



## Original Research Article

# Significance of *Citrobacter* as an Emerging Nosocomial Pathogen with Special Reference to it's Antibiotic Susceptibility Pattern in a Tertiary Care Hospital, Nellore, AP. India

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## ABSTRACT

### Keywords

*Citrobacter* species,  
Diabetes mellitus,  
Invasive medical devices,  
Trauma,  
Multidrug resistance

*Citrobacter* species are a distinct group of Aerobic gram negative bacilli of Family Enterobacteriaceae. *Citrobacter* species have been reported to cause wide range of infections with simple UTI to Septicemia. Although *Citrobacter* species once considered as less frequent nosocomial pathogen, at present they are posing a major health concern to the microbiologist & clinician because of their multiple drug resistance. The present study was carried out to enlighten the significance of *Citrobacter* as an emerging nosocomial pathogen & its multiple drug resistance. During the study period of one year from May 2014 to April 2015, 1000 clinical specimens were collected and processed from patients with different risk factors. 251 (25.1%) were culture positive for *Citrobacter* Sp. Diabetes mellitus was found to be the major risk factor in the study population of our study, followed by Invasive medical devices, Trauma etc. Antibigram of *Citrobacter* species was performed and interpreted as per CLSI guidelines. *Citrobacter* Sps exhibited maximum sensitivity to Imipenem(97.21%) followed by Meropenem( 94.02%) and least sensitivity to Ampicillin(0.79%). The present study highlighted the impact of nosocomial infections caused by *Citrobacter* species & it's multiple drug resistance nature towards commonly used antibiotics.

## Introduction

*Citrobacter* species are Aerobic, motile, Gram negative bacilli of Enterobacteriaceae commonly found in water, soil, food & intestinal tracts of humans & animals (1). Although *Citrobacter* species are less frequently isolated, they are emerging as nosocomial pathogens with multi drug resistance (2). *Citrobacter* species have been reported to cause a range of infections which

include Urinary tract infections, Wound infections, (Surgical site infections) Respiratory infections, Middle ear infections, infections of Meninges, Osteomyelitis, Peritonitis, Endocarditis, Bacteremia (1). Antibiotic resistance is emerging in the isolates of Enterobacteriaceae and in other Gram negative bacilli globally and is a major

threat to the successful treatment of infections in hospitals. (3).Patients with invasive devices are more prone for Invasive *Citrobacter* infections and are associated with high mortality with majority of patients perishing or wilting to *Citrobacter* bacteremia(4) The high mortality rate associated with *Citrobacter* infections may be due in part to ineffective empirical antibiotic therapy(4). *Citrobacter* species are often resistant to routinely used antibiotics especially extended spectrum Cephalosporins, due to overexpression of chromosomal  $\beta$ -Lactamases(5) Extended spectrum beta lactam antibiotics such as 3<sup>rd</sup> generation Cephalosporins form the major component of empirical antibiotic treatment especially in tertiary care hospital. Indiscriminate use of 3<sup>rd</sup> generation Cephalosporins has contributed to the evolution of Extended spectrum beta lactamases(ESBLs)(6) Keeping in view of these facts, this study was undertaken to enlighten the clinical significance of *Citrobacter* as an emerging nosocomial pathogen with special emphasis on its antibiotic susceptibility pattern.

## **Materials and Methods**

The present study was conducted in Central clinical Microbiology Laboratory of Narayana medical college & Hospital a tertiary care hospital, from April 2014 to March 2015. The present study was approved by the Institutional ethical committee. A total of 1000 samples received to the laboratory from various departments were processed for isolation of *Citrobacter* species. Patients' demographic data, Clinical data was collected thoroughly and noted for analysis.

The specimens include urine, pus, sputum, ET secretions & tubes, blood, CSF. All these were processed immediately as per standard CLSI guidelines.

Gram's staining was performed for the relevant specimens & then inoculated on to commonly used media such as blood agar, Macconkey agar, Chocolate agar, and incubated at 37°C for overnight or for 24 hrs. Blood samples were inoculated in to blood culture bottles containing Trypticase soya broth & incubated in BACT ALERT Automated system. Any indication of growth in BACT ALERT was further inoculated on to BA, NA and MacConkey agar incubated at 37°C overnight. Presence of Gram negative, motile bacilli with Catalase test positive and Oxidase test negative confirms the presence of Enterobacteriaceae member (7) in the processed specimen & isolation of *Citrobacter* was further confirmed by simple phenotypic biochemical tests.

## **Phenotypic identification of *Citrobacter* species**

Any growth on inoculated culture media, especially presence of late lactose fermenting colonies on MacConkey agar were subjected to simplified phenotypic biochemical tests, as described by Brenner et al (8) Tests performed for identification of *Citrobacter* species were Indole production test, Citrate utilization test, Triple sugar Iron agar test, acid production from Lactose, Sucrose, Dulcitol, reduction of Nitrates to Nitrites, positive reaction for O- Nitrophenyl beta D- galactopyranoside (ONPG).

Antibiotic sensitivity testing was performed by Kirby- bauer's disc diffusion method on Muller hinton agar with reference to 0.5 McFarland standards. Selection of antibiotics in the present study was based on their mechanism of action on *Citrobacter* isolates and also the antibiotic policy in the hospital. The results were interpreted based on CLSI guidelines as Sensitive (S), Intermediate (I) and resistant (R).

Antibiotics used in the study include Ampicillin (10µg), Amikacin (30µg), Amoxicillin+clavulanic acid (20/10µg), Aztreonam(30µg), Cefipime (30µg), Cefoperazone-sulbactam(75/30µg), Cefotaxime (30µg), Cefixime (5µg), Co-trimoxazole (20µg), Colistin, Gentamicin (µg), Imipenem(10µg), Meropenem(10µg), Ciprofloxacin(30µg), Ceftriaxone, Piperacillin/Tazobactam(100/10µg). Nitrofurantoin was used for *Citrobacter* isolates from urine.

## Results and Discussion

The present study was conducted in Central clinical Microbiology Laboratory of Narayana medical college & Hospital a tertiary care hospital, from April 2014 to March 2015.

Of the 1000 samples processed in the present study, The highest number of samples collected were from urine (600), followed by pus(200).sputum and blood includes (75) samples individually.ET tubes and secretions include (50) samples (Table-1).

Of the 1000 samples processed, 251 were positive for *Citrobacter* Sps. The prevalence of *Citrobacter* infections was high among male patients (56.58%) than females(43.4%).(Table-2).

Among 251 culture positive *Citrobacter* Sps,150 (59.76%) were from urine, 60(23.9%) from Pus,18(7.17%) from blood, 16 (6.37%) from Sputum and 07(2.79%) from ET tubes & secretions(Table- 3).

Among the major risk factors associated with *Citrobacter* infections,Diabetes mellitus was the major risk factor (41.83%), followed by in order Hypertension (17.92%), IVD's (16.73%), Trauma (8.36%), Chronic Renal failure (7.96%) and

Chronic Pulmonary disease(6.77%) (Table-4).

Antibiogram of the study clearly demonstrated maximum sensitivity to Imipenem (97.21%), followed by Meropenem (94.02%), Colistin (92.04%), Piperacilli&Tazobactam (90.83%), Amikacin (85.6%), Cefperazone+Sulbactam(84.86%).

*Citrobacter* isolates from Urine were sensitive to Nitrofurantoin (92.82%). Of the 251 *Citrobacter* isolates in the present study isolates exhibited maximum resistant to Ampicillin(99.20%) which correlates with the study done by Greeshma etal at Government Medical College, Idduki, Kerala in which 98% of *Citrobacter* isolates were resistant to Ampicillin. In current study resistance has been observed in cephalosporins such as Ceftriaxone (85%), Cefotaxime (76%) followed by resistance to Co-trimoxazole (65%), Aztreonam (65%). Resistance to commonly used Aminoglycosides like Gentamicin in our study (64%) correlates with the study done by Ashish Khanna etal, at Amritsar.(Table-5)

Presence of risk factors in the study population is found to be the major area of concern especially risk factors such as Diabetes mellitus, Trauma, Presence of Insitu Medical devices which are found to be the root cause in treating patients with *Citrobacter* infections because of multi drug resistance exhibited by these group of Enterobacteriaceae members.

The present study indicates that the *Citrobacter* species are mostly associated with Nosocomial infections. This might be due to lack of preventive measures especially when handling the patients on Catheters, CVP lines, IV catheters especially during specimen collection.

**Table.1** Showing sample distribution

Total No of Samples	URINE	PUS	SPUTUM	ET TUBES & SECRETIONS	BLOOD
1000	600	200	75	50	75

**Table.2** showing gender wise distribution of *Citrobacter* infections

TOTAL	MALES	FEMALES
251	142 (56.57)	109 (43.4)

**Table.3** Showing % of cultures positive / with growth of *Citrobacter*

Total No.Of Positive cultures for <i>Citrobacter</i>	URINE (n=600)	PUS (n=200)	SPUTUM (n=75)	ET TUBES & SECRETIONS (n=50)	BLOOD (n=75)
251(25.1%)	150(59.76)	60(23.90)	16(6.37)	07(2.78)	18(7.17)

**Table.4** Showing associated risk factors in patients with *Citrobacter* infections

RISK FACTOR	NUMBER (%)
Diabetes mellitus	105 (41.83)
Hypertension	45 (17.92)
In situ Medical Devices	42(16.73)
Trauma	21 (8.36)
Chronic Renal failure	20(7.96)
Chronic Pulmonary disease	18(7.17)

**Table.5** Antibiotic susceptibility pappern of *Citrobacter* isolates

S.NO	ANTIBIOTICS	SENSITIVE (%)	RESISTANCE(%)
1	Ampicillin	0.79	99.2
2	Amikacin	85.6	14.4
3	Amoxycillin+Clavulanic acid	66.66	33.34
4	Aztreonam	35	65
5	Cefipime	72	28
6	Cefotaxime	24	76
7	Cefixime	22.28	77.72
8	Co-trimoxazole	35	65
9	Colistin	92.04	7.96
10	Gentamicin	36	94
11	Imipenem	97.21	2.79
12	Meropenem	94.02	5.98
13	Ciprofloxacin	33	67
14	Ceftriaxone	15	85
15	Piperacillin-Tazobactam	90.83	9.17
16	Nitrofurantoin	92.82	7.18

In the present study *Citrobacter* infections were high among elderly people with Diabetes mellitus as major risk factor, this is in turn due to hospitalization of such population for prolonged periods of time &

waning of immunity in such age group. Similar results were seen in study done by Shih etal (9) In the present study 25% of isolates of *Citrobacter* were from Urine sample in patients with UTI & having

indwelling Catheters because Catheterization helps the bacteria to colonize Urinary bladder & during extensive chemotherapy this bacterium disseminates to the blood stream to cause severe Bacteraemia.

Presence of intact immune system in the host at least to some extent helps in defending against the pathogen to certain limit. But once if the host is having other than normal immune status or in immune compromised hosts the condition would be fatal. The problem is further aggravated by the emergence of Multi Drug Resistant *Citrobacter* species leading to treatment failure. Infections due to *Citrobacter* were high in the people admitted in ICU because of invasive procedures or kept on devices such as Catheters, CVP lines etc. Several reports suggest that host susceptibility & underlying illness are important predisposing factors (10,11).

The study by Whitby et al has found *Citrobacter* in 12% of acute infections. Some studies reported that *Citrobacter* species appears to be one of the contributing organisms in causing UTI (12)(13) Majority of isolates in our study were resistant to Ampicillin, Aminoglycosides like Gentamicin, Fluoroquinolones such as Ciprofloxacin(14) Imipenem was the preferred drug for the infections due to *Citrobacter* especially in hospitalised patients according to our study followed by Meropenem, Colistin & Piperacillin-Tazobactam.

In conclusion, *Citrobacter* species once considered as a rare isolate, has emerged as a major nosocomial pathogen causing diversified infections especially in hospitalized patients & at present considered as major pathogen causing UTI after

*Escherichia coli*, *Klebsiella* species, *Proteus* of Enterobacteriaceae family.

Indiscriminate use of broad spectrum antibiotics especially in tertiary care hospitals is considered as one of the reason for the emergence of new pathogens such as *Citrobacter*, posing a challenge to the microbiologist & clinician in treating patients. Multi drug resistance & ESBL production were quite high among *Citrobacter* species. Multi drug resistant *Citrobacter* species isolated in our study were sensitive to a combination of antibiotics such as Piperacilli-Tazobactam and are also sensitive to reserved group of Carbapenems such as Imipenem, Meropenem. In spite of vast sensitivity to these Carbapenems, very few of *Citrobacter* isolates in our study exhibited resistance indicating an alarm for the clinicians & microbiologists about future massive resistance for these group of reserved drugs.

Strict implementation of antibiotic policies should be inculcated especially in tertiary care hospitals. Proper disinfection of OTs, Wards, proper hand washing procedures should be implemented in order to minimize the emergence of such new pathogens, which were seldom isolated earlier. The opportunistic character of *Citrobacter* is further aggravated by the age of affected host, presence of risk factors such as Diabetes mellitus, Hypertension, Trauma etc. as mentioned in current study.

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