

## Prevalence and antimicrobial susceptibility pattern of *Citrobacter* species in various clinical samples in a tertiary care hospital

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### Abstract

**Background:** Infections with *Citrobacter* species have been increasing across worldwide. Earlier, considered to be organism of low virulence but now known to cause variety of infections with high morbidity and mortality.

**Objective:** The present study aims to determine the prevalence and antibiotic susceptibility pattern of *Citrobacter* species from various clinical samples in a tertiary care hospital.

**Material and methods:** This one year prospective observational study, conducted in the Department of Microbiology at Krishna Institute of Medical Sciences, Secunderabad from January 2018 to December 2018. The samples received to the laboratory from both outpatient and inpatient from various departments. Identification and antimicrobial susceptibility of the isolates were done by using Vitek 2 compact automated system (bioMerieux).

**Results:** In 3,337 culture positive sample, 83 (2.4%) isolates were found to be culture positive for *Citrobacter* species where *C. koseri* (n=57) is predominant followed by *C. freundii* (n=24) and *C. amalonaticus* (n=2). Majority of *Citrobacter* species were isolated from urine samples 56% followed by pus 21%, sputum 8.43%, blood 7.2% and in bile fluid 3.6%. The highest incidence was found in elderly patients of age group 51-80 year age group, more common in males (70%) than females (30%).

**Conclusion:** The present study highlight that *Citrobacter* species are an emerging pathogen with prevalence of 2.4%. It is the most common uropathogen followed by pus and sputum, and bacteraemia also. *C. freundii* was comparatively less susceptible as compared to *C. koseri*. Antibiotic policy should be implemented strictly and followed strongly so that emergence of such low virulent organism with emerging resistance can be controlled.

**Keywords:** *Citrobacter* spp.; antimicrobial; susceptibility; *Citrobacter koseri*; *Citrobacter freundii*; *Citrobacter amalonaticus*; infections; Vitek 2

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## Introduction

Infections with *Citrobacter* species have been increasing across worldwide [1]. *Citrobacter* species belong to a group of facultative anaerobic, gram-negative bacilli belongs to the family Enterobacteriaceae, tribe *Citrobacterae* [2]. It is found singly or in pairs, and are motile by peritrichous flagella which typically utilize citrate as sole source of carbon [3]. In the environment, they are frequently found in water, soil, food, and as an occasional colonizers of the intestines of human and animals. These organisms are isolated from variety of clinical specimens like urine, pus, blood, and cerebrospinal fluid [4].

There are 11 different species (*Citrobacter freundii*, *Citrobacter koseri*, *Citrobacter amalonaticus*, *Citrobacter farmeri*, *Citrobacter youngae*, *Citrobacter braakii*, *Citrobacter werkmanii*, *Citrobacter sedlakii*, *Citrobacter rodentium*, *Citrobacter gillenii*, and *Citrobacter murliniae*) among which the most commonly isolated from human clinical specimens are *C. koseri* (formerly named *C. diversus*), *C. freundii*, *C. youngae*, *C. braakii*, and *C. amalonaticus* [5].

Earlier, they are recognised as environmental contaminants or colonizers with low virulence, they are now known to cause variety of infections such as the urinary tract, wounds, soft tissue, respiratory tract, liver, bloodstream, biliary tract, peritoneum, meningitis and brain abscess [6].

Although *Citrobacter* infections occur in adults with immunocompromised state but it is now an emerging cause of UTIs, neonatal septicaemia, neonatal meningitis, brain abscess with high mortality as high approximately one-third of infants with abscesses die, and one-half sustain CNS damage [7, 8].

*Citrobacter* infections were found to represent 0.8% of Gram-negative infections in a large surveillance study conducted in American medical centres [9], while, in hospital settings, accounted for 3–6% of all isolates of Enterobacteriaceae [9]. There are reports of *Citrobacter* species being a major nosocomial pathogen with multi drug resistant antimicrobial susceptibility [10]. The present study was taken up to determine the prevalence and antibiotic susceptibility pattern of *Citrobacter* species from various clinical samples in a tertiary care hospital.

## Material and methods

This was a prospective observational study, conducted in the Department of Microbiology at Krishna Institute of Medical Sciences, Secunderabad from January 2018 to December 2018. A total of 17,544 samples received to the laboratory from both outpatient and inpatient from various departments. Out of total samples, 3,337 were culture positive. The samples included urine (clean catch & catheter catch), pus, and sputum, endotracheal tube secretions, sterile body fluids and blood. All the samples were received in the microbiology laboratory under sterile conditions and were processed as per standard microbiological guidelines.

Specimens were processed on blood agar (BA), MacConkey agar (MA) plate and urine samples were processed on cysteine lactose electrolyte deficient (CLED) agar. The inoculated culture plates were incubated at 37°C for 16-18 hours under aerobic conditions. In case of blood sample, paired samples were collected and they were inoculated in BacT Alert bottles which were incubated in BacT Alert 3D (bioMérieux).

After culture growth, bacterial identification and susceptibility was done by VITEK 2 compact (bioMérieux) automated system. Identification of the isolates were done by using Vitek GN-ID card and antimicrobial susceptibility testing was done by using GN AST 280 card.

Antimicrobials used in the VITEK AST 280 card was ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam (TZP), cefuroxime (CXM), ceftriaxone, cefepime (FPM), cefepime (FPM), ertapenem (ETP), imipenem, meropenem (MEM), amikacin, gentamycin (GM), nalidixic acid (NA), ciprofloxacin (CIP), tigecycline (TGC), nitrofurantoin (FT), colistin (CS) and cotrimoxazole (SXT). Reporting of susceptibility, intermediate and resistance based on minimum inhibitory concentration values (MIC) was done as per Clinical Laboratory Standard Institute (CLSI) guidelines 2018 [11].

## Results

In 3,337 culture positive sample, 83 (2.4%) isolates were found to be culture positive for *Citrobacter*

species where *C. koseri* (n=57) is predominant followed by *C. freundii* (n=24) and *C. amalonaticus* (n=2). Majority of *Citrobacter* species were isolated from urine samples 56% (n=47) in which clean

catch is (n=37) 44% is high as compared to catheter catch (n=10) is 12% followed by pus (n=18) 21% , sputum (n=7) 8.43%, blood (n=6) 7.2% and in bile fluid (n=3) is 3.6% as shown in Table 1.

**Table 1:** Distribution of *Citrobacter* species in various clinical samples.

Sample type	Total	Percentage	<i>C. koseri</i>	<i>C. freundii</i>	<i>C. amalonaticus</i>
Urine(Clean catch)	37	44.5%	28	9	0
Urine (Catheter catch)	10	12%	7	2	1
Pus	18	21.6%	13	4	1
Sputum	7	8.43%	3	4	0
Blood	6	7.2%	4	2	0
Bile fluid	3	3.6%	0	3	0
E.T secretion	2	2.40%	2	0	0
Total (percentage)	83		57 (68.60%)	24 (29%)	2 (2.40%)

The highest incidence was found in elderly patients of age group 51-80 year age group, more common in males (70%) than females (30%) as shown in Table 2.

**Table 2:** Age group wise distribution.

Age Group	Male	Female	Total
01-10	0	1	1
11-20	1	0	1
21-30	2	4	6
31-40	4	5	9
41-50	5	3	8
51-60	9	4	13
61-70	16	8	24
71-80	14	0	14
81-90	7	0	7
Total	58	25	83
Percentage	70%	30%	

The susceptibility pattern of various antimicrobials used in the Vitek 2 compact for each *Citrobacter* species isolated is given in Table 3 and Figure 1.

The susceptibility pattern observed in *C. koseri* is 100% in tigecycline and colistin. piperacillin-tazobactam (84%), cefoperazone-sulbactam (94%). Aminoglycosides such as amikacin and gentamycin showed good susceptibility of 96% and 98% each respectively. In carbapenems, ertapenem (91.2%), imipenem (92%), meropenem (89%) also showed good susceptibility. In cephalosporins 3<sup>rd</sup> generation ceftriaxone showed 75.4% which is pretty low as compared to 4<sup>th</sup> generation cefepime which showed 92%. In fluoroquinolones, ciprofloxacin showed 87% susceptible.

Urine isolates of *C. koseri* showed nitrofurantoin (89.4%), nalidixic acid (80.7%) and trimethoprim-sulfamethoxazole (96.4%).

The susceptibility pattern observed in *C. freundii* it was slightly reduced with piperacillin-tazobactam (75%), cefoperazone-sulbactam (83.3%). Aminoglycosides such as amikacin and gentamycin showed 79% and 70.8% susceptible. In carbapenems, ertapenem (87.5%), imipenem

(91.6%), meropenem (87.5%) also showed good susceptibility. In cephalosporins 3<sup>rd</sup> generation, ceftriaxone showed 62.5% and 4<sup>th</sup> generation cefepime which showed 87.5% In fluoroquinolones, ciprofloxacin showed 75% susceptible. High end antimicrobials such as tigecycline and colistin showed excellent susceptibility of 100%.

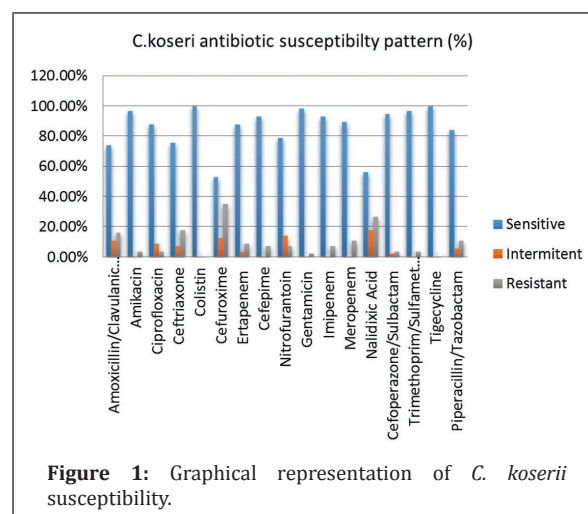
**Table 3:** Antibiotic susceptibility pattern of *Citrobacter spp.* isolates.

Antimicrobial agents	Susceptible ( <i>C. koseri</i> )	Susceptible ( <i>C. freundii</i> )
	Number(n)/Percentage(%)	Number(n)/Percentage(%)
Amikacin	55(96.4)	19(79.1)
CIP-Ciprofloxacin	50(87.7)	18(75)
CRO-Ceftriaxone	43(75.43)	15(62.5)
CS-Colistin	57(100)	24(100)
CXM-Cefuroxime	30(52.63)	14(58.3)
ETP-Ertapenem	52(91.2)	21(87.5)
FEP-Cefepime	53(92)	21(87.5)
FT-Nitrofurantoin	51(89.4)	19(79)
GM-Gentamicin	56(98)	17(70.8)
IPM-Imipenem	53(92)	22(91.6)
MEM-Meropenem	51(89)	21(87.5)
NA-Nalidixic Acid	46(80.7)	15(62.5)
SFP-Cefoperazone/Sulbactam	54(94)	20(83.3)
SXT-Trimethoprim/Sulfamethoxazole	55(96.4)	18(75)
TGC-Tigecycline	57(100)	24(100)
TZP-Piperacillin/Tazobactam	48(84)	18(75)

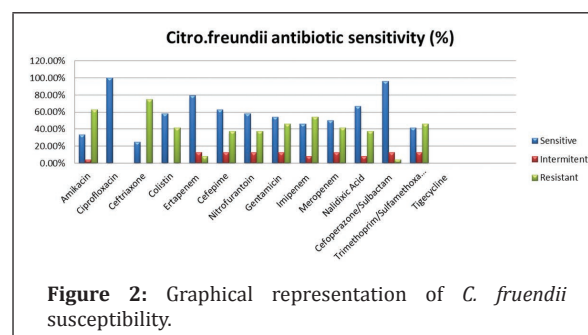
Again urine isolates in case of *C. freundii*, showed nitrofurantoin (79%), nalidixic acid (62.5%) and trimethoprim-sulfamethoxazole (75%). Tigecycline was not reported for urinary isolates.

*Citrobacter spp* inherit intrinsic resistance to certain antimicrobials. *C. koseri*, *C. freundii*, and *C. amalonaticus* are intrinsically resistant to ampicillin

whereas *C. freundii* is intrinsically resistant to amoxicillin-clavulanate, cephalosporin I and II . Therefore, these antimicrobials were excluded while reporting for susceptibility [11].



**Figure 1:** Graphical representation of *C. koserii* susceptibility.



**Figure 2:** Graphical representation of *C. freundii* susceptibility.

## Discussion

The present study indicates the importance of *Citrobacter species* in various clinical samples as they are becoming an emerging pathogen with prevalence of 2.4% in 1 year. In the present study it has emerged as a potential uropathogen 56% followed by pus 21.6%, sputum and blood with 8.4% and 7.2% respectively.

A study by Avinash et al. [2] mentioned the similar results of *Citrobacter species*, 150 (59.76%) were from urine, 60(23.9%) from Pus, 8(7.17%) from blood, 16(6.37%) from Sputum. Metri et al. [3] also concluded *Citrobacter species* as the third most common uropathogen isolated with prevalence of 15.7%.

In the present study *C. koseri* (68.60%) is the most common species isolated from urine and pus followed

by *C. freundii* (29%) and *C. amalonaticus* (2.40%) which is quite similar to the results of Dhanya et al. [12]. Similar results were also seen in study by Sami et al. [8] where *C. koseri* was the most abundant (53.2%) followed by *C. freundii* 45 (18.2%) and *C. amalonaticus* 24 (9.75%) in urine samples. Metri et al. [13] found *Citrobacter* isolate accounting to 9.4% with *C. koseri* (72.4%) as predominant species in pus (48.1%), followed by urine (24.3%), sputum (20.3%), body fluids (05.2%), blood (02.1%) which is pretty high as compared to the present study. However, on the contrary another study by Samsonis et al. [6] and Sneha et al. [1] mentioned *C. freundii* was more common (71.8%) (49%), followed by *C. koseri* (23.1%), (28%) in urine and pus isolates respectively in their study.

The majority of age group affected in the present study is elderly age group of 51-80 years with male predominance which is similar to Metri et al. [13] and Samsonis et al. [6]. The reason of this could be because of the fact that these group of population have reduced immunity, having co-morbid diseases and more prone to hospitalisation.

In the present study the most common isolate identified is *C. koseri* (68.60%) followed by *C. freundii* (29%) and *C. amalonaticus* (2.40%) therefore Antimicrobial susceptibility (AST) pattern was compared between *C. koseri* and *C. freundii* as *C. amalonaticus* isolated is too low to be commented upon.

In *C. koseri*, Beta lactam combinations showed good susceptibility piperacillin-tazobactam (84%), cefoperazone-sulbactam (94%) whereas in *C. freundii* it was slightly reduced with piperacillin-tazobactam (75%), cefoperazone-sulbactam (83.3%).

Aminoglycosides in case of *C. koseri* showed amikacin and gentamycin of good susceptibility of 96% and 98% each as compared to 79% and 70.8% in case of *C. freundii*.

In carbapenems, ertapenem (91.2%, 87.5%), imipenem (92%, 91%), meropenem (89%, 87.5%) showed good susceptibility in *C. koseri* and *C. freundii* respectively which is pretty similar to Metri et al. [3] and Avinash et al. [2].

In cephalosporins 3<sup>rd</sup> generation ceftriaxone showed 75.4% and 62.5% which is pretty low as compared

to 4<sup>th</sup> generation cefepime which showed 92% and 87.5% in *C. koseri* and *C. freundii* respectively. In fluoroquinolones, ciprofloxacin showed 87% and 75 % susceptibility in *C. koseri* and *C. freundii* respectively.

For urine isolates, nitrofurantoin (89.4%, 79%), nalidixic acid (80.7%, 62.5%) and trimethoprim-sulfamethoxazole (96.4%, 75%) in both *C. koseri* and *C. freundii*. High end antimicrobials such as tigecycline and colistin showed excellent susceptibility of 100% in both *Citrobacter* species isolated which correlates with study by Samsonis et al. [6].

Various studies observed the increased resistance in *Citrobacter* species in different parts of India but in the present study it shows considerable good susceptibility. This can be due to wide variation in specimen considered, hospital environment and hospital infection control policy.

## Conclusion

The present study highlights that *Citrobacter* species are an emerging pathogen with prevalence of 2.4%. It is the most common uropathogen followed by pus and sputum, however it is now shown to cause bacteraemia also. In this study, *C. freundii* was comparatively less susceptible as compared to *C. koseri* therefore more studies are required to keep the check on changing susceptibility pattern. The only limitation of the present study is isolation of *C. amalonaticus* is too less to comment on its susceptibility pattern. Hospital antibiotic policy should be implemented strictly so that emergence of such low virulent organism with emerging resistance can be controlled. Most importantly, strict adherence to infection prevention measures, implementation of antimicrobial stewardship programme and maintaining proper hand hygiene practices in tertiary care hospitals should be followed to prevent the transmission of this emerging pathogen.

## Conflicts of interest

Authors declare no conflicts of interest.

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