

Antibiotic Resistant Pattern of Citrobacter Species Isolated from Various Clinical Samples at VIMS Hospital, Ballari

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Received on 20.04.2019, **Accepted on** 16.05.2019

Abstract

Background: Citrobacter is a Gram negative, non-sporing bacillus belonging to the family of Enterobacteriaceae. Citrobacter species are primary inhabitants of intestinal tract, often found in human faces. They can also be found in soil, sewage, food, and animal feces. Nowadays Citrobacter species are emerging as important nosocomial pathogens due to reduced host defences leading to urinary tract infections, neonatal sepsis, pulmonary infections, brain abscess, meningitis and blood stream infections. In health care settings, Citrobacter species have become an increasing cause of concern as they are becoming multidrug resistant (MDR) leading to high morbidity and mortality in the patients.

Objectives: to assess the antibiotic resistance pattern of Citrobacter species isolated from various clinical samples at VIMS hospital, Ballari.

Materials and Methods: A retrospective study was done on results of culture and antimicrobial susceptibility of isolates of Citrobacter species from urine, pus, sputum and blood samples obtained for a duration of 6 months, at VIMS, Ballari. Samples were processed for routine microscopy and culture and Citrobacter species were identified by standard methods. Antimicrobial susceptibility testing was performed as per CSLI guidelines by Kirby-bauer disc diffusion method.

Results: Out of 82 isolates of Citrobacter spp, majority were from urine (63.4%), followed by sputum (17%), pus (13.4%) and blood (6%). The prevalence of Citrobacter infections was high among male patients (58.5%) than females (41.4%). Citrobacter koseri was the predominant species 56 (68.2%) isolated. Anti-biograms of Citrobacter isolates revealed that effective antimicrobial agent against Citrobacter isolates was imipenem (91.8% sensitive), followed by Piperacillin/Tazobactam (58.3%) and Amikacin (53.4%).

Conclusion: Citrobacter spp are emerging as one of the important pathogens causing nosocomial infections. Emergence of multi-drug resistant Citrobacter species is also alarmingly high. Hence routine monitoring of drug susceptibility pattern of the isolates in a particular area helps to identify the resistance trend prevalent in that particular area which in turn helps to suggest empirical treatment options to the clinicians.

Keywords: Citrobacter species; Gram negative bacilli; Bacterial flora; Multidrug resistance nosocomial infections; Antimicrobial resistance.

How to cite this article:

Kalpana S, Gajendra Varma. Antibiotic Resistant Pattern of Citrobacter Species Isolated from Various Clinical Samples at VIMS Hospital, Ballari. J Microbiol Relat Res. 2019;5(2):71-74.

Introduction

Citrobacter is a Gram negative, non-sporing bacillus belonging to the family of Enterobacteriaceae.¹ They are primary inhabitants of intestinal tract, often found in human faeces. They are also found in soil, sewage, food, and animal faeces.² They are implicated in a number of infections in human beings. Three species are recognised in clinical samples: *Citrobacter freundii*, *Citrobacter koseri* (Formerly *Citrobacter Diverses*) and *Citrobacter amalonaticus*.³ *Citrobacter* spp. especially *Citrobacter freundii*, is frequently causing nosocomial infections, and increasingly becoming multi-drug resistant (MDR).⁴ This may be due to reduced host defences which can allow them to cause a range of infections which include urinary tract infections, neonatal sepsis, pulmonary infections, meningitis and blood stream infections⁵. In health care settings, *Citrobacter* species have become an increasing cause of concern as they are associated with high morbidity and mortality about 30–60%.⁶ They tend to produce serious nosocomial outbreaks and that they are increasingly becoming resistant to antibiotic.⁷ Patients with invasive devices are more prone for invasive *Citrobacter* infections and are associated with high mortality with majority of patients succumbing to *Citrobacter* bacteraemia. The high mortality rate associated with *Citrobacter* infections may be due to ineffective empirical antibiotic therapy.⁸

Objectives

1. To identify the various *Citrobacter* species in clinical samples—urine, pus, sputum and blood
2. To determine the antimicrobial resistance pattern of the isolated *Citrobacter* species from urine, pus, sputum and blood samples

Materials and Methods

Various clinical samples like urine, sputum, pus and blood samples were collected from patients attending different wards in hospital or visiting

the outpatient departments at VIMS, MCH, Ballari for a duration of 6 months. All the samples were processed by Gram's stain and inoculated onto Blood agar and MacConkey agar within two hours of collection. The inoculated agar plates were incubated at 37°C aerobically overnight and examined for the presence of any growth after incubation the next day.

The isolates were identified by colony morphology, Gram's stain and various biochemical reactions,

Citrobacter species were differentiated using biochemical reactions like Indole test, H₂S production in Triple sugar iron test, Ornithine decarboxylation, Growth in KCN media, Ability to ferment Adonitol. Antibiotic susceptibility tests were performed as per CLSI recommended guidelines⁹ by Kirby-Bauer disc diffusion method¹⁰. Antibiotics used for the disc diffusion method were Ampicillin (10 µg), Amoxicillin-clavulanic acid (20 µg + 10 µg), Cotrimoxazole (25 µg),

Gentamicin (10 µg), Ciprofloxacin (5 µg), Norfloxacin (10 µg), Nitrofurantoin (300 µg), Amikacin (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Imipenem (10 µg), Piperacillin-Tazobactam (100 + 10 µg).

Results

Eighty-two *Citrobacter* species were isolated from different clinical samples during the 6 months study period. Out of 82 isolates of *Citrobacter* species, majority were from urine 52 (63.5%), followed by sputum 14 (17%), pus 11 (13.5%) and blood 5 (6%). The prevalence of *Citrobacter* species infections was high among male patients 48 (58.5%) as compared to female patients 34 (41.4%). *Citrobacter koseri* was the predominant species isolated 56 (68.3%). The other species isolated was *Citrobacter freundii*-26 (31.7%). Anti-biograms of *Citrobacter* isolates revealed that the most effective antibiotic against the isolates was Imipenem (91.8% sensitive), followed by Piperacillin/Tazobactam (58.3%) and Amikacin (53.4%).

Table 1: Distribution of *Citrobacter* species among various clinical samples

Sample	Number (%)
Urine	52 (63.5)
Sputum	14 (17)
Pus	11 (13.5)
Blood	5 (6)
Total	82 (100)

Out of 82 isolates of *Citrobacter* species, majority were from urine 52 (63.5%), followed by sputum 14 (17%), pus 11 (13.5%) and blood 5 (6%) (Table 1).

Table 2: Gender wise distribution of *Citrobacter* species

Gender	Number (%)
Male	48 (58.5)
Female	34 (41.5)

The prevalence of *Citrobacter* species infections was high among male patients 48 (58.5%) as compared to female patients 34 (41.4%) (Table 2).

Table 3: Antibiotic resistance pattern of *Citrobacter* species—number of isolates and resistance percentage (%)

A	AMC	COT	G	CIP	N	NIT	AK	CTX	CZM	PTZ	Im
76	73	62	45	53	59	56	38	68	71	34	7
92.6%	89 %	75.6%	54.8%	64.6%	72%	68.2%	46.4%	83%	86.5%	41.5%	8.6%

A-Ampicillin, AMC-Amoxycilin-clavulanic acid, COT-Cotrimoxazole, G-Gentamicin, CIP-Ciprofloxacin, N-Norfloxacin, NIT-Nitrofurantoin, AK-Amikacin, CTX-Ceftriaxone, CZM-Ceftazidime, PTZ-Piperacillin-Tazobactam, Im- Imepenem,

Discussion

In our study, the antibiogram of the *Citrobacter* species showed increased resistance to the commonly used antibiotics. 92.6% of the strains were resistant to Ampicillin. Resistance to fluoroquinolones was in the range of 64%–72%. The third generation cephalosporins resistance ranged from 80–85%. The *Citrobacter* isolates showed sensitivity to Imipenem (91.4%), followed by Piperacillin/tazobactam (58.5%) and Amikacin (53.6%).

Our study was in concordance with the study done by Avinash G¹¹ in which incidence of *Citrobacter* infections was high among male patients (56.58%) than females (43.4%).

A study done by Liu *et al.* showed that among Sixty-two *Citrobacter* isolates, 13 were *Citrobacter freundii*, 41 *C. youngae* and 8 *C. braakii* isolates which were obtained from human diarrheal patients and food sources. Antibiogram of these isolates revealed that all were resistant to Cefoxitin, and sensitive to Imipenem, Meropenem and Amikacin as seen in our study. The majority of *Citrobacter* isolates (61.3%) were multi drug resistant to three or more antibiotics among the 22 antibiotics tested.

Mohan S *et al.*¹² revealed in his study done on 146 *Citrobacter* isolates in which *Citrobacter* were isolated most commonly in pus samples in about 41.1%. The commonest species identified were *C. freundii* (49%) and *C. koseri* (28%). In our study also *Citrobacter freundii* was the most common species identified. In the antibiogram, carbapenem was the most sensitive class of antibiotic, followed

by penicillin group. In our study though imipenem showed increased susceptibility, Penicillin group of drugs showed increased resistance pattern. This may be due to inadvertent use and irrational use of antibiotics which may be one of the reasons for development of resistance to common antibiotics.

In a study done by Dhanya A and Sevitha Bhat,¹³ majority of the *Citrobacter* spp. isolated were from pus. The antibiogram of the *Citrobacter* species showed increased resistance to commonly used antibiotics, a high level of resistance to Ampicillin, fluoroquinolones, Amikacin and Gentamicin.

Similarly, a study done by Patil MA and Shah A^{14,15} showed that *Citrobacter* species was commonly isolated from pus unlike our study in which urine was the most common sample in which *Citrobacter* species were isolated. The other studies conducted showed increased resistance pattern to commonly used antibiotics used like ampicillin, ciprofloxacin, gentamicin, amikacin, third generation cephalosporins and Piperacillin tazobactam.^{16,17}

Conclusion

Citrobacter species are Gram negative, non-spore forming bacilli. They are being isolated in most of the samples like urine, pus, sputum and blood. They are emerging now a days as one of the common organisms causing nosocomial infections. They were initially being sensitive to commonly used antibiotics. But because of irrational and indiscriminate use of these antibiotics the *Citrobacter*

species are developing resistance to the routinely used antibiotics. The resistant pattern changes regularly and varies from place to place. Hence routine monitoring of drug susceptibility pattern in a particular area helps to identify the resistant trends in that area and this in turn helps clinicians to start empirical treatment. Also, regular antiseptic and hand hygiene measures in the hospital help reduce the spread of nosocomial infections in the hospitals, thus reducing the duration of the hospital stay by the patients and helps in the better outcome of the patients.

Prior publication: Nil

Support: Nil

Conflicts of interest: None declared

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