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Original Research Article

PREVALENCE OF CITROBACTER SPECIES IN VARIOUS CLINICAL SAMPLES AND THEIR ANTIBIOTIC SUSCEPTIBILITY PATTERN-A STUDY FROM A TERTIARY CARE CENTER IN SOUTH INDIA

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Abstract

Background: The genus *Citrobacte*r was discovered in 1932 by Werkman and Gillen. They are gram-negative, non-sporing, motile rods belonging to the family Enterobacteriaceae and utilize citrate as a sole source of carbon. *Citrobacter koseri* and *Citrobacter freundii* are the two important species. *C.koseri* is associated with CNS infections in newborn. Infections due to *C. freundii* are gastroenteritis, neonatal meningitis, and septicaemia. **Materials and Methods:** This cross-sectional study was conducted in Microbiology laboratory, Government Kilpauk Medical College, Chennai for a period of 6 months from June 2022 to December 2022.Institutional Ethics Committee approval was obtained vide protocol ID 815/2022 Government Kilpauk Medical College, Chennai. Various clinical samples were processed by standard microbiological techniques for isolation and characterization of Citrobacter spp based on cultural and biochemical reactions. Data were entered in Excel spread sheet and statistical analysis was done using SPSS version 21. Chi-square test was deployed to find out the significance of isolates of *Citrobacter* spp from various samples. **Result:** The number of isolates from blood, urine and pus were tabulated and the significance of it was analyzed by Chi-square test. P value was 0. 007126 and significant at $p<0.05$. Maximum number of isolates were from 41 to 50 years and the lowest number of isolates were from 51 to 60 years. Comparison of antibiotic sensitivity pattern of *Citrobacte*r species isolates from various clinical specimens (n 41) was done and imipenem was found to have the highest sensitivity irrespective of the type of infections. **Conclusion:** In this study, the prevalence of *Citrobacter* species infection was higher in comparison with other similar studies. Carbapenem resistance was found in 10% to 15% of the isolates.

INTRODUCTION

lThe genus *Citrobacter* was discovered in 1932 by Werkman and Gillen. These organisms are found in soil, water, intestinal tract of animals and humans. They are gram-negative, non-sporing, motile rods belonging to the family Enterobacteriaceae and utilize citrate as a sole source of carbon. The genus has about 11 species and their identification is based

on biochemical reactions. *Citrobacter koseri* and *Citrobacter freundii* are the two important species. *C.koseri* is associated with CNS infections in newborn. Infections due to *C. freundii* are gastroenteritis, neonatal meningitis, and septicaemia.[1] Septicaemia arising from Gram-negative organisms account for 26% to 47% of BSI in the United States and more than this estimated proportion in other parts of the world.[2,3] Among the Gram-negative organisms, Escherichia coli and Klebsiella pneumoniae are attributed as the predominant causative agents of sepsis. *Citrobacter freundii* occurs as an etiological agent of sepsis in a small proportion of cases.[4,5]

It has emerged as a common uropathogen in hospitalized patients. Indiscriminate and irrational use of antibiotics have led to the emergence of drug-resistant pathogens in healthcare-associated infections.[6] Eventhough majority of the *Citrobacter* infections are encountered in health-care associated infections it has been attributed in community acquired infections also. Among the Gram-negative infections, *Citrobacter spp* contributed to 0.8% according to a surveillance study conducted.[7] Emergence of multidrug resistant *Citrobacter spp* is a challenge to the clinicians in treating patients with this kind of infection.[8]

As the *Citrobacter Spp* is emerging as a multidrug resistant pathogen in developing countries a comprehensive cross-sectional study was undertaken to find out the prevalence of the organism in various infections and their antibiotic susceptibility pattern.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Microbiology laboratory at Government Kilpauk Medical College, Chennai for a period of 6 months from June 2022 to December 2022.Institutional Ethics Committee approval was obtained vide protocol ID 815/2022 Government Kilpauk Medical College, Chennai. Written informed consent was obtained from all the participants recruited for this study.

Various clinical samples collected for the study included blood, pus and urine. Samples were processed by standard microbiological techniques for isolation and characterization of *Citrobacter spp* based on cultural and biochemical reactions. [9] Gram-negative, motile, lactose non-fermenting, catalase positive, oxidase negative, indole+/-, A/K or A/A with H2S in Triple Sugar iron agar(TSI), Citrate utilized, Methyl red (MR)positive and Vogues-Prausker (VP) negative, Phenyl pyruvic acid negative(PPA) organisms were established as *Citrobacter.*

Inclusion criteria

- Subjects with symptoms and clinical suspicion of Sepsis
- Subjects with clinical features of urinary tract infection.
- Subjects with clinical features of skin and soft tissue infection.

Exclusion criteria

- Subjects with respiratory tract infection
- Subjects with Central nervous system infection

Statistics

Data were entered in Excel spread sheet and statistical analysis was done using SPSS version 21. Chi-square test was deployed to find out the significance of isolates of *Citrobacter* spp from various samples. The proportion of culture positivity among various age group was calculated. Antibiotic sensitivity pattern of the organism isolated from various clinical samples was tabulated and the percentage of sensitivity to the antibiotics tested was given in percentage. Comparison of antibiotic sensitivity pattern of *Citrobacter* spp isolates from various clinical specimens was done by applying Chi-square test.

RESULTS

The various samples collected for this study were blood, urine and pus from patients suspicious of sepsis, urinary tract infection and wound infection respectively. The number of samples in which the organisms were isolated is given in table 1.

Culture positivity according to the age group is depicted in Figure 1

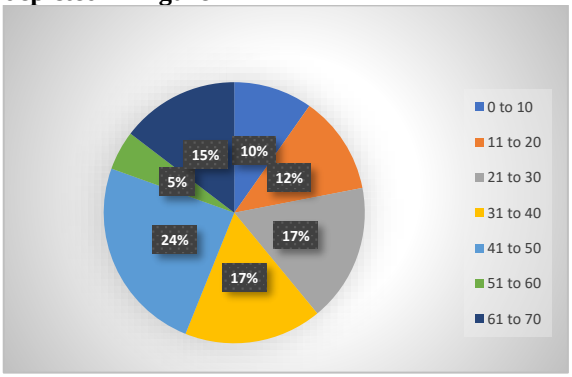

Figure 1: Culture positive in different age groups (N=41)

Maximum number of isolates were from 41 to 50 years and the lowest number of isolates were from 51 to 60 years.

Table 1: Culture positivity in various clinical samples (N=300)

Sl. No	Specimen	Culture positive	Culture negative
1	Blood n (100)	7	93
2	Pus n (100)	22	88
3	Urine n (100)	12	78

The p-value is. 007126. Significant at p < .05

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The highest number of isolates were from suspected cases of wound infection followed by urinary tract infection and sepsis.

Antibiotic susceptibility test (AST) was performed for the *Citrobacter* species isolated from blood using the antibiotic disc from different groups of antibiotics like carbapenems, aminoglycosides,3rd generation cephalosporins, fluoroquinolones, co-trimoxazole etc. The proportion of isolates sensitive to these antibiotics are tabulated in table 2.

Table 2: Antibiotic sensitivity pattern of isolates from blood n 7

Sl. No	Drugs	Sensitive		Resistant	
		Number	**Percentage %**	**Number**	**Percentage %**
1	Imipenem	6	85.71	1	14.28
2	Gentamycin	5	71.42	2	28.57
3	Ceftriaxone/Ceftazidime	4	57.14	3	42.85
4	Co-trimoxazole	3	42.85	4	57.14
5	Ciprofloxacin	3	42.85	4	57.14
6	Cefotaxime	2	28.57	5	71.42

Imipenem was found sensitive to a large number of isolates. The second drug which was sensitive to the isolates was Gentamycin. The 3rd generation cephalosporin Cefotaxime had a very low sensitivity against *Citrobacte*r species in patients with sepsis.

Urine culture was done in patients with clinical features of urinary tract infection (UTI) and it showed growth of *Citrobacter* species in 12 patients. AST was done by Kirby-Bauer disc diffusion method and the results are given in table 3.

Table 3: Antibiotic sensitivity pattern of isolates from urine n 12

Sl. No	**Drugs**	**Sensitive**		**Resistant**	
		Number	**Percentage %**	**Number**	**Percentage %**
1	Imipenem	11	91.67	1	8.33
2	Gentamycin	9	75	3	25
3	Cefotaxime	7	58.33	5	41.67
4	Ciprofloxacin	6	50	6	50
5	Amoxycillin-Clavulanic acid	6	50	6	50
6	Co-trimoxazole	5	41.67	7	58.33
7	Nitrofurantoin	4	33.33	8	66.67

Among the 12 *Citrobacter* isolates from urinary tract infection imipenem showed the highest sensitivity followed by gentamycin and cefotaxime. The spectrum of sensitivity of these isolates for nitrofurantoin was very low.

The number of isolates from pus sample collected from patients with skin and soft tissue infection was high with respect to blood and urine. The antibiotic sensitivity pattern of the *Citrobacter* species in skin and soft tissue infection is depicted in table 4.

Table 4: Antibiotic sensitivity pattern of isolates from pus (N=22)

Sl. No	**Drugs**	**Sensitive**		**Resistant**	
		Number	**Percentage %**	**Number**	**Percentage %**
1	Imipenem	20	90.90	2	9.10
2	Piperacillin- tazobactam	19	86.36	3	13.64
3	Gentamycin	18	81.81	4	18.18
4	Ciprofloxacin	18	81.81	4	18.18
5	Ceftriaxone	13	59.09	9	40.90
6.	Co-trimoxazole	13	59.09	9	40.90
7	Cefotaxime	10	45.45	12	54.54
8	Amoxycillin-Clavulanic acid	7	31.81	15	68.18

Here again imipenem was the most sensitive antibiotic followed by Piperacillin-tazobactam, Gentamycin and Ciprofloxacin. The beta lactam antibiotic in combination with beta lactamase inhibitor was least sensitive in skin and soft tissue infections.

The antibiotic sensitivity pattern of *Citrobacter* species from various infections like blood stream infections, urinary tract infection, skin and soft tissue infection were compared among various groups of antibiotics and the results are tabulated in table 5.

Table 5: Comparison of antibiotic sensitivity pattern of Citrobacter species isolates from various clinical specimens n 41

Sl.No	**Drugs**	**Blood Sensitivity %**	**Urine Sensitivity %**	**Pus Sensitivity %**
1	Imipenem	85.71	91.67	90.90

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2	Gentamycin	71.42	75	81.81
3	Cefotaxime	28.57	58.33	45.45
4	Ciprofloxacin	42.85	50	81.81
5	Co-trimoxazole	42.85	41.67	59.09

The p-value is .022794 *Significant at p < .05*

Imipenem was the most sensitive antibiotic irrespective of the type of infections. Gentamycin was the second antibiotic equally effective in sepsis, UTI, skin and soft tissue infections.

DISCUSSION

*Citrobacte*r species are normally present in soil, water, animal and human gut. Though it is less pathogenic, increased prevalence rate has been observed in the recent days. It is one of the microorganisms which is prevalent in immunocompromised patients and also emerging as a pathogen in hospital acquired infections.[10] Our study focused on the prevalence of *Citrobacte*r *species* in suspected sepsis, urinary tract infection, skin and soft tissue infection and the overall prevalence in a tertiary care hospital. According to the present study, the overall prevalence of *Citrobacter* species was 13.66%. Various studies have reported 1% to 8.23% as the prevalence rate of *Citrobacte*r spp.[11]

The prevalence rate among various age groups were studied and the results showed the highest prevalence (24%) in patients aged 41-50 years. Equal prevalence (17%) was observed in 31-40 years and 21-30 years. The prevalence rate was lowest in patients aged between 51 and 60 years. In an Indian study mean age of patients presented with infection by *Citrobacte*r spp was 35.5 years and the range were 3 days to 87 years.[12]

In our study, the prevalence of *Citrobacter* spp in Sepsis, UTI, skin and soft tissue infections are 7%, 12% and 22% respectively. In certain studies, a prevalence rate of 2.18% and 1.8% have been reported in UTI and peritonitis respectively.[13,14] However, a study carried out on epidemiology and prevalence of *Citrobacter* species has reported percentage prevalence in pus, urine and blood as 5.8, 1.7 and 2.0 percent respectively.[15] Our study is in accordance with another study in which *Citrobacter* species was isolated from 5-12% of patients with UTI.[16]

In our study, the highest prevalence of *Citrobacter* was observed in skin and soft tissue infection. This is similar to a study report which shows a maximum isolation of *Citrobacte*r species from pus followed by urine.[17] On the contrary there are reports of their higher isolation from urine than pus.

Antibiotic susceptibility test was done for all the isolates on Mueller Hinton agar by Kirby-Bauer disc diffusion method as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI).[18] In this study though the proportion of patients with sepsis sensitive to imipenem was high, 14% were resistant to carbapenems. The least sensitive antibiotic in *Citrobacter* sepsis was the 3rd generation cephalosporin Cefotaxime. Even though cefotaxime had a broad spectrum of antibacterial activity during the early periods of its discovery, most of the organisms have developed resistance over a period of time because of the irrational use of this antibiotic. Furthermore, the prevalence of drug-resistance in organisms causing bacteremia is a global threat and a study has concluded 33% of Gram-negative bacteria recovered from BSI as multi-drug resistant.[19]

In UTI due to *Citrobacter* the number one antibiotic sensitive was imipenem. About 75% were sensitive to gentamycin. Compared with cefotaxime sensitivity to *Citrobacte*r in sepsis, a higher percentage of isolates from UTI were sensitive to cefotaxime. However, 41% of the isolates from UTI were extended beta lactamase (ESBL) producers. Nitrofurantoin, a local antibiotic used in UTI was resistant to 67% of *Citrobacter* isolates. Resistance to many groups of antibiotics has been observed and many mechanisms of drug resistance have been demonstrated in *Citrobacter* spp. In a study conducted by Shobha et al., *Citrobacter* spp. was the third most common urinary pathogen and 30% of the isolates were extended spectrum beta lactamase (ESBL) producers.[20] Carbapenem resistance was found in less than 10% of the isolates from UTI. High-level carbapenem resistance was also reported in *C. freundii* due to combination of Klebsiella pneumoniacarbapenemase 2 (KPC 2) production and decreased porin expression.[21]

Our study revealed imipenem as the most sensitive antibiotic in skin and soft tissue infections too. Drugs like Piperacillin-tazobactam, gentamycin and ciprofloxacin were sensitive to more than 80% of the *Citrobacter* isolates. Resistance percentage in Amoxycillin- Clavulanic acid was found to be very high. Trimethoprim and amoxiclav extensively used in UTI and skin and soft tissue infections respectively. The real concern is the increasing rate of resistance to trimethoprim over the last 10 years and the increasing rate of resistance to amoxyclav, presumably as a result of mechanisms other than production of beta-lactamase.[22]

According to the present study imipenem is a promising drug in the isolates from blood, urine and pus samples as well. Gentamycin was the second antibiotic of choice in sepsis and UTI. In patients with skin and soft tissue infections gentamycin and ciprofloxacin were equally sensitive (81%). However, the sensitivity of ciprofloxacin in sepsis and UTI was 40% to 50% only. The 3rd generation cephalosporins were resistant to more than 40% of the isolates irrespective of the site of infection. In this study though the spectrum of sensitivity of various

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antibiotics was diverse, none of the isolate was multidrug resistant.

CONCLUSION

In this study, the prevalence of *Citrobacter* species infection was higher in comparison with other similar studies. Carbapenem resistance was found in 10% to 15% of the isolates. The implications of this study are there is an upward trend in the prevalence of *Citrobacter* species as pathogens and the emergence of carbapenem resistance among these pathogens. Moreover, the antibiotics on pipeline are negligible and the treatment options available are limited. Therefore, infection prevention and control practices along with judicious use of antibiotics based on the Institutional Antibiotic Policy can prevent the morbidities and mortalities due to infection with *Citrobacter species*.

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REFERENCES

1. Murray PR, Holmes B, Aucken HM. Citrobacter, Enterobacter, Klebsiella, Plesiomonas, Serratia, and other members of the enterobacteriaceae. Topley & Wilson's Microbiology and Microbial Infections. 2010 Mar 15.
2. Anderson DJ, Moehring RW, Sloane R, Schmader KE, Weber DJ, Fowler Jr VG, Smathers E, Sexton DJ. Bloodstream infections in community hospitals in the 21st century: a multicenter cohort study. PloS one. 2014 Mar 18;9(3):e91713
3. Fisman D, Patrozou E, Carmeli Y, Perencevich E, Tuite AR, Mermel LA, Geographical Variability of Bacteremia Study Group. Geographical variability in the likelihood of bloodstream infections due to gram-negative bacteria: correlation with proximity to the equator and health care expenditure. PLoS One. 2014 Dec 18;9(12):e114548.
4. Drelichman V, Band JD. Bacteremias due to Citrobacter Diversus and Citrobacter Freundii: Incidence, Risk Factors, and Clinical Outcome. The Journal of Urology. 1986 Apr;135(4):881-
5. Liu LH, Wang NY, Wu AY, Lin CC, Lee CM, Liu CP. Citrobacter freundii bacteremia: Risk factors of mortality and prevalence of resistance genes. Journal of Microbiology, Immunology and Infection. 2018 Aug 1;51(4):565-72.
6. Bonadio M, Meini M, Spitaleri P, Gigli C. Current microbiological and clinical aspects of urinary tract infections. European urology. 2001 Nov 14;40(4):439-45.
7. Jones RN, Jenkins SG, Hoban DJ, Pfaller MA, Ramphal R. In vitro efficacy of six cephalosporins tested against Enterobacteriaceae isolated at 38 North American medical centres participating in the SENTRY Antimicrobial Surveillance Program, 1997–1998. International journal of antimicrobial agents. 2000 Jul 1;15(2):111-8.
8. Khorasani G, Salehifar E, Eslami G. Profile of microorganisms and antimicrobial resistance at a tertiary care referral burn centre in Iran: emergence of Citrobacter freundii as a common microorganism. Burns. 2008 Nov 1;34(7):947-52.
9. Collee JG, Miles RS, Watt B. Tests for identification of bacteria. Mackie and McCartney practical medical microbiology. 1996;14:131-49.
10. Nada T, Baba H, Kawamura K, Ohkura T, Torii K, Ohta M. A small outbreak of third generation cephem-resistant Citrobacter freundii infection on a surgical ward. Japanese journal of infectious diseases. 2004 Jan 1;57(4):181-2.
11. Nayar R, Shukla I, Sultan A. Epidemiology, prevalence and identification of Citrobacter species in clinical specimens in a tertiary care hospital in India. International Journal of Scientific and Research Publications. 2014 Apr;4(4):1-6.
12. Mohanty S, Singhal R, Sood S, Dhawan B, Kapil A, Das BK. Citrobacter infections in a tertiary care hospital in Northern India. Journal of Infection. 2007 Jan 1;54(1):58-64.
13. Mohanty S, Kapil A, Das BK, Dhawan B. Antimicrobial resistance profile of nosocomial uropathogens in a tertiary care hospital. Indian journal of medical sciences. 2003 Apr 1;57(4):148-54.
14. Chao CT, Lee SY, Yang WS, Chen HW, Fang CC, Yen CJ, Chiang CK, Hung KY, Huang JW. Citrobacter peritoneal dialysis peritonitis: rare occurrence with poor outcomes. International Journal of Medical Sciences. 2013;10(9):1092. BARTON LL, WALENTIK C. Citrobacter diversus urinary tract infection. American Journal of Diseases of Children. 1982 May 1;136(5):467-8.
15. BARTON LL, WALENTIK C. Citrobacter diversus urinary tract infection. American Journal of Diseases of Children. 1982 May 1;136(5):467-8.
16. Lipsky BA, Hook III EW, Smith AA, Plorde JJ. Citrobacter infections in humans: experience at the Seattle Veterans Administration Medical Center and a review of the literature. Reviews of infectious diseases. 1980 Sep 1;2(5):746-60.
17. Nayar R, Shukla I, Sultan A. Epidemiology, prevalence and identification of Citrobacter species in clinical specimens in a tertiary care hospital in India. International Journal of Scientific and Research Publications. 2014 Apr;4(4):1-6.
18. PA W. Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing: 20th informational supplement. CLSI document M100-S20. 2010.
19. Thaden JT, Li Y, Ruffin F, Maskarinec SA, Hill-Rorie JM, Wanda LC, Reed SD, Fowler VG. Increased Costs with Multidrug Resistant Gram Negative Bloodstream Infections Are Primarily Due to Patients with Hospital-Acquired Infections. Antimicrob Agents Chemother. 2016 Dec 19.
20. Shobha KL, Gowrish Rao S, Rao S, Sreeja CK. Prevalence of extended spectrum beta-lactamases in urinary isolates of Escherichia coli, Klebsiella and Citrobacter species and their antimicrobial susceptibility pattern in a tertiary care hospital. Indian Journal for the Practising Doctor. 2007;3(6):01-2007.
21. Zhang R, Yang L, Cai JC, Zhou HW, Chen GX. High-level carbapenem resistance in a Citrobacter freundii clinical isolate is due to a combination of KPC-2 production and decreased porin expression. Journal of medical microbiology. 2008 Mar;57(3):332-7.
22. Gales AC, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, Pfaller MA, Doern GV, SENTRY Study Group Latin America T. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998). Journal of Antimicrobial Chemotherapy. 2000 Mar 1;45(3):295-303.

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