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*Original Research Article*

# PREVALENCE AND ANTI MICROBIAL SUSCEPTIBILITY PATTERN OF CITRO BACTER AS AN UROPATHOGEN INATERTIARY CARE HOSPITAL

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***Abstract***: ***Introduction:*** Urinary tract infection (UTI) is the third most common infection experienced by humans after respiratory and gastrointestinal infections. Citrobacter is prevalent worldwide as it is a component of the normal intestinal flora. The genus Citrobacter is a diverse group of facultative anaerobic, gram-negative bacilli belonging to the family Enterobacteriaceae and the tribe Citrobactereae. These organisms occur singly or in pairs and are typically motile by peritrichous flagella. They mainly utilize citrate as the sole carbon source.Citrobacter isolates are reported to be the third most frequent pathogens causing urinary tract infections in hospitalized patients after Escherichia coli and Klebsiella species, accounting for 9.4% of all isolates. This study aimed to assess the prevalence and antibiotic sensitivity pattern of Citrobacter in patients with UTIs in a tertiary care hospital. ***Aim:*** This study aims to investigate the prevalence and antimicrobial susceptibility pattern of Citrobacter as a uropathogen in a tertiary care hospital. ***Materials and Methods:*** This was an observational and prospective study conducted in the Department of Microbiology, MGM Medical College and Hospital, Kamothe, Navi Mumbai, from December 2022 to February 2024. A total of 230 urine samples were collected from UTI patients. Clean-voided midstream urine, suprapubic aspiration urine samples, and catheterized urine samples were collected under aseptic precautions. Ten milliliters of properly mixed urine was transferred into a test tube, centrifuged, and the supernatant was discarded. The sediment was mixed by tapping the bottom of the tube. One drop of the well-mixed sediment was placed onto a slide and covered with a coverslip. The samples were further processed for culture, biochemical reactions, and antibiotic susceptibility testing. ***Results:*** The prevalence rate of Citrobacter in UTI patients was 19.56%. The most common species of Citrobacter associated with UTI was Citrobacter freundii (46.66%). ***Conclusion:*** This study shows that Citrobacter can lead to severe outbreaks in healthcare settings and is becoming increasingly antibiotic-resistant in UTI cases.

*Keywords*: Urinary Tract Infections.

## INTRODUCTION

Uropathogens are microorganisms that are capable of causing infections of the urinary tract. Urinary tract infection (UTI) is the third most common infection experienced by humans after respiratory and gastrointestinal infections. [1]

*Citrobacter* is prevalent worldwide as it is a component of the normal intestinal flora. The genus *Citrobacter* is a distinct group of facultative anaerobic, Gram-negative bacilli belonging to the family

**Enterobacteriaceae** and the tribe **Citrobactereae**. They are found singly or in pairs and are typically motile by peritrichous flagella, utilizing citrate as the sole source of carbon. [2]

There are 11 different species of *Citrobacter* (*Citrobacter freundii, Citrobacter koseri, Citrobacter amalonaticus, Citrobacter farmeri, Citrobacter youngae, Citrobacter braakii, Citrobacter werkmanii, Citrobacter sedlakii, Citrobacter rodentium, Citrobacter gillenii,* and *Citrobacter murliniae*), among which the species most commonly isolated from human clinical specimens are *C. koseri* (formerly *C. diversus*), *C. freundii, C. youngae, C. braakii,* and *C. amalonaticus*. [2]

*Citrobacter* isolates are reported to be the third most common organisms causing urinary tract infections in hospitalized patients after *Escherichia coli* and *Klebsiella* species, accounting for 9.4% of all isolates. [3] Thus, laboratory diagnosis is essential to accurately differentiate between *Citrobacter* species. As these organisms catabolize glucose and numerous other carbohydrates with the production of acid and gas, biochemical tests such as oxidase, catalase, methyl red, Voges–Proskauer, and citrate utilization tests are used for species identification. Antimicrobial susceptibility testing was performed for all isolates using the Kirby–Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) M2-A9 guidelines. [2]

*Citrobacter* species are often resistant to routinely used antibiotics, especially extended-spectrum cephalosporins, due to the overexpression of beta-lactamases. [4] This study aimed to assess the prevalence and antibiotic sensitivity pattern of *Citrobacter* in patients with UTIs in a tertiary care hospital.

## MATERIALS AND METHODS

The present study was carried out at MGM Medical College and Hospital, Kamothe, Navi Mumbai.

**Processing of Specimen** [5]: Urine samples collected from patients were used for microscopy and culture.

1. **Microscopy:**
   Microscopic examination of centrifuged urine sediment was performed using the wet preparation method. Gram-stained smears were prepared from the sediment and examined when bacteria or pus cells were observed in wet preparations.
2. **Culture on Nutrient Agar (NA), Blood Agar (BA), and MacConkey Agar:**
   Uncentrifuged urine samples were inoculated using a standard calibrated sterile chromium loop (2 mm), delivering 0.002 ml of urine onto nutrient agar, blood agar, and MacConkey agar plates. The plates were incubated aerobically at 37°C for 18–24 hours. On the following day, colony counts were performed. Growth of $\geq 10^5$ CFU/ml with no more than two species of microorganisms was considered significant.
3. **Identification:**
   Isolated microorganisms were identified based on colony morphology, Gram-staining reaction, and standard microbiological procedures. Identification of pathogens was confirmed using standard biochemical tests.
4. **Antibiotic Sensitivity Testing** [6]: Antimicrobial susceptibility testing was performed on Mueller–Hinton agar using the Kirby–Bauer disc diffusion method according to CLSI guidelines (2021). Results were interpreted as sensitive, intermediate, or resistant as per CLSI standards.

## RESULTS

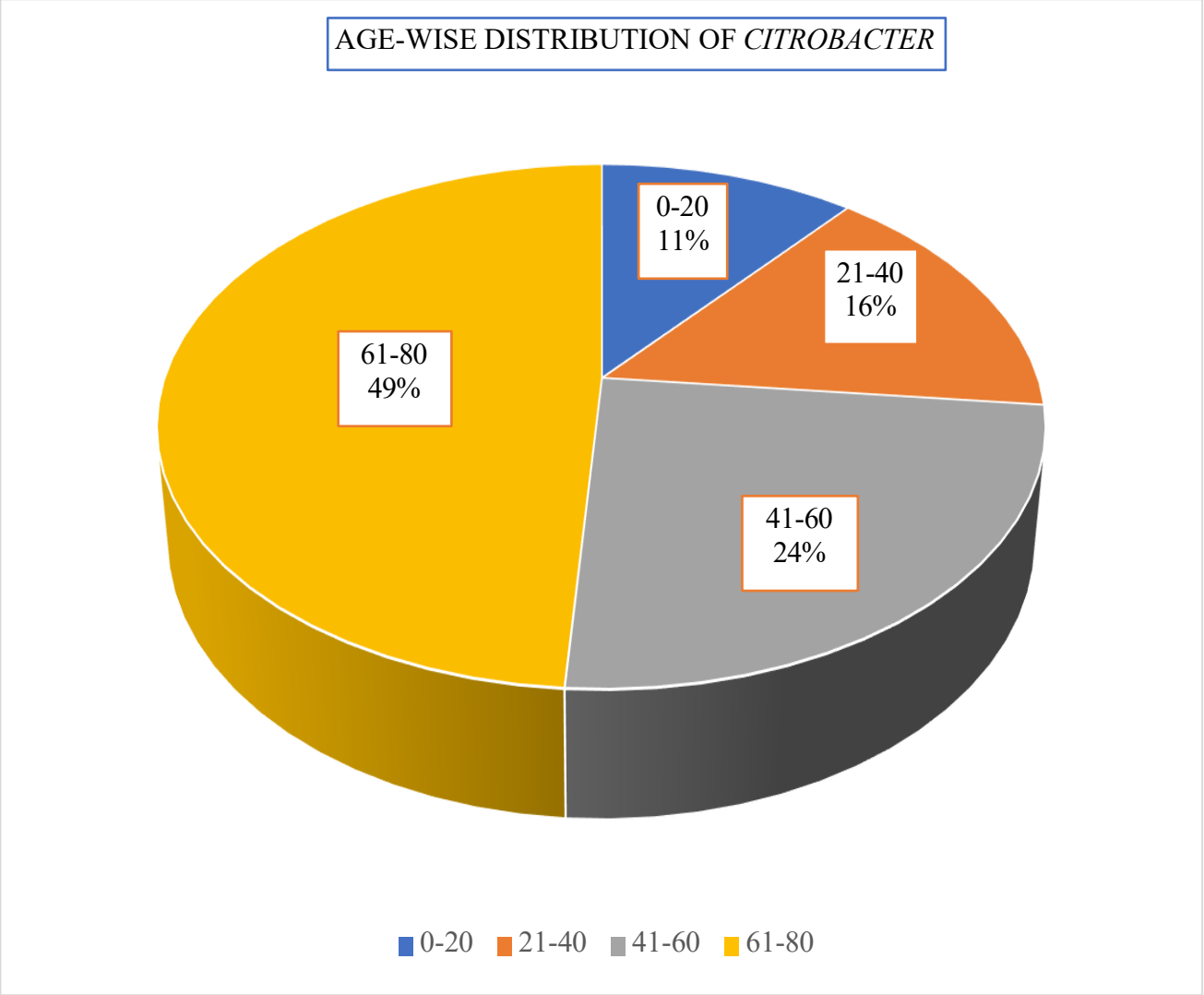
A total of 230 urine samples from UTI patients were collected from December 2022 to February 2024 at the Microbiology Laboratory of MGM Hospital, Kamothe, Navi Mumbai.

**TABLE 1- PREVALENCE RATE OF *CITROBACTER* IN UTI PATIENTS**

| TOTAL SAMPLES FROM UTI | THE NUMBER OF URINE SAMPLES SHOWING *CITROBACTER* | PERCENTAGE PREVALENCE OF *CITROBACTER* IN UTI |
|---|---|---|
| 230 | 45 | 19.56% |

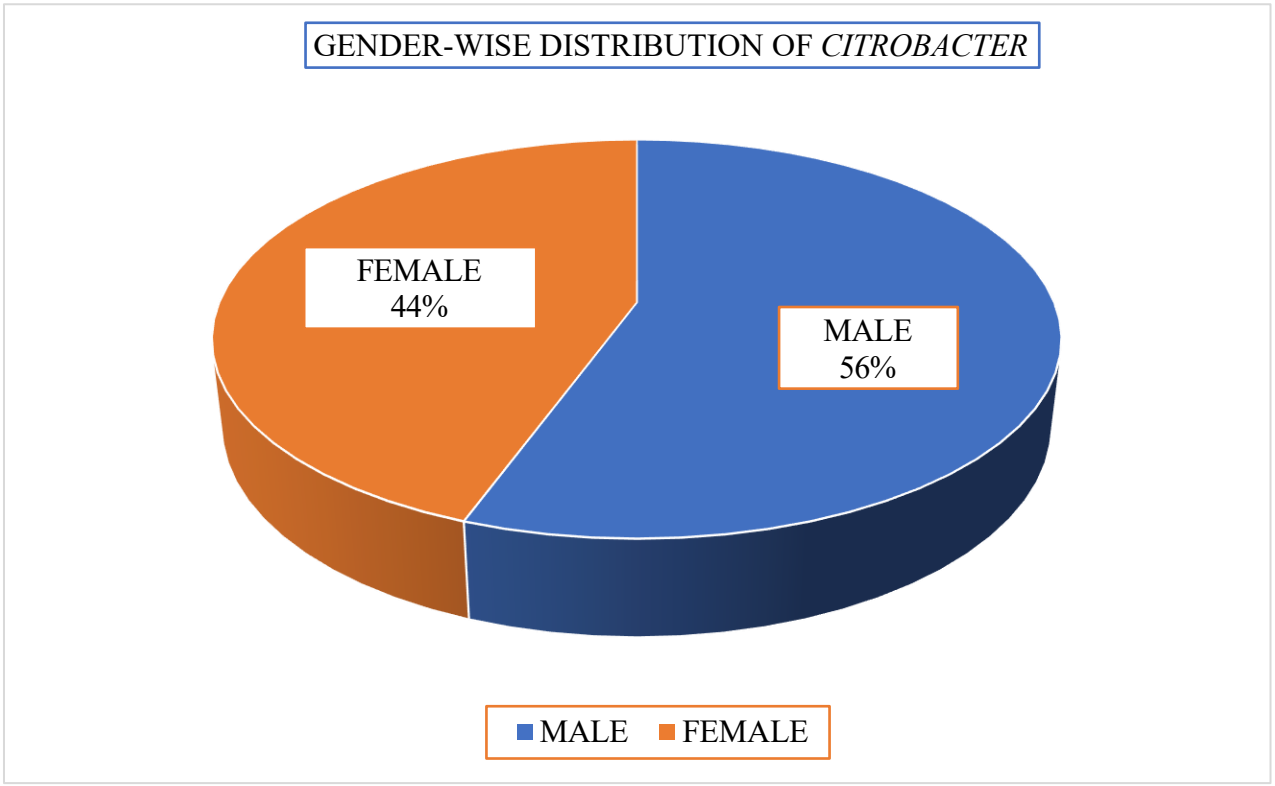
**Prevalence rate of *Citrobacter* in UTI patients is 19.56%.**

**GRAPH 1(A)- Age-wise distribution of UTI patients having *Citrobacter* (n=45)**

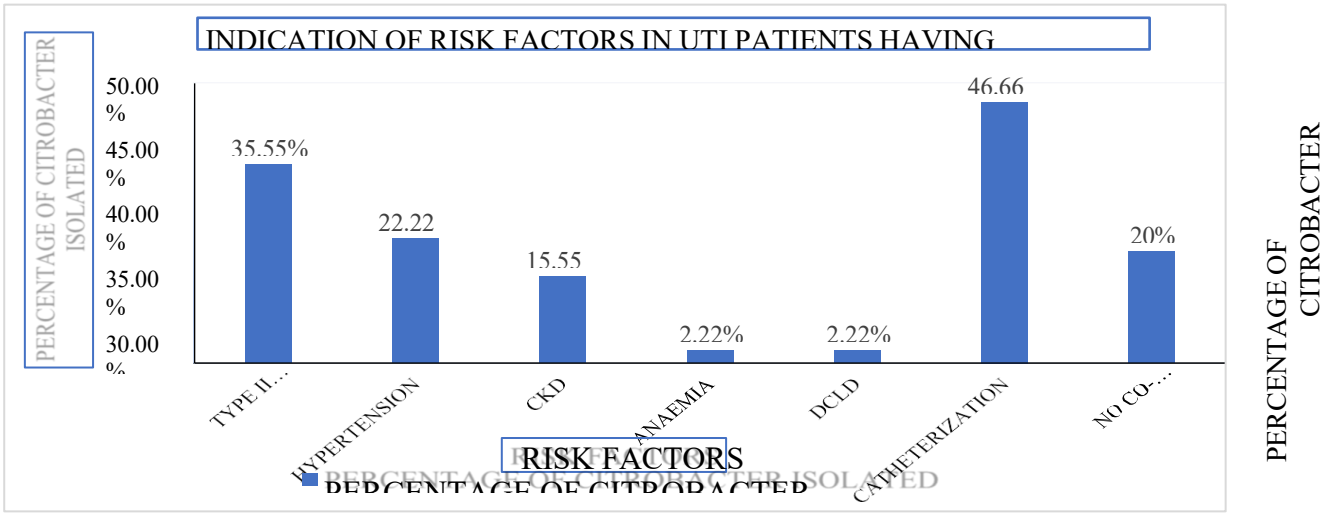



**The maximum number of UTI patients with *Citrobacter* as a causative agent belongs to the age group between 61-80 (48.88%) years of age.**

**GRAPH 2(B)- Gender-wise distribution of UTI patients having *Citrobacter* (n=45)**

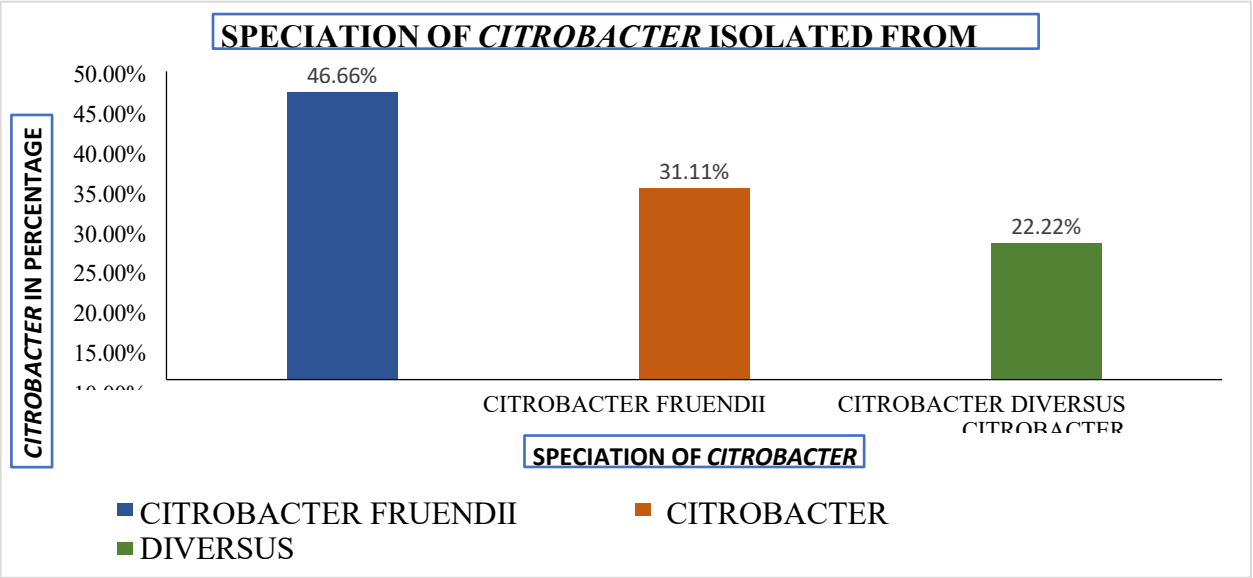



**Males (55.55%) were seen to be affected with *Citrobacter* UTI more as compared to Females. GRAPH 2(C)- Indication of Risk factors in UTI patients having *Citrobacter* (n=45)**




**Patients on Catheterization (46.66%) and Type II Diabetes Mellitus (35.55%) showed the highest risk for the development of UTI with *Citrobacter*.**

**GRAPH 2(D)- Speciation of *Citrobacter* isolated from UTI (n=45)**



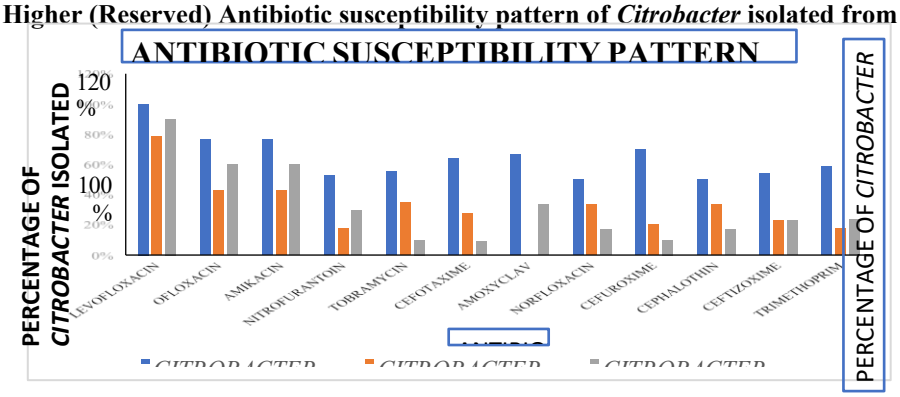

**The commonest species of *Citrobacter* associated with UTI was *Citrobacter freundii* (46.66%). GRAPH 2(A): Antibiotic susceptibility pattern of *Citrobacter* isolated from UTI patients. (n=45)**

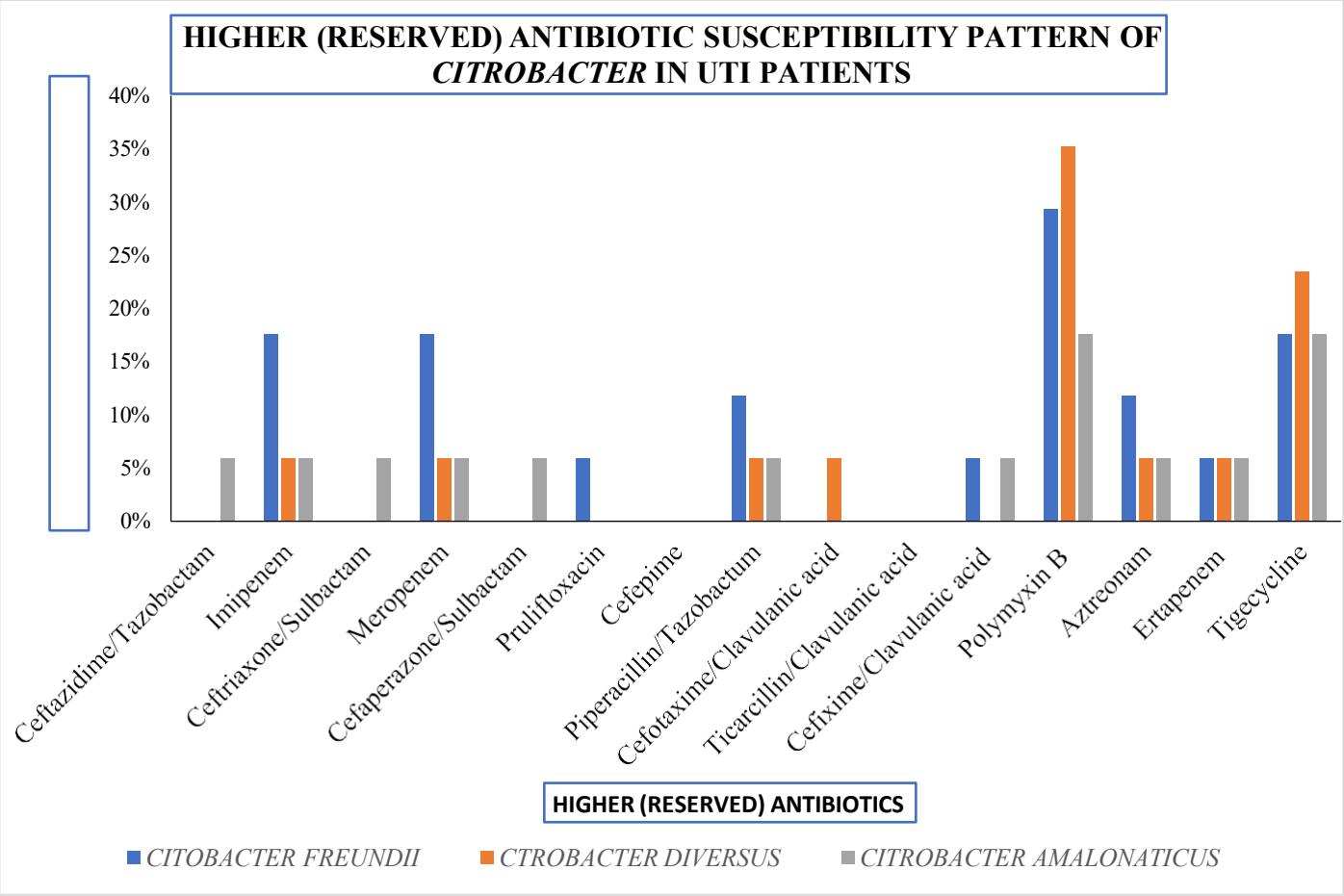
**In *Citrobacter freundii*- Highest antibiotic susceptibility was observed in Levofloxacin (100%) followed by Ofloxacin (76.19%) and Amikacin (76.19%) whereas Least antibiotic susceptibility was seen to Norfloxacin (50%) and Cephalothin (50%).**

**In *Citrobacter diversus*- Highest antibiotic susceptibility was observed in Levofloxacin (78.57%) followed by Ofloxacin (42.85%) and Amikacin (42.85%) whereas Least antibiotic susceptibility was seen to Amoxyclav (0%).**

**In *Citrobacter amalonaticus*- Highest antibiotic susceptibility was observed in Levofloxacin (90%) followed by Ofloxacin (60%) and Amikacin (60%) whereas Least antibiotic susceptibility was seen to Cefotaxime (9.09%).**

**GRAPH 2(B): Higher (Reserved) Antibiotic susceptibility pattern of *Citrobacter* isolated from UTI patients (n=45)**






**In *Citrobacter freundii*- Highest antibiotic susceptibility was observed in Polymyxin B (29.41%) followed by Meropenem (17.64%), Imipenem (17.64%) and Tigecycline (17.64%) whereas Least antibiotic susceptibility was seen to Cephalosporins.**

**In *Citrobacter diversus*- Highest antibiotic susceptibility was observed in Polymyxin B (35.24%) followed by Tigecycline (23.52%) whereas Least antibiotic susceptibility was seen to Cephalosporins.**

**In *Citrobacter amalonaticus*- Highest antibiotic susceptibility was observed in Polymyxin B (17.64%) and Tigecycline (17.64%) whereas Least antibiotic susceptibility was seen to Cephalosporins.**

## DISCUSSION

The present study was carried out at MGM Medical College and Hospital. 230 urine samples of UTI patients were collected from December 2022 to February 2024.

Out of 230 UTI patients, 45 Citrobacter were isolated from urine samples. In our study, the overall Prevalence of Citrobacter was reported as 19.56% (45/230). A study done by Hiba Sami et.al reported the prevalence rate of Citrobacter in UTI patients as 3.46% (246/7099). [1] In another study conducted by Abdulrasheed B. Abdu et.al, the prevalence rate of uropathogenic Citrobacter was found to be 30.01% (1407/5000). [7] So, in various studies, it was seen that the prevalence rate of Citrobacter in UTI patients ranged from 3.46% to 30.01%.

In our study, it was observed that the maximum number of UTI patients with Citrobacter belonged to the age group between 61-80 (48.88%) years of age. A comparable study done by Poonam AR et.al showed that the majority of the age group affected in the present study is the elderly age group of 51-80 years. [3] In another study conducted by Navin Kumar Choudhary, the prevalence of UTIs in men increases substantially after the age of 60 years due to urethral obstruction and voiding dysfunction associated with prostatic hypertrophy.[8] This shows that advanced age is one of the host characteristics that predispose to UTI patients with Citrobacter.

In our study, the maximum number of UTI patients with Citrobacter was Male (55.55%). The study was done by Nandini. M.S et al. documented a similar prevalence with 61.11% males and 38.88% females patients[9]. In another study done by Jaggi et.al[10], also showed similar

findings as our study. In all three studies, it was observed that UTI with Citrobacter was more commonly seen in males, a reason for this contradictory finding could be that in all 3 studies, a greater number of males were Catheterised as compared to females and Catheterisation has always been a significant predisposing factor for development of UTI.

In our study, Catheterization (46.66%) and Type II Diabetes Mellitus (35.55%) showed the highest risk of developing UTI with Citrobacter. In another study done by Shiva Verma et. al [11] confirmed that Diabetes and the duration of Catheterization increase the risk of developing CAUTI. This indicates Diabetes and patients on Catheterization have a greater risk of developing urinary tract infections.

In the present study, the commonest species of Citrobacter associated with UTI was Citrobacter freundii (46.66%) followed by Citrobacter diversus (31.11%) and Citrobacter amalonaticus (22.22%). In a comparative study done by Abdulrasheed B. Abdu et.al, Citrobacter freundii (60.4%) was found to be the most prevalent among the uropathogenic Citrobacter species. While, Citrobacter diversus constituted (29.9%), of Citrobacter amalonaticus (4.85%) of the isolates.[11] However, this indicates that Citrobacter freundii is the most frequently caused UTI in patients followed by Citrobacter diversus and Citrobacter amalonaticus.

In this study, it was observed that Citrobacter freundii found in patients with urinary tract infections showed the highest susceptibility to Levofloxacin (100%), followed by Ofloxacin (76.19%) and Amikacin (76.19%). On the other hand, the least susceptibility was observed for Norfloxacin (50%) and Cephalothin (50%). Interestingly, in the case of higher antibiotics (reserve), the highest susceptibility was seen in Polymyxin B (29.41%), followed by Meropenem (17.64%), Imipenem (17.64%), and Tigecycline (17.64%). Meanwhile, the least susceptibility was seen in Cephalosporins.

In the case of Citrobacter diversus, the antibiotic that showed the highest susceptibility was Levofloxacin (78.57%), followed by Ofloxacin (42.85%) and Amikacin (42.85%). On the other hand, the antibiotic that showed the least susceptibility was Amoxyclav (0%). When it comes to higher (reserve) antibiotic susceptibility patterns, Polymyxin B (35.29%) showed the highest susceptibility, followed by Tigecycline (23.52%). The least susceptibility was seen with Cephalosporins.
Regarding Citrobacter amalonaticus, the bacteria showed the highest susceptibility to Levofloxacin (90%), followed by Ofloxacin (60%) and Amikacin (60%). On the other hand, Cefotaxime exhibited the least

susceptibility (9.09%). When it comes to higher (reserve) antibiotics, Polymyxin B and Tigecycline showed the highest susceptibility (17.64%), whereas Cephalosporins exhibited the least susceptibility. In another study done by Poonam AR et.al, the susceptibility pattern observed in C.freundii urine isolates in the case of nitrofurantoin (89.4%, 79%), nalidixic acid (80.7%, 62.5%) and trimethoprim-sulfamethoxazole (96.4%, 75%) in both C. koseri and C. freundii. High-end antimicrobials such as tigecycline and colistin showed excellent susceptibility of 100% in both Citrobacter species isolated. [3]

Several studies have reported a rise in antibiotic resistance among Citrobacter species in different regions of India. However, the current study shows a significant level of susceptibility. The variation in the specimens considered, hospital environments, and hospital infection control policies could contribute to the difference.[3]

## CONCLUSIONS

Overall Prevalence rate of Citrobacter in UTI patients is 19.56% in our study.

Patients between the ages of 61-80 years are at a higher risk of developing Citrobacter in their urine.

**Male predominance was seen in our study.**
Citrobacter is more likely to cause UTI in patients who have undergone Catheterization (46.66%) or have Type II Diabetes Mellitus (35.55%).

The most common species of Citrobacter that is associated with urinary tract infections (UTI) is Citrobacter freundii, which accounts for (46.66%) of cases.

In Citrobacter freundii, the highest antibiotic susceptibility was observed in Levofloxacin (100%), followed by Ofloxacin (76.19%) and Amikacin (76.19%). The least antibiotic susceptibility was seen in Norfloxacin (50%) and Cephalothin (50%).

In Citrobacter diversus, the highest antibiotic susceptibility was observed in Levofloxacin (78.57%) followed by Ofloxacin (42.85%) and Amikacin (42.85%). The least antibiotic susceptibility was seen in Amoxyclav (0%).

In Citrobacter amalonaticus, the highest antibiotic susceptibility was observed in Levofloxacin (90%), followed by Ofloxacin (60%) and Amikacin (60%). The least antibiotic susceptibility was seen in Cefotaxime (9.09%).

In Citrobacter freundii, the highest susceptibility to antibiotics was observed with Polymyxin B (29.41%), followed by Meropenem (17.64%), Imipenem (17.64%), and Tigecycline (17.64%). The least susceptibility to antibiotics was seen with Cephalosporins.

In Citrobacter diversus, the highest susceptibility was observed to Polymyxin B (35.29%) followed by Tigecycline (23.52%). The least susceptibility was seen to Cephalosporins.

In Citrobacter amalonaticus, the highest susceptibility was observed for Polymyxin B (17.64%) and Tigecycline (17.64%), while the least susceptibility was seen for Cephalosporins.

Urinary tract infections (UTIs) are most common and can affect up to 10% of people at some point in their lifetime. It is important to detect and treat the infection early using the appropriate antibiotics to eliminate the pathogen, prevent urosepsis, and reduce the chances of kidney damage. [1]

Microorganisms belonging to the genus Citrobacter rarely cause infections in healthy individuals. However, they can lead to severe outbreaks in healthcare settings and are becoming increasingly antibiotic-resistant. Unfortunately, there are very few studies conducted in India on Citrobacter and its antibiotic sensitivity patterns and further studies are required.[12]

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