



## EMERGENCE OF EXTENDED SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING CITROBACTER - A NOSOCOMIAL PATHOGEN OF GREAT CONCERN

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### ABSTRACT

**Background:** Multidrug resistant *Citrobacter* species are emerging as nosocomial pathogens. Among the *Citrobacter* species, *C. freundii* is the most frequent isolate recovered from various clinical samples.

**Aims and objectives:** i) To determine the frequency of isolation of *Citrobacter* from various clinical samples. ii) To analyse the antibiogram of *Citrobacter* species with special reference to ESBL production. **Method:** The prospective study was carried out for a period of one year. The clinical samples were subjected to culture and sensitivity as per standard conventional methods and automated Vitek 2 compact system. **Results:** The frequency of recovery of *Citrobacter* species was 6.44%, of which the predominant was *C. freundii* (86%). Isolation was maximum from indoor patients (78.83%), the commonest sample being pus (25.9%) followed by urine (22.5%). The ESBL production was detected in 30.03% cases. **Conclusion:** Nosocomial infections due to ESBL producing *Citrobacter* species calls for the need of implementation of effective infection control measures, thus limiting the burden of this superbug.

**KEYWORDS :** *Citrobacter* species, *Citrobacter freundii*, multidrug resistance, ESBL

### INTRODUCTION

Genus *Citrobacter*, the member of Enterobacteriaceae family, are Gram negative bacilli, facultative anaerobes, motile, commonly found in soil, water, food & intestinal tracts of humans & animals.<sup>1</sup> In the recent years, *Citrobacter* species have gained importance as a clinical pathogen with multidrug-resistance, causing opportunistic, nosocomial and community acquired infections.<sup>2,3</sup> A local or systemic violation in the host defenses can permit them to cause a range of infections.<sup>4</sup> *Citrobacter* species associated with hospital acquired infections may involve urinary tract system, respiratory system, hepatobiliary system, intestine, endocardium, bone, skin and soft tissue, central nervous system and the bloodstream.<sup>5,6</sup> Among *Citrobacter* species, *C. freundii* has been the most frequent isolate recovered from various clinical samples.<sup>6</sup> Invasive *Citrobacter* infections are associated with high morbidity and mortality, most commonly being encountered in blood stream infections.<sup>7</sup> The nosocomial potential of *Citrobacter* species is attributed to breach in the sterile practices and indiscriminate usage of antibiotics.<sup>8</sup> The emergence of multidrug resistant *Citrobacter* has become a therapeutic challenge, limiting the therapeutic options, especially the Extended spectrum beta-lactamase (ESBL) producing isolates. The underlying mechanism of resistance may be due to the overexpression of chromosomal beta-lactamases.<sup>4</sup> ESBL belongs to Ambler molecular class A and Bush Jacob Medeiros functional class 2be. All the ESBL producing isolates are resistant to ampicillin, first, second and third generations of cephalosporins and monobactams. However, they are sensitive to cephamycin, fourth generation of cephalosporins, combination of beta-lactam and beta-lactamase inhibitor antibiotics, and carbapenemes.<sup>9</sup>

The present study has been carried out to analyze the frequency of isolation of *Citrobacter* species from the clinical samples and to determine the antimicrobial susceptibility profile which has posed the difficulties in the management of infection caused by this superbug.

### MATERIALS AND METHOD

The prospective study was carried out in Microbiology department of a tertiary care hospital in western U.P., over a

period of one year, from March 2021 to February 2022. The study group included all the clinical samples received for culture sensitivity, irrespective of age and gender.

**Sample processing:** All the samples such as pus, tissue, blood, genitourinary, respiratory samples etc. were processed as per standard guidelines.<sup>9,10</sup> These samples were subjected to culture and sensitivity testing by conventional methods and automated Vitek 2 compact system (BD). After twenty-four hours of aerobic incubation at 37°C, growth on Blood agar showed grayish-white colonies, 2-3 mm in size, circular, smooth, low convex with entire edge. MacConkey agar showed late lactose fermenting colonies. The isolates obtained were Catalase positive, Oxidase negative, fermentative on Hugh-Leifson test, reduced nitrate to nitrite. These findings were presumptive of our isolates belonging to family Enterobacteriaceae. Further identification of *Citrobacter* isolates upto species level was performed by phenotypic biochemical tests<sup>11</sup> or Vitek 2 compact system.

Antimicrobial sensitivity test was done as per CLSI guidelines 2020, by Kirby Bauer's disc diffusion method on Muller Hinton agar and by automated Vitek 2 compact system.<sup>12</sup> The isolates which were resistant to any of the third generation cephalosporins such as Ceftazidime or Ceftriaxone, were further confirmed for ESBL production by double disc diffusion method using Ceftazidime (30µg) and Ceftazidime - Clavulanate (30 µg/10 µg) disc. A ≥ 5-mm increase in a zone diameter for Ceftazidime-Clavulanate vs the zone diameter of the Ceftazidime when tested alone is identified as ESBL.<sup>12</sup>

### RESULTS

Total numbers of samples processed during the study period were 14876, of which 4548 were culture positive. Among total culture positive cases, *Citrobacter* species were isolated in 293(6.44%) cases (figure 1), of which 252(86%) isolates were *Citrobacter freundii* and 41(14%) were *Citrobacter koseri*.

*Citrobacter* species were predominantly isolated from indoor cases (78.83%), thus suggesting the potential of the concerned pathogen to cause nosocomial infections. The frequency of isolation of *Citrobacter* was found to be higher in males

(59.04%) as compared to females (40.96%). Number of cases were maximum in elderly age groups (> 60 years) followed by 50-60 years, and 21-40 years (table 1).

Out of total 293 cases, maximum numbers were recovered from pus 76 (25.9%) followed by urine 66 (22.5%), endotracheal secretions 38 (12.9%), sputum 31 (10.6%), blood 25 (8.5%) (table 2). Antibigram of the isolates showed maximum sensitivity to Polymixin B and Colistin (100%), followed by Tetracycline (34.82%), Amoxicillin-clavulanate (31.06%), Meropenem (27.65%), Imepenem (25.6%), Amikacin (24.58%), Piperacillin-tazobactam (21.17%). Among the urinary isolates of *Citrobacter* (n=66), sensitivity to Nitrofurantoin and Norfloxacin was detected in 48.5% and 41.6% cases respectively.

Maximum resistance was found against Ampicillin (100%), followed by Cephazolin (94.19%), Aztreonam (91.80%), Ceftriaxone (91.12%), Ceftazidime (90.44%), Cefepime (86.34), Cotrimoxazole (85.66%) and Chloramphenicol (84.98%)(table 3).

ESBL production was seen in 30.03% cases, of which 28% were indoor and 2.03% were outdoor cases (figure 2).

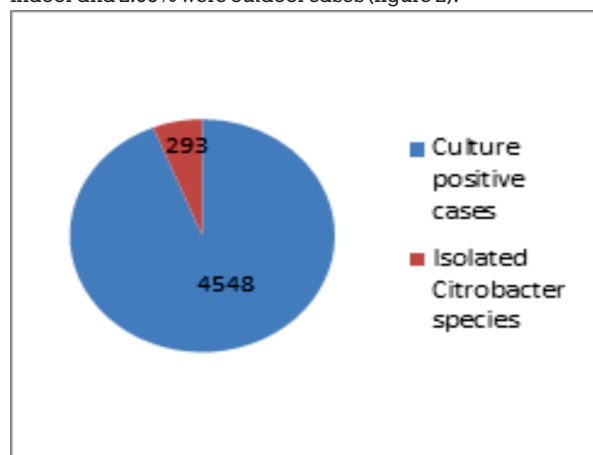


Figure 1. Frequency of isolation of *Citrobacter* species in culture positive cases (n=4548)

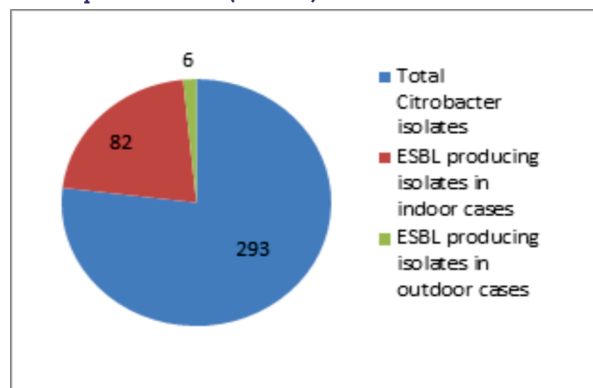


Figure 2. ESBL producing *Citrobacter* isolates among indoor and outdoor cases

Table 1. Age and Gender wise distribution of culture positive cases of *Citrobacter* (n=293)

Age (year)	Total (293)	Male(173)	Female(120)
0-10	11	7	4
11-20	19	11	8
21-30	58	36	22
31-40	48	28	20
41-50	31	17	14
51-60	61	36	25
>60	65	38	27

Table 2. Sample wise distribution of *Citrobacter* species

Sample	Frequency (n=293)	%
Pus	76	25.93
Urine	66	22.52
Endotracheal secretion	38	12.96
Sputum	31	10.58
Blood	25	8.53
Tissue	13	4.43
Bronchial wash	12	4.09
High vaginal swab	11	3.75
Tracheal aspirate	9	3.07
Pleural fluid	9	3.07
Synovial fluid	3	1.02

Table 3. Antibiotic Resistance Pattern among clinical isolates of *Citrobacter*

Antibiotic (disc concentration)	Resistant (n=293)	%	Sensitive (n=293)	%
Ampicillin (10µg)	293	100	00	0.0
Cephazolin (30 µg)	276	94.19	17	5.81
Ceftriaxon (30 µg)	267	91.12	26	8.88
Ceftazidime (30 µg)	265	90.44	28	9.56
Cefepime (30 µg)	253	86.34	40	13.66
Amoxicillin-clavulanate (20 /10 µg)	202	68.94	91	31.06
Amoxicillin-sulbactam (10/10 µg)	230	78.5	63	21.50
Piperacillin-tazobactam (100/10 µg)	231	78.83	62	21.17
Amikacin (30 µg)	221	75.42	72	24.58
Gentamycin (10 µg)	240	81.91	53	18.09
Ciprofloxacin (5 µg)	237	80.88	56	19.12
Levofloxacin (5 µg)	236	80.54	57	19.46
Imepenem (10 µg)	218	74.40	75	25.60
Meropenem (10 µg)	212	72.35	81	27.65
Tetracyclin (30 µg)	191	65.18	102	34.82
Chloramphenicol (30 µg)	249	84.98	44	15.02
Trimethoprim-sulfamethoxazole (1.25/23.75 µg)	251	85.66	42	14.34
Aztreonam (30 µg)	269	91.80	24	8.20
Polymixin B (300U)	00	00	293	100
Colistin (10 µg)	00	00	293	100

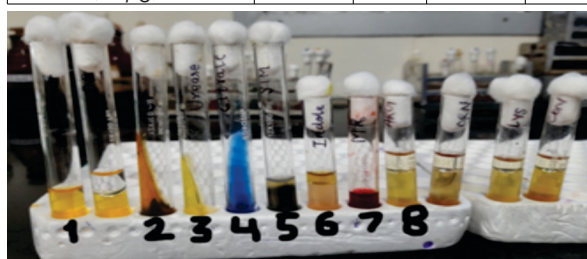


Image 1. Biochemical identification of *Citrobacter freundii*

1-Hugh Leifson test: fermentative; 2-Triple sugar iron agar test: K/A with H<sub>2</sub>S and gas; 3-Urea hydrolysis test negative; 4-Citrate utilization test positive; 5-SIM motility media: motile; 6-Indole test negative; 7-Methyl red positive; 8-decarboxylase test i.e. Arginine, Ornithine, Lysin : not decarboxylated.

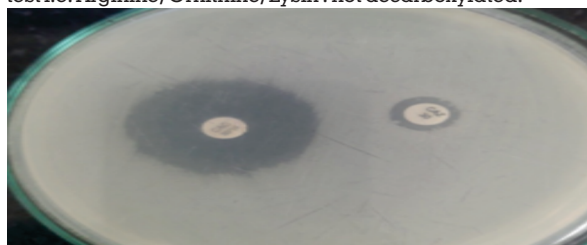


Image 2. Detection of ESBL production by double disc

diffusion method using Ceftazidime (CAZ 30µg) and Ceftazidime – Clavulanate (CAC 30 /10 µg) disc. A  $\geq$  5-mm increase in a zone diameter for CAC vs the zone diameter of the CAZ when tested alone is identified as ESBL

## DISCUSSION

Citrobacter has been an emerging nosocomial pathogen with special emphasis on its antibiotic susceptibility pattern, exhibiting multidrug resistance. Citrobacter isolates producing the Extended spectrum beta lactamases has been posing hinderance in the management of infection, which could lead to morbidity and mortality.<sup>1</sup>

In our study, the frequency of isolation of Citrobacter species was found to be 6.44% which was higher than that reported by Khanna A et al (4.70%) in their study done in 2012.<sup>4</sup> However, our findings was much lower than that reported by Bhise M et al. (31.3%)<sup>7</sup> and Avinash G et al. (25.1%)<sup>8</sup> in their studies done in India.

In the present study Citrobacter freundii was the commonest species isolated (image 1). Similar findings have been reported in other studies also,<sup>4,6,13</sup> while studies done by Metri B et al.<sup>14</sup> and Mohanty S et al.<sup>15</sup> have reported C. koseri as the predominant species.

Out of total Citrobacter isolates, majority (78.83%) were isolated from nosocomial cases. Similar observations were also made by various authors in their studies.<sup>4,14,15</sup>

In reference to the isolation of Citrobacter species, males (59.04%) outnumbered females (40.96%) with the ratio of 1.4:1. The male predominance was also reported in other studies.<sup>1,8</sup>

Most of our isolates were recovered from pus (25.9%) and urine (22.5%) which have been supported by various other studies as well.<sup>1,4,6,8,13,14,15</sup> These findings give us an insight of wound infections and urinary tract infections attributed to Citrobacter.

The antibiogram of the Citrobacter isolates revealed multidrug resistance, especially the resistance to oxyminogroup of antibiotics. The ESBL production was seen in 30.03% of cases (image 2), out of which indoor cases were 28% and outdoor were 2.03%. This rate of ESBL production was much lower than reported by Khanna et al. in their study, where they have found 61.64% ESBL production in indoor cases and 30.70% in outdoor cases.<sup>4</sup> Higher rate of ESBL production was also detected by Rizvi M et al. (62%)<sup>16</sup> and Uma A et al. (82.50% in indoor cases).<sup>17</sup> On the other hand, in a study carried out by Kanamori H et al.<sup>18</sup> ESBL production was detected in 19.3% of cases. Our study observed the potential of ESBL producing isolates to cause healthcare associated infections. Thus, alerting us for the strict adherence to hand hygiene and aseptic clinical practices. The underlying factors for the emergence of MDR Citrobacter may be due to excessive usage of antimicrobial agents especially broad spectrum, extended hospital stay, surgical procedure and instrumentation.

## CONCLUSION

The emergence of ESBL producing Citrobacter species in hospital settings calls for the need of implementation of effective infection control measures, thus limiting the burden of this nosocomial pathogen. Correct identification and antimicrobial susceptibility testing can help in the management of the infections caused by multidrug resistant isolates.

## LIMITATIONS

1. The result of the study would have been more conclusive if it had been carried out for a prolonged duration.
2. Due to financial constraints, molecular techniques could

not be carried out in our setting.

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