isolation



rates of 46.2% from urine, 16.3% from respiratory specimens, 15.8% from blood, 12.1% from pus, and 9.3% from sterile body fluids [9]. Risk factors for infection include prolonged hospitalization, prior antibiotic exposure, indwelling medical devices (e.g., catheters, ventilators), underlying comorbidities (e.g., diabetes, cancer, burns), and immunosuppression [10].

Geographical variation in prevalence has been documented. Studies report prevalence rates of 2–7.5% in Latin America [11], 12.7% in China [12], 1.6% in Iran [13], and 10.5% in Pakistan (Abbottabad) [14].

Alarming, *Citrobacter* spp. are emerging as multidrug-resistant nosocomial pathogens, particularly in developing countries. They often exhibit resistance to penicillins, cephalosporins, aminoglycosides, and fluoroquinolones, the mainstay therapies for UTIs, while showing variable sensitivity to agents like cefazolin and amoxiclavulanate. Furthermore, their association with extended-spectrum β -lactamase (ESBL) production and multidrug resistance emphasizes the need for close monitoring of their susceptibility profiles. Once considered commensals, *Citrobacter* spp. must now be recognized as clinically significant uropathogens with increasing therapeutic challenges.

Methodology

This retrospective cohort study was conducted in the Microbiology Laboratory of Shalamar Hospital, Lahore, after approval from the Shalamar School of Allied Health Sciences Institutional Review Board (SSAHS-IRB/AL/18/2023).

Study Design and Sampling

Samples were collected using a non-probability convenience sampling technique. Data were obtained from patients presenting to Shalamar Hospital with clinical suspicion of urinary tract infection (UTI) between January 2021 and December 2022. The study included male and female patients across all age groups, while individuals with recent antibiotic exposure (≤ 3 days), recurrent UTIs, or missing clinical data were

excluded.

Microbiological Processing

Urine cultures were processed according to standard operating procedures. Plates were incubated at 37 °C for 18–24 hours. Colony growth was examined for size, color, shape, translucency, surface elevation, and margins. Gram staining was performed, followed by assessment of cultural characteristics including lactose fermentation, odor, and growth patterns.

Bacterial identification was carried out using the oxidase test and a panel of biochemical tests: Triple Sugar Iron (TSI), indole, citrate utilization, and urease.

Antimicrobial Susceptibility Testing

Susceptibility testing was carried out on Mueller–Hinton agar following the Kirby–Bauer disk diffusion procedure. Inocula were standardized to 0.5 McFarland density, spread over agar plates, and incubated at 37 °C for 18–24 h after disc placement. Results were interpreted according to CLSI recommendations (2020).

The following antibiotics were tested against *Citrobacter* spp.: amoxicillin-clavulanic acid, amikacin, ampicillin, ciprofloxacin, gentamicin, cefotaxime, cefoxitin, nitrofurantoin, fosfomycin, imipenem, meropenem, co-trimoxazole, and piperacillin-tazobactam.

Data Analysis: All analyses were carried out in IBM SPSS Statistics version 20. Descriptive statistics were used: categorical variables were summarized as proportions, and continuous variables were described using means and standard deviations. A threshold of $p \leq 0.05$ was applied to determine statistical significance.

Results

A total of 7074 urine samples were processed during the study period. Significant bacteriuria was observed in 1979 samples (27.9%).

Citrobacter spp. accounted for 63 isolates (3.1%), while non-*Citrobacter* organisms represented 1916 isolates (96.9%) (Table 1).

Table 1. Isolation pattern of Uropathogens from urine cultures

Organisms isolated	No. of organisms	Isolation rate
<i>Citrobacter</i> spp.	63	3.1%
Non- <i>Citrobacter</i> spp.	1916	27.0%
Negative	5095	72.0%
Total	7074	100%

Among all culture-positive samples, the most frequent monomicrobial isolates were *Escherichia coli* (1000, 50.5%), *Enterococcus* spp. (409, 20.6%), *Klebsiella* spp. (201, 10.3%), *Pseudomonas* spp. (87, 4.4%), *Citrobacter* spp. (63,

3.1%), and *Candida* spp. (49, 2.6%). Polymicrobial growth was observed in 170 cases, most commonly *E. coli* with *Staphylococcus* spp. (100, 5.0%) and *E. coli* with *Streptococcus* spp. (70, 3.6%) (Table 2, Figure 1).

Table 2. Distribution of Uropathogens among culture-positive samples

Uropathogens	Frequency	Percentage
<i>Escherichia coli</i>	1000	50.5%
<i>Enterococcus</i> spp.	409	20.6%
<i>Klebsiella</i> spp.	201	10.3%
<i>E. coli</i> + <i>Staphylococcus</i> spp.	100	5.0%
<i>Pseudomonas</i> spp.	87	4.4%
<i>E. coli</i> + <i>Streptococcus</i> spp.	70	3.6%
<i>Citrobacter</i> spp.	63	3.1%
<i>Candida</i> spp.	49	2.6%
Total	1979	100%

Gender distribution of *Citrobacter* isolates

Among the 63 *Citrobacter* isolates, 35 (55.6%)

were from females and 28 (44.4%) from males, yielding a female-to-male ratio of 1.25:1 (Table 3).

Table 3. Gender-wise distribution of *Citrobacter* spp. isolates

Gender	Frequency	Percentage
Female	35	55.6%
Male	28	44.4%
Total	63	100%

Age distribution

The mean age of patients with *Citrobacter*-positive UTIs was 53.7 years (range: 2 months–100 years). Isolation rates were highest among elderly female patients, whereas the lowest rates were observed in younger adults.

Antimicrobial susceptibility

Antibiotic susceptibility testing revealed a high rate of multidrug resistance. The most effective agents against *Citrobacter* spp. were amikacin (90.5% sensitivity), meropenem (90%), imipenem (89%), and gentamicin (80.1%). In contrast, the highest resistance was observed against amoxicillin (95%) and ciprofloxacin (74%) (Table 4).

Table 4. Antimicrobial susceptibility profile of *Citrobacter* spp.

Antibacterial agent	Sensitive (%)	Resistant (%)
Ciprofloxacin	26.0	74.0
Ceftriaxone	42.9	57.0
Cefuroxime	47.2	52.8
Co-trimoxazole	61.1	38.8
Nitrofurantoin	70.1	29.9
Piperacillin–tazobactam	71.4	28.6
Fosfomycin	73.9	26.0
Gentamicin	80.1	19.9
Amikacin	90.5	9.5
Imipenem	89.0	11.0
Meropenem	90.0	10.0

Discussion

A total of 7,074 urine samples were received in the Microbiology Laboratory of Shalamar Hospital for culture and sensitivity testing. Among all the tested samples, 1,979 (27.9%) cultures showed significant growth of bacteria. The present study is in agreement with many similar studies that have shown the overall prevalence of UTI as 19.6% and 30.45%, respectively [15]. In our study, the prevalence of *Citrobacter* species as uropathogens was found to be 3.1%, and this rate is in accordance with a similar study conducted in Khyber Teaching Hospital (KTH), Peshawar, which documented the frequency of *Citrobacter* as 4.4% [16]. This result was lower than that observed in a previous study carried out at the Microbiology Laboratory of the Virgen de las Nieves University Hospital in Granada, which reported the frequency of *Citrobacter* as 10.3% [17]. However, it was higher than the study conducted at the Department of Microbiology at Safdarjung Hospital, New Delhi, India, which showed the total prevalence rate of 1.4% [18]. The proportion of UTI cases in females was lowest in the adult age group (under 40 years) and highest in the older age group (60–80 years). The possible explanation for this high number in older females could be urinary catheterization, prolonged hospital stay, prior use of antibiotics, and immunosuppression. In our study, 55.6% of all isolates were from women (female-to-male ratio of 1.25:1). Some other studies have also documented

the same findings. For example, a study conducted at Safdarjung Hospital, New Delhi, India, showed that the vast majority (97.6%) of all samples were received from females, while only 2.4% were from males [18]. In this study, the results revealed that *Citrobacter* isolates were most susceptible to Amikacin, Meropenem, and Imipenem. Khan I, et al., carried out an experimental study at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, and showed that 100% of *Citrobacter* were sensitive to Fosfomycin [19]. However, this susceptibility rate is higher than in our study, in which the susceptibility rate of Fosfomycin was 73.9%. Our study revealed that the *Citrobacter* isolates were most resistant to Ciprofloxacin (74%), Ceftriaxone (57%), and Cefuroxime (52.7%). In another study by Shinu P, et al., at the M.M. Institute of Medical Sciences and Research, Ambala, Haryana, India, the most resistant anti-biotics to *Citrobacter* were Ciprofloxacin (21.6%) and Gentamycin (16.25%) [20]. Imipenem, Meropenem, and Nitrofurantoin demonstrated high in vitro activity against *Citrobacter* isolates. While carbapenems remain valuable for severe or complicated infections, Nitrofurantoin may serve as an appropriate oral option for ambulatory patients. The high resistance rates observed in our study are likely attributable to inappropriate antibiotic prescribing practices, including suboptimal dosing and incomplete treatment courses. There are some limitations in our study, which include the fact that *Citrobacter* was

not identified at the species level. Therefore, no information is presented regarding the resistance trends in isolated *Citrobacter* spp.

Conclusion

This study demonstrates that *Citrobacter* species, though traditionally considered of low virulence, are emerging as clinically significant uropathogens with concerning resistance trends. In particular, high resistance to commonly prescribed antibiotics such as fluoroquinolones and cephalosporins underscores the need for cautious empirical therapy. To mitigate the rise of resistant *Citrobacter* infections, tertiary care hospitals should enforce antibiotic stewardship programs, strengthen infection prevention practices, and emphasize

rational antibiotic prescribing. Routine surveillance and species-level identification are also recommended to guide more effective therapeutic strategies.

Funding

The authors did not receive any financial assistance, grants, or external funding for the design, execution, or publication of this study.

Conflict of Interest

All authors affirm that there are no financial or non-financial conflicts of interest that could have influenced the conduct or reporting of this study.

References

1. Soujanya BR, Banashankari G. Phenotypic detection of virulence factors of uropathogenic Enterobacteriaceae. *J Pure Appl Microbiol.* 2023;17(2). doi:10.22207/JPAM.17.2.22.
2. Minardi D, d'Anzeo G, Cantoro D, Conti A, Muzzonigro G. Urinary tract infections in women: etiology and treatment options. *Int J Gen Med.* 2011;4:333-43. doi:10.2147/IJGM.S11767. PMID:21674026; PMCID:PMC3108201.
3. Adugna B, Sharew B, Jemal M. Bacterial profile, antimicrobial susceptibility pattern, and associated factors of community- and hospital-acquired urinary tract infection at Dessie Referral Hospital, Northeast Ethiopia. *Int J Microbiol.* 2021;2021:5553356. doi:10.1155/2021/5553356. PMID:34589128; PMCID:PMC8476241.
4. Bouza E, San Juan R, Muñoz P, Voss A, Kluytmans J, et al. A European perspective on nosocomial urinary tract infections I: report on microbiology workload, etiology and antimicrobial susceptibility (ESGNI-003 study). *Clin Microbiol Infect.* 2001;7(10):523-31. doi:10.1046/j.1198-743x.2001.00326.x. PMID:11683792.
5. Kumari R, Dey JB, Jana A, Ghosh T, Tudu NK. *Citrobacter* emerging as a common uropathogen in pediatric population. *Int J Sci Study.* 2018;6(4):29-33. doi:10.17354/ijss/2018/200.
6. Yuan C, Yin Z, Wang J, Qian C, Wei Y, Zhang S, et al. Comparative genomic analysis of *Citrobacter* and key genes essential for the pathogenicity of *Citrobacter koseri*. *Front Microbiol.* 2019;10:2774. doi:10.3389/fmicb.2019.02774. PMID:31866966; PMCID:PMC6908497.
7. Lee R, Choi SM, Jo SJ, Lee J, Cho SY, Kim SH, et al. Clinical characteristics and antimicrobial susceptibility trends in *Citrobacter* bacteremia: an 11-year single-center experience. *Infect Chemother.* 2019;51(1):1-9. doi:10.3947/ic.2019.51.1.1. PMID:30941932; PMCID:PMC6446011.
8. Liu L, Zhang L, Zhou H, Yuan M, Hu D, Wang Y, et al. Antimicrobial resistance and molecular characterization of *Citrobacter* spp. causing extraintestinal infections. *Front Cell Infect Microbiol.* 2021;11:737636. doi:10.3389/fcimb.2021.737636.
9. Mohanty S, Singhal R, Sood S, Dhawan B, Kapil A, Das BK. *Citrobacter* infections in a tertiary care hospital in Northern India. *J Infect.* 2007;54(1):58-64. doi:10.1016/j.jinf.2006.01.015. PMID:16815552.
10. Fonton P, Hassoun-Kheir N, Harbarth S. Epidemiology of *Citrobacter* spp. infections among hospitalized patients: a systematic review and meta-analysis. *BMC Infect Dis.* 2024;24:662. doi:10.1186/s12879-024-09575-8.
11. Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Cisneros JM, Peña C, et al. Risk factors and prognosis of nosocomial bloodstream infections caused by ESBL-producing *Escherichia coli*. *J Clin Microbiol.* 2010;48(5):1726-31. doi:10.1128/JCM.02353-09. PMID:20181897; PMCID:PMC2863889.
12. Ding JG, Sun QF, Li KC, Zheng MH, Miao XH, Ni W, et al. Retrospective analysis of nosocomial infections in the ICU of a tertiary hospital in China during 2003–2007. *BMC Infect Dis.* 2009;9:115. doi:10.1186/1471-2334-9-115. PMID:19630992; PMCID:PMC2722662.
13. Khoshbakht R, Salimi A, Shirzad Aski H, Keshavarzi H. Antibiotic susceptibility of bacterial strains isolated from urinary tract infections in Karaj, Iran. *Jundishapur J Microbiol.* 2012;6(1):86-90. doi:10.5812/jjm.4830.
14. Rizvi M, Fatima N, Shukla I, Malik A. Epidemiology of extended spectrum beta-lactamases in *Serratia* and *Citrobacter* species in North India. *Indian J Pathol Microbiol.* 2010;53(1):193-4. doi:10.4103/0377-4929.59237. PMID:20090276.
15. Sami H, Sultan A, Rizvi M, Khan F, Ahmad S, Shukla I, et al. *Citrobacter* as a uropathogen: its prevalence and antibiotic susceptibility pattern. *CHRISMED J Health Res.* 2017;4(1):23-6. doi:10.4103/2348-3334.196037.
16. Khan S, Taj R, Rehman N, Ullah A, Khan I. Incidence and antibiogram of β -lactamase-producing *Citrobacter freundii* recovered from clinical isolates in Peshawar, Pakistan. *Pak J Zool.* 2020;52(5):1877-82. doi:10.17582/journal.pjz/20181118151126.



17. Jiménez-Guerra G, Borrego-Jiménez J, Gutiérrez-Soto B, Expósito-Ruiz M, Navarro-Marí JM, Gutiérrez-Fernández J. Susceptibility evolution to antibiotics of *Enterobacter cloacae*, *Morganella morganii*, *Klebsiella aerogenes* and *Citrobacter freundii* in UTIs: an 11-year surveillance study. *Enferm Infecc Microbiol Clin*. 2020;38(4):166-9. doi:10.1016/j.eimc.2019.07.010. PMID:31606242.
18. Gupta A, Sharma B. Recent trends in uropathogenic infections in patients of a tertiary care center, New Delhi, India. *MIR J*. 2023;10(1):39-44. doi:10.18527/2500-2236-2023-10-1-39-44.
19. Khan IU, Mirza IA, Ikram A, Ali S, Hussain A, Ghafoor T. In vitro activity of fosfomycin tromethamine against ESBL-producing urinary tract bacteria. *J Coll Physicians Surg Pak*. 2014;24(12):914-7. PMID:25523727.
20. Shinu P. Antimicrobial resistance, phenotypic characteristics, and biofilm production in *Citrobacter freundii* isolates from urinary tract infections. *J Pharmacol Pharmacother*. 2023;13(4):375-81. doi:10.1177/0976500X221147747.

