

# The Antibiotic Resistance Pattern in *Citrobacter* Species: An Emerging Nosocomial Pathogen in a Tertiary Care Hospital

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## ABSTRACT

Members of the genus, *Citrobacter*, a gram negative, non-spore forming rod which belongs to the family, Enterobacteriaceae are emerging as important nosocomial pathogens. The study was carried out to determine the frequency and the anti-microbial sensitivity pattern of the *Citrobacter* species among the various gram negative isolates which were obtained from different clinical samples. Out of the total 1830 gram negative bacilli which were isolated over a period of one and half year, from February 2010 to August 2011, from various clinical samples

which were submitted to the microbiology laboratory of the Sri Guru Ram Das Hospital, eighty six (4.70%) isolates belonged to the *Citrobacter* species, of which 58 were *Citrobacter freundii* and 28 were *Citrobacter koseri*. Sixty three (73.26%) out of 86 *Citrobacter* isolates were multi-drug resistant. There was a highly significant difference in the resistance of the *Citrobacter* isolates to all the antimicrobials including beta lactams, quinolones and aminoglycosides, among the hospital and outdoor isolates. ESBL production was seen in 61.64% of the hospital and in 30.70% of the outdoor isolates.

**Key Words:** *Citrobacter freundii*, *Citrobacter koseri*, Antimicrobial resistance, ESBL, MBL

## INTRODUCTION

Antibiotic resistance is emerging in the isolates of Enterobacteriaceae and in other gram negative bacilli in many parts of the world and this is a major threat to the successful treatment of infections in hospitals [1]. Nosocomial infections are major public health concerns because of the substantial morbidity and the mortality which are associated with them. Although *Citrobacter* spp. are infrequent nosocomial pathogens, a local or systemic breach in the host defenses can allow them to cause a range of infections. These include urinary tract infections, neonatal sepsis, pulmonary infections, brain abscess, meningitis and blood stream infections. The *Citrobacter* species are emerging as important nosocomial pathogens. The *Citrobacter* infections are associated with a high mortality rate. They are often resistant to the routinely used antibiotics, especially the extended spectrum cephalosporins, due to the overexpression of chromosomal beta lactamases [2]. Keeping these facts in mind, this study was conducted to know the prevalence of the *Citrobacter* spp. amongst gram negative bacilli, its antibiotic resistance pattern and ESBL/MBL production in this part of the country.

## MATERIAL AND METHODS

A total of 1830 consecutive gram negative bacilli which were recovered from clinical specimens during the study period from 1-2-2010 to 30-8-2011 were included in this study. These were isolated from various clinical samples like urine, blood, pus (pus aspirate, chest tubes, IV cannula and catheter tips). The isolates of the *Citrobacter* spp. were further identified by their colony characteristics; Gram's staining, and biochemical reactions by standard methods [3]. The anti-microbial sensitivity testing of the isolates of *Citrobacter* spp. 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) guidelines [4]. The isolates which were

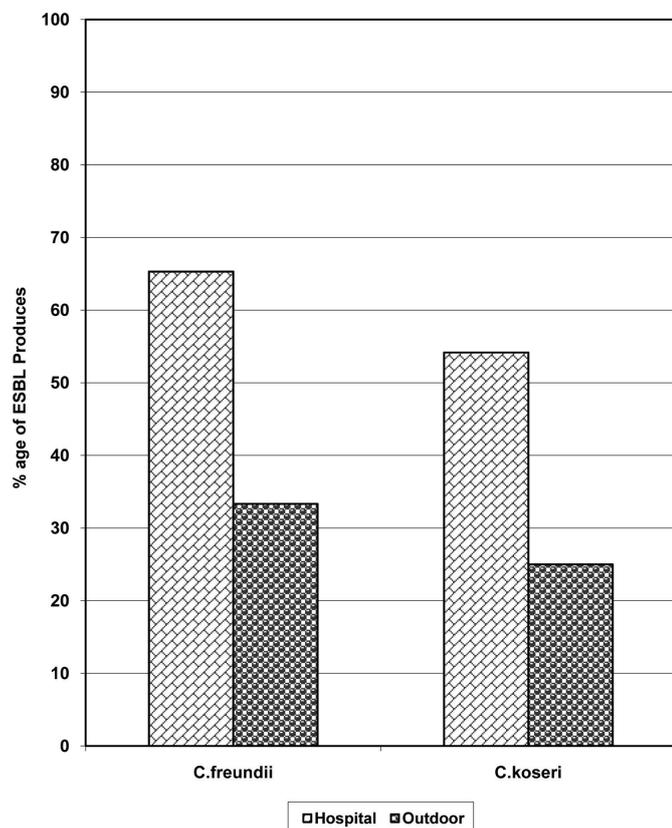
resistant or intermediately susceptible to any of the third generation cephalosporins were further processed for ESBL detection by the double disc potentiation method [4] by using a disc of cefotaxime (30µg)/ ceftazidime (30µg), and combination discs of cefotaxime 30µg and clavulanic acid 10µg and of 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. MBL detection was done by using imipenem and imipenem-EDTA discs [5] [Table/Fig-2].

## RESULTS

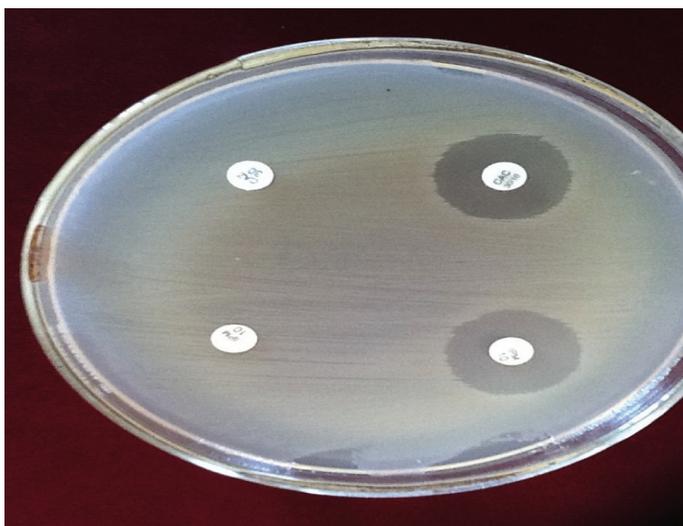
Out of the 1830 gram negative isolates, 86 belonged to the *Citrobacter* species (*Citrobacter freundii*-58, *Citrobacter koseri*-28). Among the 86 *Citrobacter* isolates, 73 (84.88%) were obtained from nosocomial samples, whereas 13 (15.12%) were obtained from outdoor specimens. A majority of these isolates were recovered from pus samples (44.19 %), followed by urine (36.05%) and blood (8.14%). [Table/Fig-3] Further statistical analysis showed a highly significant difference ( $p < 0.001$ ) in the resistance of the *Citrobacter* species to all the antimicrobials including beta lactams, aminoglycosides and fluoroquinolones among the hospital and the outdoor isolates [Table/Fig-4]. ESBL production was seen in 45 (61.64%) of the hospital and in 4(30.70%) of the outdoor isolates. The ESBL producing *Citrobacter freundii* and *Citrobacter koseri* among the hospital isolates and the outdoor isolates is shown in the graph. [Table/Fig-1]. MBL production was seen in only 2(4.08) isolates.

## DISCUSSION

The present study indicates that the *Citrobacter* spp. are mostly associated with nosocomially acquired infections. Of the total gram



**[Table/Fig-1]:** Graph Showing ESBL Production Among Hospital And Outdoor Isolates Of Citrobacter Freundii And Citrobacter Koseri



**[Table/Fig-2]:** Extended spectrum beta lactamase detection were confirmed by CLSI confirmatory method by using disc of ceftazidime 30µg/disc and ceftazidime and clavulanic acid (30 µg/10 µg/disc) (Himedia, Mumbai) (1st Row), and MBL detection by Imipenem and Imipenem and EDTA (2nd Row).

Sample	No of Samples	%age of Samples
Pus	38	44.19
Urine	31	36.05
Respiratory	9	10.47
Blood	7	8.14
Sterile body fluid	1	1.16

**[Table/Fig-3]:** Distribution Of Citrobacter Species Among Various Clinical Samples

negative bacilli, 4.70% were of *Citrobacter* spp. and this rate of isolation was much higher than that which was reported by Ali et al [6] i.e. 1.35% of the *Citrobacter freundii* out of a total of 812

Antibiotic (disc concentration)	<i>C. freundii</i>		<i>C. koseri</i>	
	Hospital (n = 49)	Outdoor (n = 9)	Hospital (n = 24)	Outdoor (n = 4)
Gentamicin (10µg)	33 (67.35)	2 (22.22)	14 (58.73)	1 (25.00)
Amikacin(30µg)	15 (30.61)	-	5 (20.83)	-
Ciprofloxacin(5µg)	35 (71.43)	1 (11.11)	15 (62.50)	-
Norfloxacin(10µg)	39 (79.59)	3 (33.33)	13 (54.17)	-
Piperacillin(30µg)	15 (30.61)	-	4 (16.67)	-
Cephalexin(30µg)	39 (79.59)	4 (44.44)	16 (66.66)	2 (50.00)
Cefotaxime(10µg)	33 (67.35)	3 (33.33)	15 (62.50)	1 (25.00)
Ceftazidime(10µg)	34 (69.39)	-	12 (50.00)	-
Ceftriaxone(30µg)	24 (48.98)	-	9 (37.50)	-
Cefdinir(5µg)	35(71.43)	1(11.11)	14(58.33)	1(25.00)
Imipenem(30µg)	2(4.08)	-	-	-
Meropenem(10µg)	2(4.08)	-	-	-
Piperacillin/ Tazobactam(100-10µg)	3(6.12)	-	-	-

**[Table/Fig-4]:** Antibiotic Resistance Pattern among Hospital and Outdoor Isolates of Citrobacter Spp. (in %age)

gram negative bacilli. This might be because of a lack of preventive measures during the handling of the patients in our institution, thus leading to the spread of this organism among the patients, especially the old, debilitated patients who were on IV catheters, CVP lines, urinary catheters, etc. A majority of the isolates were recovered from pus samples (44.19%), which was higher than that which was reported by Patil et al [7] (21.1%) and Shah et al [8] (22.85%). In our study, about 36.05% isolates were recovered from urinary samples, whereas other authors had reported the isolation of about 65.71% *Citrobacter* species from urinary samples [8]. The antibiotic resistance pattern showed a significantly higher level of resistance among the hospital isolates as compared to that among the outdoor isolates. Similar results have been reported in other studies also [7,9]. The high level of antibiotic resistance among the hospital isolates in the present study may be due to the fact, that ours was a tertiary care hospital with a widespread use of broad spectrum antibiotics, which might have led to the selective survival advantage of these pathogens. In our study, *Citrobacter freundii* was the commonest species which was isolated, followed by *Citrobacter koseri*, as was the case in other studies also [10]. ESBL production was found in 45 (61.64%) of the hospital and in 4 (30.70%) of the outdoor isolates by the double disc potentiation method 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 [4]. The rate of the ESBL production among the *Citrobacter* species in our study was higher than that which was reported by other authors [6,11], which was 36.36% and 19.3% respectively. However, it was comparable with the finding of Rizvi et al [12] (62%) and lower than that which was reported by Uma A et al [13] who had reported it to be 86.50% amongst the hospital isolates. Excessive antibiotic exposure (especially the extended spectrum cephalosporins), extended hospital stay, recent surgery, admission to the ICU and instrumentation have been identified as the risk factors for the selection of the ESBL producing strains. The rate of the ESBL production was also much higher in the outdoor patients, which could have been due to the fact, that in our setup, extended spectrum cephalosporins were being prescribed injudiciously to the outpatients.

## CONCLUSION

*Citrobacter* species are emerging as important nosocomial pathogens. *Citrobacter freundii* was the commonest species which was isolated at our hospital. Multi-drug resistance and ESBL production are quite high amongst the *Citrobacter* species. The multidrug resistant *Citrobacter* species that were isolated in our study were mostly sensitive to a combination of piperacillin/tazobactam and sulbactam/ cefoperazone. So, greater caution is required in the selection of the antibiotics which have to be used, to avoid treatment failure. Carbapenems like imipenem and meropenem should be kept as reserve drugs, in case the organism becomes resistant to these combinations. This bacterium can become resistant to these drugs at any time and so, the indiscriminate, inadequate and the prophylactic use of these antibiotics should be avoided. Infection control measures like disinfection of the wards and equipments, barrier precautions against colonized patients and proper hand hygiene measures should be strictly adhered to, to prevent the spread of this pathogen. The antibiogram is a cost effective, easy and rapid typing tool in a low resource country like ours, where molecular typing tools are not easily accessible. Only in case of emergency, empirical therapy which is based on the antibiotic sensitivity pattern of that institute should be given to the patients.

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