

Original Article

ANTIBIOTIC SENSITIVITY PATTERN OF *CITROBACTER* SPP. ISOLATED FROM PATIENTS WITH URINARY TRACT INFECTIONS IN TERTIARY CARE HOSPITAL IN SOUTH INDIA

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ABSTRACT

**Objectives:** Urinary tract infection (UTIs) caused by *Citrobacter* species have been described in 5 to 12% of bacterial urine isolates in adults. The genus *Citrobacter* is a distinct group of aerobic Gram-negative bacilli from the Enterobacteriaceae family. We report here the emergence of *Citrobacter* as an increasingly common urinary pathogen in patients attending this medical college.

**Methods:** The study was carried out over a period of 2 years from January 2010 to December 2011. Urine specimens from both outpatients and inpatients of our hospital were processed. Urine samples which yielded the growth *Citrobacter* spp. were included in the study

**Results:** *E. coli* was the most common organism isolated followed by *Klebsiella* spp. and *Citrobacter* spp. The most active antimicrobial agent against *Citrobacter* isolates was piperacillin-tazobactam (55% sensitive), closely followed by amikacin (47%).

**Conclusion:** The emergence of this usually rare organism as the third most common urinary pathogen, which is resistant to commonly available antibiotics is alarming. Such studies will guide clinicians to choose accurate empirical treatment options and will help to reduce the mortality and morbidity rates from infections.

**Keywords:** Anti-microbial resistance, Antibiotics, *Citrobacter*, Drug resistance, Urinary tract infections.

INTRODUCTION

Urinary tract infection (UTI) continues to be the commonest nosocomial infection according for approximately 40% of all hospital acquired infections and it is one of the most important causes of morbidity and mortality [1, 2]. UTIs caused by *Citrobacter* species have been described in 5 to 12% of bacterial urine isolates in adults [3]. The genus *Citrobacter* is a distinct group of aerobic Gram-negative bacilli from the Enterobacteriaceae family [4]. *Citrobacter* species are primary inhabitants of intestinal tract, often found in human feces [5]. They are also found in soil, sewage, food, and animal feces [6, 7].

These organisms are isolated from variety of clinical specimens like urine, pus, blood, and cerebrospinal fluid [8]. Organisms of genus *Citrobacter* are Gram-negative straight rods, found singly or in pairs, and are motile by peritrichous flagellae. They are facultative anaerobes, oxidase-negative, and typically utilize citrate as sole source of carbon. The genus *Citrobacter* comprises 11 different species. Among these, *Citrobacter koseri* (previously known as *C. diversus*) and *C. freundii* are the commonest species implicated in infections. *Citrobacter* species can cause variety of infections like, respiratory tract infections, urinary tract infection, blood stream infections, wound and burns infections, meningitis, endocarditis, and peritonitis [9]. We report here the emergence of *Citrobacter* as an increasingly common urinary pathogen in patients attending this medical college.

MATERIALS AND METHODS

Study population, design, and setting

The current study was conducted in the Department of Microbiology, Shri B M Patil Medical College, Bijapur, from January 2010 to December 2011.

Ethical clearance and consent

As it was a retrospective study, ethical clearance and consent was not obtained.

Patient evaluation

A total of 1434 urine specimens from both outpatients and inpatients of our hospital having one or more urinary symptoms,

like burning during micturition, fever, pyuria, frequency of urine, dysuria, hematuria, flank pain, suprapubic discomfort, etc., were processed.

Sample collection and isolation, identification of the organism

Mid-stream urine sample in early morning was collected in wide mouth sterile container [10]. All urine samples were examined by routine microscopic examination by wet mount of urine sediment after centrifuging urine for 10 minutes at 1000 revolution per minute (rpm).

Presence of pus cells, red blood cells (RBCs), epithelial cells, casts, and crystals were noted as supportive findings of urinary infection. Simultaneously all urine samples were cultured over routine culture media; Mac Conkey agar and Cysteine lactose electrolyte deficient (CLED) agar with a sterile standard loop. These plates were incubated at 37°C for 2 consecutive days.

Culture results were interpreted according to the standard criteria and a growth of  $10^5$  colony forming units/ml was considered as significant bacteriuria [11][11]. Cultures with more than three colonies were discarded, as contaminants and their antibiotic susceptibility were not tested. Species were identified by conventional biochemical tests according to standard microbiological techniques [10].

Antimicrobial susceptibility testing

All isolates were tested for antimicrobial susceptibility on Mueller Hinton agar by the standard disc diffusion method recommended by the Clinical and Laboratory Standards Institute (CLSI)[12]. agents (disks) were obtained from Hi Media laboratories, Pvt Ltd, Mumbai. Appropriate quality control strains were used to validate the results of the antimicrobial disk. *E. coli*, ATCC 25922, and *Pseudomonas aeruginosa*, ATCC 27853, were used as quality control strains [12].

Statistical analysis

Statistical analysis was performed with SPSS 14 software. Categorical data was analyzed using Chi-Square test with a 0.05 significance level.

## RESULTS AND DISCUSSION

The education and application of personal hygiene are important in that *Citrobacter* strains are excreted as fecal wastes. Epidemics occurring in hospitals are closely related to the fact that hospital staff carry the bacterium in their hands and gastrointestinal systems. *Citrobacter* strains are mostly isolated from the infections of urinary and respiratory systems as nosocomial infection causes [13].

Urinary tract infection by *C. koseri* has been reported to be 12.0% in 1969 and the prevalence rate is rising. Invasive procedures like, catheterization helps them to colonize urinary bladder and during intensive chemotherapy this bacterium disseminates to the blood stream to cause severe bacteremia. Intact immunity helps to control the pathogen to certain extent but when the patients are immunocompromised, the situation is grave. The problem is further intensified by the emergence of multidrug resistance *Citrobacter* sp. resulting into treatment failure [14].

In the present study, *Citrobacter* infections were high among elderly people; this is because of the fact that these groups constitute large proportion of our hospital populations and reduced immunity in these people to fight against infection in general. Similar results were seen in the study conducted by Shih et al [15].

In this study piperacillin-tazobactam was the most active drug, closely followed amikacin. Least active antibiotics were ampicillin

and amoxyclav. Amoxicillin and ampicillin are often used as oral therapy for gram -negative UTIs, but the high rate of in vitro resistance demonstrated in this study and others suggests that they should not be used. Trimethoprim and amoxyclav are also often prescribed; of concern is the increasing rate of resistance to trimethoprim over the last 10 years and the more recent increase in resistance to amoxyclav, presumably as a result of mechanisms other than production of beta -lactamase [16].

**Table 1: Frequency of different organism isolated from UTI**

Organism	Number	Percentage
<i>E. coli</i>	314	45.6
<i>Klebsiella</i> spp.	124	17.9
<i>Citrobacter</i> spp.	108	15.7
Other Gram negatives bacilli	38	5.5
Gram positive cocci	61	8.9
Fungi	44	6.4
Total	689	100

Of the 1434 urine samples received for culture during the study period, 12 samples contained more than one species, significant bacteriuria was found in 48.1 % samples. Among these *E. coli* was the most common organism isolated (Table 1) followed by *Klebsiella* spp. and *Citrobacter* spp.

**Table 2: Age and sex wise distribution of *Citrobacter* spp from UTI patients**

Age in years	Male (n=71)		Female (n=37)		Total (n=108)	
	Number	%	Number	%	Number	%
1-20	6	8.1	4	10.8	10	9.26
21-50	24	35.1	23	62.2	47	43.5
> 51	41	57.7	10	27.0	51	47.2

In all age groups, except those aged more than 51 years, females were more frequently affected than males. Among females, frequency of UTI was more among 21-50 years age group and among males elderly patients were more commonly affected.

**Table 3: Antimicrobial susceptibility of uropathogenic *Citrobacter* isolates**

Antibiotics	Sensitive	Sensitive(%)
Ampicillin	3	2.9
Co -trimoxazole	20	18.5
Norfloracin	28	25.9
Ciprofloxacin	40	37
Gentamicin	25	23.1
Nalidixic acid	10	9.26
Amoxyclav	8	7.41
Amikacin	51	47.2
Cephalexin	8	7.41
Cefoparazone -salbactam	35	32.4
Piperacillin-tazobactam	59	54.6
Ofloxacin	46	42.6
Nitrofurantoin	39	36.1

The most active antimicrobial agent against *Citrobacter* isolates were piperacillin-tazobactam (55% sensitive), closely followed by amikacin (47 %). Amikacin was more effective aminoglycoside than others. Least active antibiotics were ampicillin (3%) and cephalaxin.(7%)

Majority of the isolates showed multidrug resistance in the current study and were found to be resistant to cephalaxin, norfloracin, ciprofloxacin, and the aminoglycosides [Table 3]. This has important implications as patients in a tertiary care hospital like ours receive cephalosoprins, aminoglycosides, fluoroquinolone, or a combination of these drugs as empirical therapy or as definitive treatment. Since good in vitro activity was shown by piperacillin-tazobactam it may be considered as first line therapy for ambulatory patients. The high rates of antibiotic resistance observed in the present study may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogens.

In our study, most of the isolates were from inpatients, similar observations have been made by previous studies [14,17], the probable reason for this are as follows. The hospital staff carry the bacterium in their hands and gastrointestinal systems and invasive procedures like catheterization helps these organisms to colonize urinary bladder [13,14].

## CONCLUSION

The *Citrobacter* isolates resistant to multiple anti-microbial agents have emerged, making it an emerging nosocomial pathogen. The emergence of this usually rare organism as the third most common urinary pathogen, which is resistant to commonly available

antibiotics is alarming. The indiscriminate use of antimicrobial agents is possibly the main reason responsible for this situation. Therefore, such studies will guide clinicians to choose accurate empirical treatment options and will help to reduce the mortality and morbidity rates from infections. It is more significant to prevent the resistance development in micro-organisms and to lend the accurate information to clinicians in terms of the use of antibiotics in appropriate period.

#### CONFLICT OF INTERESTS

Declared None

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