



## CITROBACTER SPECIES: AN EMERGING NOSOCOMIAL PATHOGEN AND ITS ANTIBIOTIC RESISTANCE PATTERN AT A TERTIARY CARE CENTRE

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### ABSTRACT

**BACKGROUND & OBJECTIVES:** 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 Urinary tract Infections to Septicaemia. 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. AIMS AND OBJECTIVES:-a) To characterize the infections caused by Citrobacter species. b) To study the occurrence of risk factors for infection with Citrobacter species c) To assess the antibiotic susceptibility pattern of Citrobacter species

**MATERIALS AND METHODS:** During the study period of one year from May 2017 to April 2018, 800 clinical specimens were collected and processed from patients with different risk factors. Citrobacter species isolated and were speciated using biochemical reactions. Antibiogram of Citrobacter species was performed and interpreted as per CLSI guidelines

**RESULTS:** Of the 800 samples 250 Citrobacter species were isolated .There was a highly significant difference in the resistance of the Citrobacter isolates to all the antimicrobials including beta lactams, quinolones and aminoglycosides. ESBL production was seen in 64% of the hospital

**CONCLUSION:** The present study highlighted the impact of Nosocomial infections caused by Citrobacter species & its multiple drug resistance nature towards commonly used antibiotics..

**KEYWORDS :** Citrobacter species, Risk factors, Antibiotic resistance. ESBL

### 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 [3]. 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. [4]. Patients with invasive devices are more prone for Invasive *Citrobacter* infections and are associated with high mortality with majority of patients perishing or willing to *Citrobacter* bacteraemia[5] 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 –Lactamases[5] Extended spectrum beta lactam antibiotics such as 3rd generation Cephalosporins form the major component of empirical antibiotic treatment especially in tertiary care hospital. Indiscriminate use of 3rd 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.

### Material and Methods:-

A total of 800 consecutive gram negative bacilli which were recovered from clinical specimens during the study period from April 2017 to May 2018 were included in this study. These were isolated from various clinical samples like urine, blood, pus, sputum

and miscellaneous samples .Patient's demographic data, clinical data was collected thoroughly and noted for analysis .The isolates of the *Citrobacter species* were further identified by their colony characteristics; Gram's staining, and biochemical reactions by standard methods [7]. Any growth on inoculated culture media, especially presence of late lactose fermenting colonies on MacConkey agar were subjected to simplified phenotypic biochemical tests. 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).The anti-microbial sensitivity testing of the isolates was performed on Mueller Hinton agar by the Kirby Bauer method by using the disc diffusion technique and the results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) 2018 guidelines [7]. The isolates which were resistant or intermediately susceptible to any of the third generation cephalosporins were further processed for ESBL detection by the double disc potentiation method [7] by using a disc of cefotaxime (30µg)/ ceftazidime (30µg), and combination discs of cefotaxime 30µg and clavulanic acid 10µg and of ceftazidime 30µg plus clavulanic acid 10µg. *Klebsiella pneumoniae* ATCC 700603 was used as the ESBL positive control. ESBL production was inferred if the inhibition zone increased by 5 mm towards the cefotaxime plus clavulanic acid disc or towards the ceftazidime plus clavulanic acid disc in comparison to the third generation cephalosporin disc alone.[8]

### Results and discussion:-

Of the 250 samples processed in the present study, the highest number of samples collected were from urine (100), followed by pus (70). sputum and blood includes (25) samples individually. ET tubes and secretions include (30) samples (Table-1). Of the 800 samples processed, 250 were positive for *Citrobacter* Species. The prevalence of *Citrobacter* infections was high among male patients (56.57%)

than females (43.43%). (Table-2). Among 250 culture positive *Citrobacter* Species, 100 (41.66%) were from urine, 70 (11.66%) from Pus, 50 (8.33%) from blood, 20 (3.33%) from Sputum and 10 (1.66%) from miscellaneous (Table- 3). Antibiogram of the study clearly demonstrated maximum sensitivity to Imipenem (97.21%), followed by Piperacillin & Tazobactam (90.83%), Amikacin (85.6%) *Citrobacter* isolates from Urine were sensitive to Nitrofurantoin (82.82%). Of the 250 *Citrobacter* isolates in the present study isolates exhibited maximum resistant to Ampicillin (99.20%) which correlates with the study done by Greeshma et al [9] 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%), Resistance to commonly used Aminoglycosides like Gentamicin in our study (64%) correlates with the study done by Ashish Khanna et al [10], at Amritsar. (Table-5) ESBL Production was seen amongst 64% of *Citrobacter* isolates which was higher as compared to Shobha et al. and Mehar Rizvi et al. 2009.[11,12]

**Table.1 Showing sample distribution**

Total number of samples	Urine	Pus	Blood	Sputum	Miscellaneous
800	300	250	120	80	50

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

Total	Male	Females
250	140(56.57)	110(43.43)

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

Total number of positive cultures for <i>Citrobacter</i>	Urine n= 300	Pus n=250	Blood n=120	Sputum n=80	Miscellaneous n=50
250	100	70	50	20	10

**Table.4 Antibiotic Resistance pattern of *Citrobacter* isolates**

Sr no	Antibiotics	Resistance (%)	Sensitive (%)
1	Ampicillin	99.2	0.79
2	Amikacin	14.4	85.6
3	Cefotaxime	24	76
4	Ciprofloxacin	67	33
5	Imipenem	2.79	97.21
6	Piperacillin tazobactam	9.17	90.83
7	Co-trimoxazole	65	35
8	Cefotaxime +clavulanic acid	33.34	66.66
9	Gentamycin	64	36
10	Nitrofurantoin	17.18	82.82

**Table.5 ESBL pattern of *Citrobacter* isolates**

Total	ESBL	NON -ESBL
250	160(64%)	90(36%)

**Figure showing ESBL Detection**



**Table.6 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)

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%) 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 In situ 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 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 Shah et al [13] 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]. 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 Piperacillin-Tazobactam.

**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 quiet high among *Citrobacter* species. Multi drug resistant *Citrobacter* species isolated in our study were sensitive to a combination of antibiotics such as Piperacillin-Tazobactam and are also sensitive to reserved group of Carbapenems such as Imipenem. 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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