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Original Research Article

ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE TRENDS IN CITROBACTER FREUNDII AND CITROBACTER KOSERI

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Abstract

Background: Citrobacter species, notably Citrobacter freundii and Citrobacter koseri, are opportunistic pathogens linked to hospital-acquired infections, particularly in immunocompromised patients. These species are known for their ability to produce extended-spectrum beta-lactamases (ESBLs) and carbapenemases, contributing to growing concerns over antibiotic resistance. Understanding their resistance patterns is crucial for effective treatment. This study aimed to assess the antibiotic resistance profiles of C. freundii and C. koseri isolated from clinical samples. **Materials and Methods:** This prospective observational study was conducted over 1 year and 10 months, involving 150 Citrobacter isolates from clinical samples (pus, urine, blood, sputum, and tracheal aspirates) collected in a hospital setting. Isolates were identified using standard biochemical methods, and antimicrobial susceptibility was tested by the Kirby-Bauer disc diffusion method. The presence of ESBL production was assessed using the combination disc method, while carbapenemase production was detected using the Modified Hodge Test (MHT). Data were analyzed using simple percentage calculations. **Results:** Of the 150 isolates, C. koseri accounted for 61.33%, and C. freundii for 38.67%. Pus samples yielded the highest number of isolates. C. freundii exhibited significant resistance to ampicillin, ciprofloxacin, and ceftazidime, while C. koseri was more susceptible to most antibiotics, including meropenem and amikacin. ESBL production was observed in 32% of isolates, with C. freundii being more prevalent. Additionally, 4.7% of isolates were carbapenemase producers, all identified as C. freundii. **Conclusion:** This study highlights the diverse resistance patterns of C. freundii and C. koseri, particularly the higher resistance of C. freundii to several antibiotics. The findings emphasize the importance of continuous surveillance and antibiotic stewardship to manage these infections.

INTRODUCTION

Citrobacter species are abundant in the environment, and have traditionally been considered low-virulence pathogens, producing infections less frequently.[1] Citrobacter species are gram-negative, facultative anaerobic bacilli that are members of the tribe Citrobactereae and family Enterobacteriaceae. It can be found single or in pairs, and its motile peritrichous flagella usually use citrate as its only carbon source.[2] Hospital infections can be caused by the bacterium Citrobacter. It is frequently associated with brain infections, pneumonia, gastrointestinal infections, bloodstream infections, and urinary tract infections. Newborns may also be affected, developing joint infections, sepsis, and meningitis.[3] As per a comprehensive surveillance research carried out in US healthcare facilities, Citrobacter infections accounted for 0.8% of Gram-negative infections and 3–6% of all Enterobacterales isolates in hospital settings.[4]

Among the eleven species—Citrobacter freundii, Citrobacter koseri, Citrobacter amalonaticus, Citrobacter farmeri, Citrobacter youngae, Citrobacter braakii, Citrobacter werkmanii, Citrobacter sedlakii, Citrobacter rodentium, Citrobacter gillenii, and

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Citrobacter murliniae—Citrobacter koseri (previously named C. diversus), C. youngae, C. braakii, and C. amalonaticus are the most frequently isolated from human clinical specimens.[5] The two main opportunistic pathogens, C. koseri and C. freundii, are clearly responsible for the majority of Citrobacter infections, with over 80% of patients having underlying medical conditions such as diabetes, cardiovascular disease, renal disease, leukaemia, neurologic disease, or urinary tract abnormalities.[6,7]

Studies on antibiotic resistance in Citrobacter isolates have been developing along with the increasing amount of data on hospital infections caused by Citrobacter spp., including studies on carbapenemase-producing and Amp C betalactamase strains.[8]

The aim of this study was to identify the spectrum of the two most prevalent Citrobacter species, Citrobacter freundii and Citrobacter koseri, from clinical samples and their susceptibility pattern. Since these bacteria are commonly associated with hospital infections, especially in immunocompromised patients, understanding their resistance pattern to various antibiotics is essential. The findings from this study will aid clinicians in selecting effective antibiotics and improving patient care.

MATERIALS AND METHODS

The present study was prospective observational study conducted over a period of 1 year and 10 months. All the clinical samples received in the department of microbiology were processed as per standard guidelines. A total of 150 isolates of Citrobacter species were identified from various clinical samples. Citrobacter spp. was identified by the conventional biochemical methods.[9]

Antimicrobial susceptibility testing was done for all the isolates using Kirby-Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) M2-A9.[10]

ESBL production was detected phenotypically using the combination disc method with ceftazidime/clavulanate and plain ceftazidime discs. A positive result was confirmed if there was a ≥5 mm increase in the zone of inhibition around the ceftazidime/clavulanate disc compared to the plain ceftazidime disc, indicating the presence of ESBL production.[11]

The Modified Hodge Test (MHT) was performed to detect carbapenemase production. An inoculum of E. coli (ATCC 25922), which is susceptible to carbapenems, was streaked on an agar plate. A carbapenem disc (e.g., imipenem) was placed at the center of the plate. The test strain was then streaked in a straight line from the edge of the carbapenem disc to the edge of the plate. After incubation, the plate was examined for the formation of a "cloverleaf" pattern of growth around the streaked area, which would indicate a positive result for carbapenemase production.[12]

Samples on standard microbiological culture media demonstrating Citrobacter species growth were included in the study. Samples with co infections where Citrobacter species were not the predominant bacteria and growth from patients receiving antibiotics prior to sample collection or those with incomplete clinical data in requisition form were excluded from the study.

Data was analysed by using simple percentage method.

RESULTS

Out 150 isolates, C.koseri (61. 33%) was found to the most common Citrobacter species followed by C. freundii (38.67%). In the present study, majority of samples yielded Citrobacter freundii (84.48%)was found to be from in patients of our hospital. In the present study both species were predominantly observed among male patients (63.3%) followed by female patients(63.3%). In the present study, C. freundii was more common in the 31–40 age range, but C. koseri typically more common in younger age groups, particularly those between 21 and 40. Both species' overall incidence tends to decline with age, with those over 70 years old seeing the lowest prevalence. [Table 1]

Table 1: Age wise distribution of isolates.

Age in years	C. freundii	C.koseri
1-10	7(12.1%)	5(5.4%)
11-20	1(1.7%)	4(4.3%)
21-30	6(10.3%)	17(18.5%)
31-40	21(36.2%)	38(41.3%)
41-50	9(15.5%)	10(10.9%)
51-60	7(12.1%)	11(12%)
61-70	6(10.3%)	6(6.5%)
>70	1(1.7%)	1(1.1%)
Total	58(100%)	92(100%)

In our study, Both species were frequently isolated from pus samples, with C. koseri being slightly more prevalent. C. freundii was found in 21 samples (36.2%) and C. koseri in 31 samples (33.7%). C. freundii was more commonly found in urine samples compared to C. koseri . C. freundii was isolated in 17 samples (29.3%) and C. koseri in 21 samples (22.8%). C. freundii was present in 8 samples (13.8%) and C. koseri in 27 samples (29.3%). C. koseri was significantly more prevalent in sputum

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samples, indicating its stronger association with respiratory infections. [Table 2]

Table 2: Sample wise distribution of isolates

Sample	C. freundii	C.koseri
Pus	21(36.2%)	31(33.7%)
Urine	17(29.3%)	21(22.8%)
Sputum	8(13.8%)	27(29.3%)
Blood	8(13.8%)	10(10.9%)
Tracheal aspirate	2(3.4%)	2(2.2%)
BAL fluid	2(3.4%)	1(1.1%)

In our study, Citrobacter. koseri found to be more susceptible to different classes of antibiotics than C. freundii overall, especially to Piperacillin/Tazobactam, Meropenem, and Co-Trimoxazole. It was observed that C. freundii exhibits higher resistance to Ampicillin, Ciprofloxacin, and Ceftazidime, which is consistent with its higher beta-lactamase production.

All the strains of C.koseri exhibited susceptibility to Meropenem. Meropenem and Amikacin are reliable options for both species. Susceptibility pattern was found to be low against various third generation cephalosporins in the study. But cefoxitin, second generation cephalosporin remained as more susceptible to both species. [Table 3]

Table 3: Antibiotic susceptibility of citrobacter species

Antibiotic	C. freundii(n=58)	C.koseri(n=92)
Amikacin	47(81.03%)	81(88.04)
Ampicillin	9(15.5%)	21(22.8)
Piperacillin/Tazobactum	45(77.5%)	90(97.8)
Gentamicin	39(67.2%)	75(81.5)
Ciprofloxacin	22(37.9%)	67(72.8)
Meropenam	51(87.9%)	92(100)
CO-Trimaxazole	38(65.5%)	90(97.8)
Ceftazidime	31(53.4%)	72(78.2)
Ceftriazone	26(44.8%)	57(61.9)
Cefotaxime	29(50%)	42(45.7)
Cefoxitin	45(77.5%)	89(96.8)

In the present study, 48(32%) strains of Citrobacter species were found to be ESBL producing. Citrobacter freundii (24.67%) was observed as most common ESBL producing strain than C. koseri (7.33%). Majority of ESBL producing strains found to be susceptible to Meropenem and Amikacin. Among ESBL producing strains, consistent resistant was observed towards Ampicillin, Ciprofloxacin, and Ceftazidime.

A total of seven strains were found to carbapenamase producing and accounted for 4.7%. All carbapenamase producing strains were C. freundii. Carbapenemase producing Citrobacter strains were resistant to all cefalosporins, Piperacillin/ Tazobactum and Amikacin. All the carbapenamase producing Citrobacter strains were resistant to all tested antibiotics in the study.

DISCUSSION

In the present study, a total of 150 Citrobacter species were isolated from various clinical samples processed during study period. Citrobacter koseri (61.33%) was found more often than Citrobacter freundii (38.67%) in the present study, in addition younger patients had a greater prevalence. The age group of 31–40 years old had the highest prevalence of C. koseri (41.3%), followed by the age group of 21–30 years old (18.5%), indicating a preference for younger individuals. On the other hand, C. freundii was most prevalent from people between the ages of 31 and 40 (36.2%), followed by people between the ages of 41 and 50 (15.5%), suggesting that it tends to affects middle-aged people. Patients over 70 years old had the lowest prevalence for both species, which is consistent with studies showing a decrease in the frequency of Citrobacter infections in aged population.5 Numerous variables, including host immunity, comorbidities, and exposure to hospital environments, may have an impact on the age distribution pattern of C. freundii and C. koseri. The opportunistic pathogen C. freundii is frequently identified from patients who have underlying illnesses such as diabetes, renal disease, and cancers, which are more common in middle-aged people.6 Furthermore, effective clinical care and antibiotic selection for Citrobacter infections can be facilitated by an awareness of age-related susceptibility patterns.[4]

In the present study, majority of isolates were from pus samples (34.7%). In the study by Dhanya A and Sevitha Bhat, Citrobacter species were predominantly isolated from pus samples, accounting for 47.2% of the isolates.13 This finding is in line with the present study, where pus samples constituted 36.2% of C. freundii and 33.7% of C. koseri isolates. These studies collectively highlight the significance of Citrobacter species in wound infections. Second most common sample yielded the growth was urine samples(25.3%). This is in agreement with the study

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conducted by Metri et al. (24.3%).2 On the other hand, a large number of research studies conducted globally have demonstrated that the majority of Citrobacter species isolates are found in urine samples.[2,14,15]

In the present study, susceptibility pattern of Citrobacter freundii and Citrobacter koseri are two significant bacteria linked to various clinical infections in hospital and community. It was observed that C. koseri was more susceptible to a wider range of antibiotics, C. freundii exhibited significant resistance to various antibiotics particularly to ampicillin, ciprofloxacin, and ceftriaxone. Both C. freundii and C. koseri showed a high level of meropenem susceptibility, with C. koseri being 100% sensitive. This result is consistent with earlier research showing that carbapenems are effective against Citrobacter species because of their broad-spectrum action and capacity to evade several typical resistance mechanisms, including the generation of beta-lactamases.[16]

In our study, overall ESBL production among Citrobacter species was 32 %. The prevalence of ESBL production is extremely high compared to the previous study by Kanamori et al. As per the results of Kanamori et al., ESBL production among Citrobacter species was 19.3 %.[17] In our research, we found that C. freundii had a higher prevalence of ESBL-producing strains than C. koseri. This is consistent with other studies that found ESBL production in a significant percentage (20% to 60%) of C. freundii isolates.[16,18] In contrast study by Kanamori et al. showed C. koseri as common producer of ESBL production.[17]

In our study carbapenamase producing strains were 4.7%. All the strains were Citrobacter freundii. According to studies, the frequency of C. freundii strains that produce carbapenemase varies greatly by the geographical however in some hospital settings, it has been detected in as many as 10–30% of clinical isolates.[19,20] C. koseri is typically less frequently linked to the development of carbapenemase than C. freundii. According to certain studies, the prevalence of carbapenemase production in C. koseri is very low, ranging from 3% to 15%.[16,21]

The present comprises of following limitations, Citrobacter freundii and Citrobacter koseri were the two species of Citrobacter that were the subject of the study. Because of its narrow focus, the study does not fully depict the variety of Citrobacter species that might be involved in clinical infections. Furthermore, only traditional biochemical procedures were employed for identification, which could have resulted in the omission of certain strains that could have been recognised through the use of more sophisticated molecular methods.

CONCLUSION

This study highlights the antibiogram of Citrobacter freundii and Citrobacter koseri, with pus samples yielding the highest number of isolates. C. koseri demonstrated higher susceptibility to the tested antibiotics particularly towards piperacillin/tazobactam, meropenem, and co-trimoxazole, whereas C. freundii exhibited greater resistance, towards the tested antibiotics. Notably, all carbapenemase-producing strains were C. freundii, the findings emphasize the need for continuous surveillance, antibiotic stewardship, and targeted antimicrobial therapy to effectively manage Citrobacter infections and mitigate the spread of resistant strains.

REFERENCES

1. Lee R, Choi S-M, Jo SJ, Lee J, Cho S-Y, Kim S-H, et al. Clinical Characteristics and Antimicrobial Susceptibility Trends in Citrobacter Bacteremia: An 11-Year Single-Center Experience. Infect Chemother. 2019;51:1–9. doi: 10.3947/ic.2019.51.1.1.
2. Metri BC, Jyothi P. Antibiotic sensitivity pattern of Citrobacter spp . Isolated from patients with urinary tract infections in tertiary care hospital in South India. Int J Pharm Pharmaceut Sci. 2015; 7(1):14–16
3. Ribeiro T. G., Izdebski R., Urbanowicz Paweland Carmeli Y., Gniadkowski M., Peixe L., Urbanowicz P., et al. (2021). Citrobacter telavivum sp. nov. with chromosomal mcr-9 from hospitalized patients. Eur. J. Clin. Microbiol. Infect. Dis. 40, 123–131. doi: 10.1007/s10096-020-04003-6.
4. Negrete-González C, Turrubiartes-Martínez E, Briano-Macias M, Noyola D, Pérez-González LF, González-Amaro R, et al. Plasmid carrying blaCTX-M-15, blaPER-1,and blaTEM-1 genes in Citrobacter spp. from regional hospital in Mexico. Infect Dis(Auckl). 2022;15:11786337211065750. Doi: 10.1177/11786337211065750.
5. Liu L, Wang N, Wu AY, Lin C, Lee C, et al. Citrobacter freundii bacteremia: Risk factors of mortality and prevalence of resistance genes. J Microbiol Immunol Infect 2018; 51(4):565–572.
6. Ramachandran K., Patel Y., Shetty A. P., Shanmuganathan R. (2022). Citrobacter koseri as a rare cause of hematogenous pyogenic spondylodiscitis in young adult – A case report. J. Orthop. Rep. 1, 51–54. doi: 10.1016/j.jorep.2022.03.005.
7. Khan S., Taj R., Rehman N., Ullah A., Khan I., Rahman S. (2020). Incidence and antibiogram of β Lactamases-producing citrobacter freundii recovered from clinical isolates in Peshawar, Pakistan. Pak. J. Zool. 52, 1877–1882. doi: 10.17582/journal.pjz/20181118151126.
8. Iredell J, Brown J, Tagg K. Antibiotic resistance in Enterobacteriaceae: Mechanisms and clinical implications. BMJ. 2016;352:h6420. doi: 10.1136/bmj.h6420.
9. Collee JG, Fraser AG, Marmion BP, Simmons ACollee JG, Miles RS, Watt B. Tests for the identification of bacteria Mackey and McCartney Practical Medical Microbiology. 200614th ed. New Delhi, India Elsevier:131–49.
10. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Fifteenth Informational Supplement. CLSI Document M100-S15. 2005 Wayne, PA Clinical and Laboratory Standards Institute.
11. Jarlier V, et al. Detection of extended-spectrum beta-lactamase production by a combination disk test. J Clin Microbiol. 1988;26(10):2185-2187
12. Lee K, et al. Modified Hodge test for the detection of carbapenemase-producing bacteria: evaluation of a phenotypic test for surveillance. J Clin Microbiol. 2001;39(9):3582-3587.
13. Dhanya A, Sevitha Bhat. Clinicomicrobiological study of infections due to Citrobacter species. J Evol Med Dent Sci. 2015; 4(42):7327–7331
14. Hawaldar R., Sadhna S. Prevalence and drug resistance pattern of Citrobacter sps – A retrospective study. Indian J. Microbiol. Res.2019: 6, 142–145. doi: 10.18231/j.ijmr.2019.030

ISSN (O): 2687-5365; ISSN (P): 2753-6556

15. Tadesse S., Mulu W., Genet C., Kibret M., Belete M. A. Emergence of high prevalence of extended-spectrum beta-lactamase and carbapenemase-producing enterobacteriaceae species among patients in Northwestern Ethiopia Region. BioMed. Res. Int. 2022: 1–9. doi: 10.1155/2022/5727638.
16. Borer, A., & Gilad, J. Citrobacter species: A review of the microbiological features, clinical relevance, and management strategies. Clinical Microbiology Reviews.2012: 25(4), 784-794.
17. Kanamori H, Yano H, Hirakata Y, Endo S, Arai K, Ogawa M, Shimojima M, Aoyagi T, Hatta M, Yamada M, Nishimaki K, Kitagawa M, Kunishima H, Kaku M. High prevalence of extended-spectrum β-lactamases and qnr determinants in Citrobacter species from Japan: dissemination of CTX-M-2. J Antimicrob Chemother. 2011 Oct;66(10):2255-62. doi: 10.1093/jac/dkr283. Epub 2011 Jul 6. PMID: 21733965.
18. Zhang, Y., et al. Prevalence of ESBL-producing Citrobacter species in a Chinese hospital: A 6-year retrospective study. Infection and Drug Resistance.2016: 9, 133-139.
19. Wang, Q., et al. Antimicrobial resistance and carbapenemase production in Citrobacter species: A hospital-based surveillance study in China. Journal of Global Antimicrobial Resistance.2018: 14, 113-118.
20. Toleman, M. A., et al. Carbapenemase-producing Citrobacter species: Clinical implications and management strategies. Journal of Antimicrobial Chemotherapy.2014: 69(9), 2263-2270.
21. Jacob, M. A., et al. Prevalence of carbapenemase-producing Citrobacter species in a hospital-based study: An emerging problem in nosocomial infections. Journal of Clinical Microbiology.2016 54(3), 689-696

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