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Original Article

# Citrobacter Emerging as a Common Uropathogen in Pediatric Population

**Ritu Kumari[1], Jayanta Bikash Dey[2], Anshuman Jana[3], Tapashi Ghosh[4], Nikhil Kumar Tudu[4]**

[1]Post Graduate Trainee, Department of Microbiology, Bankura Sammilani Medical College and Hospital, Bankura, West Bengal, India, [2]Head and Professor, Department of Microbiology, Bankura Sammilani Medical College and Hospital, Bankura, West Bengal, India, [3]Assistant Professor, Department of Microbiology, Bankura Sammillani Medical College and Hospital, Bankura, West Bengal, India, [4]Associate Professor, Department of Microbiology, Bankura Sammillani Medical College and Hospital, Bankura, West Bengal, India

## Abstract

**Introduction:** There is a changing scenario of the epidemiology of uropathogen in the hospital setup. *Citrobacter* species being the inhabitants of intestinal tract is emerging as one of the leading uropathogens in pediatric age group.

**Purpose:** The study was undertaken to look for the prevalence of *Citrobacter* species responsible for uropathogen with its antibiogram, in pediatric age group.

**Materials and Methods:** A cross-sectional study was carried out over a period of 1½ year. A total of 1890 clean-catch midstream urine samples were processed in clinical microbiology laboratory during the study period. Isolates were identified using standard isolation methods, and antibiogram was done using Kirby–Bauer diffusion method as per the Clinical and Laboratory Standards Institute guidelines. Extended-spectrum beta-lactamase (ESBL), metallo-beta-lactamase, and AmpC were tested for the multidrug-resistant (MDR) isolates.

**Results:** 90% belonged to Gram-negative pathogens, among which highest growth isolated was *Escherichia coli* 289 (41%) followed by *Citrobacter* spp. 122 (17.3%) and *Pseudomonas* spp. 105 (15%). Antibiograms of *Citrobacter* spp. isolates revealed that effective agent against *Citrobacter* spp. isolates was imipenem (89.7% sensitive), followed by amikacin (85.4%), gentamicin (72%), and piperacillin/tazobactam (68.4%). Among oral drugs, most sensitive is levofloxacin (52%) and nitrofurantoin (51.2%). Of the 122 strains in pediatric population, 74 (61%) were found to be MDR of which 62 (51%) were ESBL producers and 81 (66.4%) were AmpC producers.

**Conclusion:** Our study showed that the prevalence of *Citrobacter* as a uropathogen in pediatric population has increased becoming the 2nd most common uropathogen (17.3%). The *Citrobacter* isolates resistant to multiple antimicrobial agents have emerged, making it an emerging nosocomial pathogen. In pediatric age group, urinary tract infection soon progresses to urosepsis and renal damage, so search for this pathogen as well as its antibiogram is essential. Adoption of hospital infection control practices and a good antibiotic policy may prevent their spread.

**Key words:** Antibiotic sensitivity, *Citrobacter* species, Multidrug resistance, Pediatrics, Urinary tract infection

## INTRODUCTION

Urinary tract infections (UTIs) in children are usually associated with high morbidity and long-term complications if they are not treated in the beginning. The incidence varies according to age, races, and sex of children.[1,2] UTI is one of the most common infections of childhood. It distresses the child, concerns the parents, and may cause permanent kidney damage.[3]

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The genus *Citrobacter* is a distinct group of facultative anaerobic Gram-negative bacilli from the *Enterobacteriaceae* family.[4] The genus *Citrobacter* comprises 11 different species. Among these, *Citrobacter koseri* (previously known as *Citrobacter diversus*) and *Citrobacter freundii* are the most common species implicated in infections. *Citrobacter* species can cause variety of infections such as respiratory tract infections, UTI, bloodstream infections, wound and burns infections,

**Corresponding Author:** Ritu Kumari, Flat No. 306, S.D Apartment, Nageshwar Colony, Boring Road, Patna – 800 001, Bihar, India. Phone: +91-9732846321. E-mail: ritudisu@gmail.com

meningitis, endocarditis, and peritonitis.[5] We report here the emergence of *Citrobacter* as an increasingly common urinary pathogen in pediatrics patients attending this medical college.

As a pathogen of UTI, most common etiologic pathogens are *Escherichia coli* (93.3%) followed by *Proteus* spp., *Klebsiella* spp., *Citrobacter* spp., *Staphylococcus aureus*, and others.[6]

An association with virulence markers such as the serum resistance, the cell surface hydrophobicity, and the killing in the polymorphonuclear leukocytes, which had been studied in *E. coli*, was found to exist in *Citrobacter* spp., leading to its pathogenicity.[7]

Recently, the isolation of this pathogen in hospital settings across the globe is increasing, and multidrug-resistant (MDR) strains are emerging. These strains present a challenge for clinicians and clinical microbiologists alike because of their increased propensity to cause not only nosocomial infections but also community-acquired infection also. The aim of this study was to assess the prevalence and antibiotic sensitivity pattern of *Citrobacter* spp. in pediatric population with UTIs in a tertiary care hospital.

## MATERIALS AND METHODS

This is a cross-sectional study conducted over a period of 1 year and 6 months (June 2016–January 2017) in the Department of Microbiology, Bankura Sammilani Medical College and Hospital, Bankura, West Bengal.

### Inclusion Criteria

A total of 1890 freshly voided midstream specimens of urine under the 12 years were submitted to the clinical microbiology laboratory for processing.

### Exclusion Criteria

Inadequate urine samples, urine bag collected samples, specimens collected more than 2 h before submission, specimens submitted in leaking or unsterile containers, and specimens revealing the growth of more than two types of bacteria on culture were excluded from the study.

Semiquantitative urine culture using a calibrated loop was used to inoculate Blood agar and MacConkey Plates (HiMedia Laboratories Pvt., Ltd., Mumbai).[8]

After aerobic incubation at 37°C for 24 h, the petri plates were inspected for growth of bacteria in the form of colonies. Those, which showed no colony, were noted down as no growth. The media, which showed the appearance of colonies, was processed following the standard bacteriological procedures. Colony characteristics of each type were recorded. Gram-staining was done from the colonies, and based on the findings of that, identification of bacterial isolates was done by conventional biochemical tests for identification The significant pathogens were identified by standard biochemical procedures.[9]

Inoculum for each isolate was prepared by direct colony suspension in nutrient broth, and the turbidity of the broth was adjusted to a 0.5 McFarland standard suspension, which contains approximately 1–4 × 108 colony-forming units/ml. The Mueller-Hinton agar (MHA) plates were inoculated and then incubated at 37°C for overnight. On the next day, MHA plates were examined, the zone of inhibition was noted by measuring with a ruler held against the back of petri plate, and the sensitivity pattern of the bacterial isolates to various antibiotics was noted. Antimicrobial susceptibility testing was done for all the isolates using Kirby–Bauer disc diffusion method on MHA (HiMedia Laboratories Pvt., Ltd., Mumbai) recommended by Clinical and Laboratory Standards Institute (CLSI) M2-A9.[10]

The antimicrobials tested for the Gram-negative bacteria were amikacin (30 μg), gentamicin (10 μg), ofloxacin (5 μg), levofloxacin (5 μg), ceftriaxone (30 μg), cefoperazone (CP) (75 μg), CP-sulbactam (CPS) (75 μg, 1:1), cefotaxime (30 μg), ceftazidime (30 μg), nitrofurantoin (300 μg), amoxicillin-clavulanate (30 μg), piperacillin-tazobactam (100:10 μg), and imipenem (10 μg) which were obtained from HiMedia, India. MDR was defined as resistance to more than two groups of drugs.

### Detection of Extended-Spectrum and AmpC Beta-Lactamase

The isolates which were resistant or intermediately susceptible to any of the third-generation cephalosporins were further processed for extended-spectrum beta-lactamase (ESBL) detection by the double-disk potentiation method[11] using a disc of cefotaxime (30 μg)/ceftazidime (30 μg, and combination discs of cefotaxime 30 μg and clavulanic acid 10 μg and of ceftazidime 30 μg plus clavulanic acid 10 μg. *Klebsiella pneumoniae* ATCC 700603 was used as the ESBL-positive control. ESBL production was inferred if the inhibition zone increased by 5 mm toward the cefotaxime plus clavulanic acid disc or toward the ceftazidime plus clavulanic acid disc in comparison to the third-generation cephalosporin disc alone.

Organism resistant to cefoxitin, CPS, and piperacillin-tazobactam combination in addition to other cephalosporins were considered to be AmpC producers (CLSI 2003).[12]

### Detection of Metallo-Beta-Lactamases (MBLs)

Imipenem-resistant isolates were tested for MBL production using modified Hodge test and double-disc synergy test using ethylenediaminetetraacetic acid.[13]

The data were tabulated and statistical analyses of the data were done using SPSS Statistics 20.0, and qualitative and quantitative data were expressed as frequency and percentage. Association between two or more qualitative variables was analyzed using Chi-square test. A two-sided $P < 0.05$ was considered to be statistically significant.

## RESULTS

Of the total 1890 pediatric samples, growth was seen in 705 samples. Among the positive urine cultures, 90% belonged to Gram-negative pathogens. Among which highest growth isolated was *E. coli* 289 (41%) followed by *Citrobacter* spp. 122 (17.3%) and *Pseudomonas* spp. 105 (15%) Shown in Table 1 [$\chi^2 = 95.34$, d.f = 5, $P = 0.000$].

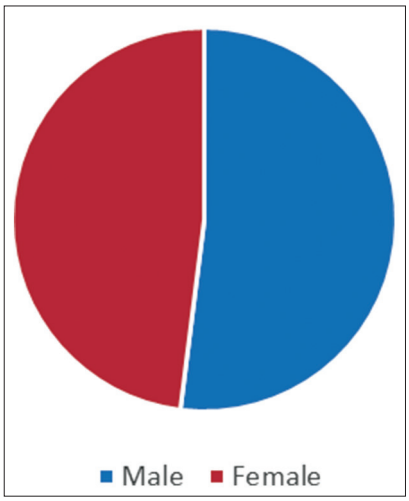


**Figure 1: Gender distribution of *Citrobacter* species in children**

In pediatric population, of the 620 Gram-negative isolates, 122 belonged to the *Citrobacter* species (*C. freundii* - 30 and *C. koseri* - 92). Among the 122 *Citrobacter* isolates, 96 (78.68%) were obtained from indoor samples, whereas 24 (19.6%) were obtained from outdoor specimens.

Among pediatric group, the *Citrobacter* spp. isolates showed slight male predominance (52%) [Figure 1]. Maximum number of cases was seen in the age group of 6–12 years of age, where males were 190 and females were 120 and majority of female culture positive cases were in age group of 2–5 years [Table 2].

Antibiograms of *Citrobacter* spp. isolates revealed that effective agent against *Citrobacter* spp. isolates was imipenem (89.7% sensitive), followed by amikacin (85.4%), gentamicin (72%), and piperacillin/tazobactam (68.4%). Among oral drugs, most sensitive is levofloxacin (52%) and nitrofurantoin (51.2%) [Figure 2].

Of the 122 *Citrobacter* strains in pediatric population, 74 (61%) were found to be MDR, of which 62 (51%) were ESBL producers and 81 (66.4%) were AmpC producers. Most of the ESBL producing strains were isolated from inpatients. No MBL was identified in this study.

## DISCUSSIONS

The education and application of personal hygiene are important in that *Citrobacter* strains are excreted as fecal wastes. Infections occurring in hospitals are closely related to the fact that hospital staff carries the bacterium in

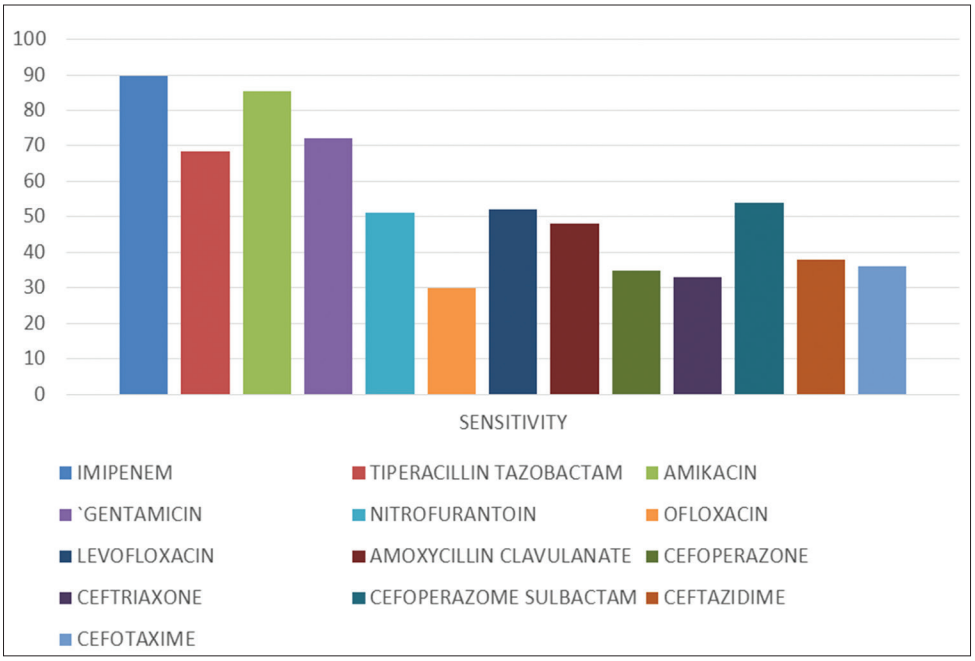


**Figure 2: Antibiogram of *Citrobacter* species isolated from urine of children**

**Table 1: Distribution of pathogens**

| Bacteria | Total population (%) | Pediatrics (%) |
|---|---|---|
| *E. coli* | 982 (48) | 289 (41) |
| *Citrobacter* spp. | 200 (9.7) | 122 (17.3) |
| *Klebsiella* spp. | 344 (16.7) | 85 (12) |
| *Pseudomonas* spp. | 280 (13.6) | 105 (15) |
| Other GNB | 42 (2) | 19 (2.7) |
| GPC | 207 (10) | 85 (12) |
| Total | 2056 | 705 |

*E. coli: Escherichia coli*

**Table 2: Age- and gender-wise distribution of children**

| Age (years) | Sex | | Total (%) |
|---|---|---|---|
| | Male (%) | Female (%) | |
| <2 | 12 (19) | 11 (18.6) | 23 (18.9) |
| 2–5 | 18 (28.6) | 27 (45.8) | 45 (36.9) |
| 6–12 | 33 (52.4) | 21 (35.6) | 54 (44.2) |
| Total | 63 | 59 | 122 |

their hands and gastrointestinal systems. *Citrobacter* strains are mostly isolated from the infections of urinary and respiratory systems as pathogens.[14] Invasive procedures such as catheterization help them to colonize urinary bladder, and during intensive chemotherapy, this bacterium disseminates to the bloodstream to cause severe bacteremia. The problem is further intensified by the emergence of MDR *Citrobacter* spp. resulting into treatment failure.[15]

*Citrobacter* has emerged as the 2nd leading uropathogen in pediatric population in our study, with increasing trend of 17.3%, resistant to the commonly used antibiotics which are alarming. The prevalence of *Citrobacter* isolates in pediatric age group reported by Khatoon *et al.* reported as 9.5%, Gautam *et al.* as 12.1%, and Metri *et al.* as 9.4%. In our study, we found the prevalence to be 17.3% which is much more higher in contrary to other studies so far.[5,16,17]

*Citrobacter* species isolated in our study are *C. koseri* and *C. freundii* with *C. koseri* being the predominant one. This study correlates well with the study of Hiba *et al.*[18]

Antibiograms of *Citrobacter* spp. isolates revealed that effective agent against *Citrobacter* spp. isolates were imipenem (89.7% sensitive), followed by amikacin (85.4%), gentamicin (72%), and piperacillin/tazobactam (68.4%). Among oral drugs, most sensitive is levofloxacin (52%) and nitrofurantoin (51.2%).

Of the 122 strains in pediatrics, 74 (61%) were found to be MDR of which 62 (51%) were ESBL producers and 81 (66.4%) were AmpC producers. Most of the ESBL producing strains were isolated from inpatients. The rate of the ESBL production among the *Citrobacter* species in our study was higher than that which was reported by other authors,[19,20] which was 36.36% and 19.3%, respectively. However, it was comparable with the finding of Rizvi *et al.*[21] (62%) and lower than that which was reported by Uma *et al.*[22] who had reported it to be 86.50% among the hospital isolates. Excessive antibiotic exposure (especially the extended-spectrum cephalosporins), extended hospital stay, recent surgery, admission to the intensive care unit, and instrumentation have been identified as the risk factors for the selection of the ESBL-producing strains.

Sensitivity to imipenem showed in our study was 89.7% which in contrary to various studies which shows 100% sensitivity[23] but corresponds well the study of Preethishree *et al.*[24] Carbapenems are important antibiotics for the treatment of healthcare-associated infections and have a special role in treating infection with ESBL-producing organisms. The emergence and spread of resistance to carbapenems will end all the treatment options available for treating MDR pathogens.

The strength of this study was that it would be helpful for better patient care in pediatric population, precautionary actions to prevent the emergence and spread of resistant microorganisms can be undertaken. The limitation of the study was that it is phenotypic identification. It would have been better if molecular-based method was used for confirmation of identification, and bacterial resistance was identified on genetic level.

## CONCLUSION

The magnitude of *Citrobacter* as a pathogen of urine in pediatric population has shown increase in our study, becoming the 2nd most common uropathogen. This pathogen once a commensal of gastrointestinal tract and environment cannot be considered a commensal anymore being 17.3% of the total uropathogen. The *Citrobacter* isolates resistant to multiple antimicrobial agents have emerged, making it an emerging nosocomial pathogen and it requires keen attention as it is not only resistant to the commonly used antibiotic but also MDR. It is alarming that 10.3% of *Citrobacter* is resistant to imipenem, suggesting carbapenemase production. As in pediatric population, UTI soon progresses to urosepsis and renal damage, so search for this pathogen as well as its antibiogram is essential as judicious use of empirical therapy will be dangerous. Adoption of hospital infection control practices and a good antibiotic policy may prevent their spread.

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