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Anti-microbial resistance profile of *Citrobacter* species in a tertiary care hospital of southern India

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| **¤****Abstract** |   |  |

**Background:** Recently, the isolation of this pathogen in hospital settings is increasing and multidrug-resistant strains are emerging; these strains present a challenge for clinician and the clinical microbiologist because of their increased occurrence in nosocomial infection. The current study was done to find out the antibiotic sensitivity pattern of Citrobacter species from various clinical specimens. **Materials and Methods:** Samples were collected from patients in accordance with standard protocols. Citrobacter species were identified by conventional biochemical tests. Antibiotic susceptibility of the isolates was done by disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations. **Results:** Out of 563 isolates of Citrobacter, majority were from pus (48.1%), followed by urine (24.3%), sputum (20.3%), body fluids (05.2%), blood (02.1%). C. koseri was the predominant species [391 (70%)] isolated. Infection was nosocomialy acquired in 493 (87.4%) patients. The mean age was 39.5 years. Anti-biograms of Citrobacter isolates revealed that effective agent against Citrobacter isolates was imipenem (91.8% sensitive), followed by piperacillin/tazobactam (58.3%) and amikacin (53.4%). **Conclusion:** Citrobacter isolates resistant to multiple anti-microbial agents have emerged, including strains resistant to imipenem, making it an emerging nosocomial pathogen. Therefore, the results of this study suggest that surveillance of anti-microbial resistance in Citrobacter is necessary. Antibiotic policy should be formulated in the hospital. Depending on the antibiotic sensitivity pattern of the Citrobacter isolates, antibiotics should be used, and proper infection control measures should be strictly followed to prevent spread of this pathogen.

**Keywords:** *Anti-microbial resistance, antibiotics, citrobacter*

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| **¤ Introduction** |   | Top |

The genus *Citrobacter* is a distinct group of aerobic Gram-negative bacilli from the Enterobacteriaceae family. [[1]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref1)*Citrobacter* species are primary inhabitants of intestinal tract, often found in human feces. [[2]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref2) They are also found in soil, sewage, food, and animal feces. [[3]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref3),[[4]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref4) These organisms are isolated from variety of clinical specimens like urine, pus, blood, and cerebrospinal fluid. [[5]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref5)

Organisms of genus *Citrobacter* are Gram-negative straight rods, found singly or in pairs, and are motile by peritrichous flagellae. [[6]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref6) They are facultative anaerobes, oxidase-negative, and typically utilize citrate as sole source of carbon. [[7]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref7)The genus *Citrobacter* comprises 11 different species. Among these, *Citrobacter* *koseri* (previously known as *C. diversus*) and *C. freundii* are the commonest species implicated in infections. [[7]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref7),[[8]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref8) *Citrobacter* species can cause variety of infections like, respiratory tract infections, urinary tract infection, blood stream infections, wound and burns infections, meningitis, endocarditis, and peritonitis. [[9]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref9),[[10]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref10),[[11]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref11)

Recently, the isolation of this pathogen in hospital settings across the globe is increasing, and multidrug-resistant strains are emerging; these strains present a challenge for clinician and the clinical microbiologist because of their increased occurrence in nosocomial infection. In India, there are very few literatures, which have dealt with *Citrobacter*and its antibiotic sensitivity pattern. Therefore, the current study was envisaged to find out the antibiotic sensitivity pattern of the isolates.

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| **¤ Materials and Methods** |   | Top |

**Study setting, samples, and bacterial identification**

The study was conducted in the Department of Microbiology, Shri B M Patil Medical College, Bijapur, India during January 2007 to December 2011.

**Inclusion criteria**

Only those patients from whom *Citrobacter* species were isolated during routine diagnostic testing were included in the study.

**Exclusion criteria**

Those patients from whom *Citrobacter* species were not isolated during routine diagnostic testing were not included in the study.

Samples were collected from patients in accordance with standard protocols. [[12]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref12) *Citrobacter* species were identified by conventional biochemical tests according to standard microbiological techniques. [[13]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref13)

A total of 563 isolates of *Citrobacter* spp. were isolated from different clinical specimens. Out of the 563 *Citrobacter*isolates, 263 were from pus, 137 from urine, 106 from sputum, 28 from body fluids, 17 from stool, and 12 from blood samples.

**Anti-microbial susceptibility test**

Antibiotic susceptibility of the isolates was done by disc diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) recommendations. [[14]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref14) Anti-microbials used were ampicillin (10 μg), amoxyclav (20/10 μg), tetracycline (30 μg), gentamicin (10 μg), co-trimoxazole (1.25 μg trimethoprim/23.75 μg sulfomethoxazole) ciprofloxacin (5 μg), cephalexin (30 μg), amikacin (30 μg), ofloxacin (5 μg), netilmicin (30 μg), imipenem (10 μg), ceftazidime (30 μg), piperacillin/tazobactam (100/10 μg), tobramycin (10 μg), norfloxacin (10 μg), nitrofurantoin (300 μg). The discs were obtained from Himedia Laboratories. Standard strains of *Escherichia**More Details* *coli* ATCC 25922,*Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853 were used as controls.

**Statistical analysis**

Statistical analysis was performed with SPSS 14 software. Categorical data was analyzed using Chi-Square test.

Two different hypothesis were set up for examining

* Gender differences and distribution rates of *Citrobacter* strains
* Distribution rates of *Citrobacter* strains according to sample
* Distribution rates of *Citrobacter* strains according to department
* Type of infection (hospital-acquired and community-acquired)

In H 0 hypothesis, there is no association between two assets. In H 1 hypothesis, however, an association exists between two sets. In this, if the *P* value was <0.05, the association was considered significant.

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| **¤ Results** |   | Top |

A total of 563 isolates of *Citrobacter* species were obtained over duration of five years. These included isolates from sample of pus, urine, sputum, body fluids, and blood [[Table 1]](http://www.indianjmedsci.org/viewimage.asp?img=IndianJMedSci_2011_65_10_429_109259_b1.jpg). Out of 563 *Citrobacter* species isolated, 391 (69.6%) were*C. koseri* and 172 (30.1%) were *C. freundii*. Three hundred and eighty-six (68.6%) were isolated from male patients and 177 (31.4%) from female patients. Out of total 563 patients, infection was nosocomialy-acquired in 493 (87.4%) patients and 70 (12.6%) were community-acquired. The mean age was 39.5 years. Of all samples with *Citrobacter*, 256 (45.6%) were obtained from surgery department, 127 (22.6%) from medicine department [[Table 2]](http://www.indianjmedsci.org/viewimage.asp?img=IndianJMedSci_2011_65_10_429_109259_b2.jpg). Anti-biograms of*Citrobacter* isolates to 16 anti-microbial agents including imipenem, cephalosporins, aminoglycosides, and fluoroquinolones are presented in [[Table 3]](http://www.indianjmedsci.org/viewimage.asp?img=IndianJMedSci_2011_65_10_429_109259_b3.jpg). The most effective agent against *Citrobacter* isolates was imipenem (91.8% sensitive), followed by piperacillin/tazobactam (58.3%) and amikacin (53.4%). Amikacin was more effective aminoglycoside than others. More than 70% of all isolates of *Citrobacter* were resistant to several anti-microbial agents, in particular ampicillin, cephalexin, amoxyclav, and co-trimoxazole (96.4%, 95.6%, 89.7%, and 89.5%, respectively).

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| http://www.indianjmedsci.org/articles/2011/65/10/images/IndianJMedSci_2011_65_10_429_109259_b1.jpg | Table 1: Distribution of *Citrobacter* isolates in various clinical specimens[**Click here to view**](http://www.indianjmedsci.org/viewimage.asp?img=IndianJMedSci_2011_65_10_429_109259_b1.jpg) |

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| http://www.indianjmedsci.org/articles/2011/65/10/images/IndianJMedSci_2011_65_10_429_109259_b2.jpg | Table 2: Distribution of *Citrobacter* isolates in various clinical departments[**Click here to view**](http://www.indianjmedsci.org/viewimage.asp?img=IndianJMedSci_2011_65_10_429_109259_b2.jpg) |

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| http://www.indianjmedsci.org/articles/2011/65/10/images/IndianJMedSci_2011_65_10_429_109259_b3.jpg | Table 3: Antibiotic susceptibility pattern of *Citrobacter* isolates[**Click here to view**](http://www.indianjmedsci.org/viewimage.asp?img=IndianJMedSci_2011_65_10_429_109259_b3.jpg) |

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| **¤ Discussion** |   | Top |

In this study, *Citrobacter* infections were more among elderly people; this is because of the fact that these groups constitute large proportion of our hospital populations and reduced immunity in these people to fight against infection in general. Similar results were seen in the study conducted by Shih *et al*. [[10]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref10)

*C. koseri* was the predominant species isolated. Out of 563 isolates, 391 (70%) were *C. koseri* and 172 (30%) were *C. freundii.* In evaluating the serotypes of the *Citrobacter*, *C. koseri* has been reported as most predominant isolate in most of the studies. [[3]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref3),[[15]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref15),[[16]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref16) Similarly, we found *C. koseri* in 70% of our patients; however, Gill, *et al*., [[1]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref1) have reported*C. freundii* to be the commonest species isolated.

The education and application of personal hygiene are important in that *Citrobacter* strains are excreted as fecal wastes. Epidemics occurring in hospitals are closely related to the fact that hospital staff carries the bacterium in their hands and gastrointestinal systems. *Citrobacter* strains are mostly isolated from the infections of urinary and respiratory systems as nosocomial infection causes. [[2]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref2)

In majority of the patients, infections were hospital-acquired (87.6%), which is consistent with other studies. [[3]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref3),[[9]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref9),[[17]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref17)The high rate of nosocomial infections might be attributed to an aging population, an increase in the number of seriously-ill patients, and the greater number of invasive interventions being used. These factors, combined with overuse of antibiotics and underutilization of infection control strategies, might have resulted in an increase in the number of nosocomial infections.

In this study, majority of the isolates were from pus (48.1%), followed by urine (24.3%), sputum (20.3%), body fluids (05.2%), blood (02.1%). Similarly, in the study conducted by Shetty, *et al.*, [[18]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref18) majorities of the isolates were recovered from pus. However, in the study conducted by Mohanty, *et al.*, [[19]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref19) majorities of the isolates were from urine, followed by sputum and pus. In this study, majority of the isolates were from pus (48.1). The reasons for this is unclear, it may simply reflect the fact that influx of the patients to surgical dept is much more than that of OBG dept, and also because of the increased antibiotic usage in treating wound infection and emerging resistance among these organisms.*Citrobacter* species have been reported as cause of many kinds of human infections, but bacteremia due to organism remains uncommon. In this study, only 2.1% of the isolates caused bacteremia, which is similar to study conducted by Shih, *et al*. [[10]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref10)

Drug-resistant *Citrobacter* present a challenge for clinician and the clinical microbiologist because of their increased occurrence in nosocomial infection. Antibiotic susceptibility pattern was studied for all isolates of *Citrobacter.* The analysis of drug resistance pattern showed that, among 563 isolates of *Citrobacter*, maximum numbers (96.4%) were resistant to ampicillin and lowest to imipenem (8.2%). Resistance was observed to commonly used antibiotics such as ampicillin, ciprofloxacin, gentamicin, netilmicin, amikacin, co-trimoxazole, and tetracycline. The greater prevalence of resistance to commonly used antibiotics has also been reported by other studies. [[3]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref3),[[15]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref15),[[16]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref16) The presence of multidrug resistance may be related to the dissemination of antibiotic resistance among hospital isolates of *Citrobacter*. Among fluoroquinolones, resistance to various agents was in the range of 65.1% to 75.2%. Resistance to cephalosporins (cephalexin, ceftazidime) and piperacillin-tazobactam were 95.6%, 76.4%, and 41.7%, respectively. In other study, resistance to broad-spectrum cephalosporins (ceftriaxone and ceftazidime), piperacillin, and piperacillin-tazobactam has been reported to be between 39% and 48%. [[9]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref9) Among aminoglycosides, amikacin was found to have an edge over gentamicin, netilmicin, and tobramycin with sensitivity of 53.4%, 24.5%, 25.4%, and 31.8%, respectively. Similar observations have been made by previous group of workers. [[18]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref18),[[19]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri" \l "ref19)

In this study, maximum sensitivity (91.8%) was observed in imipenem. This result is consistent with previous studies on imipenem resistance in *Citrobacter.*[[7]](http://www.indianjmedsci.org/article.asp?issn=0019-5359;year=2011;volume=65;issue=10;spage=429;epage=435;aulast=Metri#ref7) Sensitivity to imipenem was much higher to the sensitivity to other antibiotics, and difference is statistically significant ( *P* < 0.05), but this drug should not be used indiscriminately and should kept as a reserve drug, as otherwise resistance to this drug may occur posing threat to treatment. In the treatment of infections with anti-microbial agents, the use of antibiotics, especially those with large-spectrum, should be avoided as possible. In patients with immunodeficiency, multiple trauma or treated with large surgical interventions or implanted with alien materials, antibiotic treatment regime should be well-planned in the treatment of infections. In these patients, as a result of long-term antibiotic administration, resistance of various bacteria may be able to develop to antibiotics. It is a must that in patients in whom small-spectrum anti-microbial agents are sufficient, the administration of large-spectrum antibiotics should be avoided.

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| **¤ Conclusion** |   | Top |

The *Citrobacter* isolates resistant to multiple anti-microbial agents have emerged, including strains resistant to imipenem, making it an emerging nosocomial pathogen. Therefore, such studies will guide clinicians to choose accurate empirical treatment options and will help to reduce the mortality and morbidity rates from infections. It is more significant to prevent the resistance development in micro-organisms and to lend the accurate information to clinicians in terms of the use of antibiotics in appropriate period. Considering the correlation between the use of antibiotics and resistance development, the control of *Citrobacter* infections will be likely with the reasonable use of antibiotics.

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