Original article

Journal of International Medicine and Dentistry 2015; 2(1): 36-46

JOURNAL OF INTERNATIONAL MEDICINE AND DENTISTRY
To search.....to know.....to share
JIMD
ISSN No. 2350-045X

Clinico-microbiological study of Citrobacter isolates from various clinical specimens and detection of β-lactamase production

Greeshma Hareendranath[1], Saldanha Dominic R M[2], Vishwas Saralaya[3]

Abstract:

Citrobacter species **have been reported to cause a wide spectrum of infections in humans and invasive infections are associated with a high mortality rate, with 33 to 48% of patients succumbing to Citrobacter bacteraemia. The high mortality rate associated with Citrobacter infections may be due in part to ineffective empirical antibiotic therapy. Citrobacter has been found to produce SHV and TEM derived Extended spectrum beta lactamases in addition to chromosomal inducible AmpC beta - lactamases which could be contributing to increasing drug resistance. The aims of the study were to detect the prevalence of Citrobacter infections with its associated risk factors, antibiotic susceptibility patterns and determination of beta-lactamase activity- both extended spectrum beta - lactamase and AmpC beta-lactamase activity among Citrobacter isolates. The isolates were identified by standard microbiological procedures. ESBL detection was by double disc diffusion method and AmpC beta-lactamase detection was done using Cefotaxime and Cefoxitin discs.** ***C. braakii*** **(33.3%) was the commonest genomospecies identified followed by** ***C. freundii*** **(21.3%) and** ***C. amalonaticus*** **(16.66 %) among 150 Citrobacter isolates. Diabetes mellitus was the major risk factor. Imipenem (100%)was most effective whereas 98% showed resistance to Ampicillin; carbapenems and fourth generation Cefipime showed better sensitivity than third generation cephalosporins. The study highlights the need for informed antibiotic treatment guided by routine antimicrobial susceptibility and knowledge of the ESBL status of the isolate, the outcome of which undoubtedly will be better patient care.**

Key words: Citrobacter, Extended spectrum beta lactamase, AmpC beta lactamase

Introduction:

Citrobacter species are aerobic gram negative bacilli commonly found in water, soil, food and the intestinal tracts of animals and humans.[1] Although *Citrobacter* species is less frequently isolated, they are emerging as a nosocomial multidrug resistant pathogen.[2]

Citrobacter species have been reported to cause a wide spectrum of infections in humans (especially among the aged, immunocompromised, and debilitated) including urinary tract infections, respiratory tract infections, surgical wound infections, bone infections, peritonitis, endocarditis, meningitis and bacteremia.[1] Invasive *Citrobacter* infections are associated with a high mortality rate with 33 to 48% of patients succumbing to *Citrobacter* bacteremia.[3] The high mortality rate associated with *Citrobacter* infections may be due in part to ineffective empirical antibiotic therapy.[3]

Extended (or expanded) spectrum beta-lactam antibiotics such as third generation cephalosporins (3GC) form the major component of the empiric antibacterial armamentarium in most clinical setups and especially in tertiary care centres . Extensive use of 3GC has contributed to the evolution of extended spectrum beta-lactamases (ESBLs).[4] *Citrobacter* has been found to produce SHV and TEM derived ESBL in addition to chromosomal inducible AmpC beta - lactamases.[4]

The aims of the study were to detect the prevalence of Citrobacter infections and the associated risk factors, study the antibiotic sensitivity patterns and determine beta-lactamase activity- both extended spectrum beta - lactamase and AmpC beta-lactamase activity among *Citrobacter* isolates.

Materials & Methods:

The study was conducted between August 2010 and January 2012 in the Department of Microbiology, Kasturba Medical College, Mangalore. Specimens received at the Diagnostic Microbiology laboratory from inpatients including adult and paediatric patients of KMC Hospital, Attavar and University Medical Centre, Mangalore was taken up for the study.

Clinical and microbiological data was obtained with regard to each patient including the following information : age, sex, duration of stay in hospital, presenting history including any underlying diseases, and probable risk factors, clinical diagnosis, previous antibiotic or anti-cancer therapy, surgical procedures, trauma, exposure to indwelling devices, other laboratory investigations, chest x-ray findings, the clinical course and outcome.

All specimens received at the laboratory including urine, blood, sputum, endotracheal aspirate, bronchial lavage or washings, pus, body fluids (ascitic, pleural, peritoneal, synovial, etc), biomedical devices were processed immediately and standard microbiological procedures performed.

On receiving the sample, information provided by the patient and the adequacy of specimens required, was noted. The macroscopic appearance of the specimens-whether it is clear, turbid (due to pus cells or bacteria) or contains blood, colour and also the pH was noted. The samples were then further analyzed and growth identified by standard microbiological procedures. Standard procedures for the identification of Gram negative bacilli and Citrobacter were followed.[5, 6]

Phenotypic identification of Citrobacter species [7]:

All isolates were subjected to simplified phenotypic tests as described by Brenner et al[7]. Tests useful in separating *Citrobacter* genomospecies included indole, citrate (using Simmons citrate medium), H_2S production in Triple sugar iron medium, (TSI), arginine deaminase and ornithine decarboxylase (OD) activities, motility, malonate utilisation, fermentation of glucose, acid production from lactose, sucrose, dulcitol, salicin, raffinose, cellobiose, esculin, melibiose, and glycerol, reduction of nitrates and positive reaction for O-nitrophenyl beta-D-galactopyranoside (ONPG).

All tests were performed by conventional methods using commercial media. Sugar fermentation reactions were performed with Andrade's indicator. Esculin hydrolysis was performed on bile esculin agar slants. Tests were read and final results were recorded.

Antibiotic susceptibility testing was done by Kirby-Bauer's disk diffusion method.[8] The antibiotic discs were chosen based on their action on *Citrobacter* species and also the antibiotic policy in the hospitals. The antimicrobial discs which were used included Amikacin (30µg), Ampicillin (10µg), Ampicillin / Sulbactam (10 /10 µg), Cefipime (30 µg), Amoxicillin / Clavulanic acid (20/10µg), Cefoperazone / Sulbactam (75 /30 µg), Ceftazidime (30µg), Ceftriaxone (30µg), Cefuroxime (30 µg), Cefotaxime (30µg), Chloramphenicol (30µg), Ciprofloxacin (5 µg), Co-trimoxazole (1·25 / 23·75µg), Gentamicin (10µg), Imipenem (10µg), Meropenem (10µg), Nalidixic acid (30µg), Norfloxacin (10µg), Ofloxacin (5µg), Aztreonam (30µg), Piperacillin / Tazobactam (100 /10µg), Cefoxitin (30µg), Cefixime(5µg). Nalidixic acid and Norfloxacin were used only for urinary isolates. Results were interpreted according to CLSI guidelines.

Detection of ESBL:

Citrobacter isolates were tested for ESBL production by a double disc diffusion method of Jarlier et al.[9] Results were interpreted according to CLSI guidelines. *E.coli* ATCC 25922 was used as control strain.

Test for detection chromosomal β-lactamases (AmpC β- lactamase):[10,11]

A lawn culture of Citrobacter strain was exposed to two discs, Cefotaxime (30µg) and cefoxitin (30µg) arranged in pairs. Cefotaxime is a weak inducer, whereas cefoxitin is a potent inducer of AmpC β - lactamase. The discs were arranged in such a manner so that the distance between them was approximately twice the radius of the zone of inhibition produced by Cefotaxime tested on its own. After overnight incubation if induction of AmpC β - lactamase had taken place, the radius of the zone of inhibition around the Cefotaxime disc was flattened on the side nearest to the Cefoxitin disc.

Results:

One hundred and fifty *Citrobacter species* were isolated from different clinical samples during the 18 months study period from August 2010 to January 2012. *Citrobacter species* accounted for 1.95 % of the total positive cultures for gram negative isolates.

The total of 150 Citrobacter isolates included 39 isolates from urine, 105 isolates from exudates and 6 isolates from blood (**Table I**). The main genomospecies were *C. braakii* (33.3%), *followed by C. freundii* (21.3%), *C. amalonaticus* (16.66 %), *C. youngae* (10%), *C. sedlakii* (8%), *C. koseri* (7.33%), *C. farmeri* (2%), *C. werkmanii* (1.33%) (**Table II**). The prevalence of Citrobacter infection was more among male patients in all age groups. Most of them had chronic illness like diabetes mellitus, in-situ medical devices and pulmonary disease. Various risk factors were present in these patients as shown in **Table III** and **Graph I**. Diabetes mellitus as the risk factor was seen in 25.33% of patients whereas in-situ medical devices in the form of urinary catheter, cannula and intubation were seen in 20%.

Majority of the cases in our study were associated with monomicrobial infection. **Table IV** shows that 92% of the cases were associated with monomicrobial infection i.e., with *Citrobacter spp*. only while 8% of the cases were polymicrobial. *Staphylococcus aureus* was the most common organism associated followed by *E.coli, and Pseudomonas.*

The antibiotic sensitivity pattern of *Citrobacter* species isolated in this study is given in **Table V**. All the isolates were sensitive to Imipenem (100%), 98% of the *Citrobacter* isolates were resistant to Ampicillin; carbapenems and fourth generation Cefipime showed better sensitivity than third generation cephalosporins.

In our study, 6 (4%) isolates out of 150 were ESBL producers and 8 (5.33%) isolates were AmpC producers.

Discussion:

In our study, we attempted to identify 150 isolates of Citrobacter to species level which has been obtained during the study period from August 2010 to January 2012 (18 months) by Brenner's method.[7] The isolates were obtained from clinical specimens which included urine, blood, pus / swab, CSF, sputum, bronchial aspirate, fluids and biomedical devices. Schaberg et al while observing major trends in microbial etiology of nosocomial infections found that Citrobacter constituted about 1-2% of the nosocomial infections. This correlates with the proportion observed in our study.

In our study, *C. braakii* was the commonest species isolated which was

Table I: Proportion of Citrobacter isolates from various clinical specimens

Specimens	Total number of positive culture samples	Number positive for *Citrobacter species* (%)
Urine	2800	39 (1.39)
Exudate	4515	105 (2.33)
Pus/Swab	1536	57
Sputum	1525	26
Bronchial aspirate	595	12
Fluids	512	7
CSF	52	0
Others*	295	3
Blood	561	6 (1.069)
Total	7876	150 (1.95)

*Others include Central line tip, Catheter tip, Endotracheal tip, Suction tip

not the case in other studies[12, 13, 14] where *C. freundii* was the commonest species isolated, followed by *C. koseri*. The other species that follow *C. braakii* in frequency were *C. freundii* (21.3%) and *C. amalonaticus* (16.66%) which was again different from the earlier report [15] in which *C. freundii* was followed by *C. braakii* and *C. koseri*. Our study clearly reveals that the predominant species for this area is *C. braakii* followed by *C. freundii* and *C. amalonaticus.* These findings may have implications in epidemiology, treatment and prevention.

A total of 50 *C. braakii* species which was the main genomospecies isolated in our study were obtained from urine (13), blood (2), pus/swab (21), bronchial aspirate (3), sputum (7), fluids (3) and other miscellaneous specimens (1). Carlini A et al reported *C. braakii* as a cause of acute peritonitis in peritoneal dialysis patients.[16] *C. braakii* refers to the genomospecies 6 of the *C. freundii* complex. There are no detailed studies of infections caused by the newly formed specific genetic species. Gupta R et al reported a case of *C. braakii* infection in a renal transplant patient receiving immunosuppressive therapy. Immunosuppressive therapy in renal transplant recipients predisposes to infection by unusual pathogens, and this should be suspected when lack of a clinical

Table II: Specimen-wise distribution of *Citrobacter species*

Species	Urine	Blood	Pus/ Swab	Bronchial aspirate	Fluids	sputum	Others	Total (%)
C. amalonaticus	9	0	8	1	2	4	1	**25 (16.66)**
C. youngae	3	0	10	1	0	1	0	**15 (10)**
C. braakii	13	2	21	3	3	7	1	**50 (33.33)**
C. sedlakii	2	0	6	2	0	2	0	**12 (8)**
C. freundii	9	4	7	3	1	8	0	**32 (21.33)**
C. koseri	3	0	5	0	0	2	1	**11 (7.33)**
C. werkmanii	0	0	0	1	0	1	0	**2 (1.33)**
C. farmeri	0	0	0	1	1	1	0	**3 (2)**
Total	**39**	**6**	**57**	**12**	**7**	**26**	**3**	**150**

response to conventional antibiotics is observed.[17]

In older age groups, patients had some sort of underlying diseases like diabetes mellitus, chronic pulmonary diseases, malignancies, hepatic or renal diseases. These patients admitted to ICU were either instrumented or given antibiotics. Infections in neonates were also associated with manipulative procedures (instrumentation) or treatment with broad spectrum antibiotics. This indicates that the underlying disease conditions and treatment given in the hospital, either the antibiotic exposure or instrumentations might have predisposed these patients to Citrobacter infections. Several reports suggest that host susceptibility and underlying diseases are important predisposing factors. [18, 19]

From the present study, it appeared that diabetes mellitus (25.33%) is an important risk factor. Other risk factors included in-situ medical devices (20%), chronic pulmonary infections (14.66%), malignancies (10.66%), trauma (10.66%) and hypertension (9.33%). Earlier studies have shown in-situ medical devices causing nosocomial urinary tract infections and also devices or invasive procedures causing bacteremias.[19] Citrobacter constituted about 39 (1.39%) of the total urinary isolates. The study by Whitby et al has found Citrobacter in 12% of acute infections. As reported by another study[20], *Citrobacter species* appears to be one of

Table III: Risk factor variables in patients with Citrobacter infections

Risk factors involved	Number (%)
Diabetes mellitus	38 (25.33)
Hypertension	14 (9.33)
Chronic pulmonary disease	22 (14.66)
In-situ medical devices	30 (20)
Chronic renal failure	8 (5.33)
Liver disease	1 (0.6)
Malignancy	16 (10.66)
Trauma	16 (10.66)
Immunosuppressed state	6 (0.04)

the contributing organisms to urinary tract infections. Earlier studies had pointed that there is a strong correlation between neoplasia (primarily of the genitourinary tract), chronic indwelling catheter and diabetes mellitus with significant culture of Citrobacter in urine. Grant et al point to the opportunistic nature of Citrobacter in chronically ill patients with low host resistance.[21] In our study, females accounted for 22 cases (56.41%) whereas males accounted for 17 cases (43.58%). The female urogenital anatomy may be the reason which contributes to more number of cases.

In our study, Citrobacter constituted about 105 isolates (2.33%) from exudate samples. Stone et al conducted a prospective study in which *Citrobacter* isolates were found in 5% of surgical wound infections.[17] We got 57 isolates from pus and 38 from respiratory tract secretions in our study and *C. braakii* was mainly isolated from these specimens.

The frequency of bacteraemia due to Citrobacter in our study was less. Bacteraemia due to these organisms remains uncommon as shown by Drelichmann V et al.[19] In our study, the bacteraemias were all monomicrobial. Out of the six cases, 2 were from the paediatric age group; *C. freundii* was responsible for 4 cases and *C. braakii* in 2 cases. This was almost consistent with the previous study.[19] The major sources for bacteraemia were the urinary tract followed by biomedical devices, respiratory tract, gastrointestinal tract, wound infections and so on in the descending order. Studies have found Citrobacter bacteraemia common in elderly patients.[19] In our study, Citrobacter bacteraemia was predominant in the adult age group. Five of these patients were admitted in ICU and had endotracheal intubation and also previous antibiotic therapy. A study found hepatic, biliary, and pancreatic disease, recent surgery and procedures, catheter and previous antibiotic therapy as important risk factors.[22]

Antibiotic susceptibility patterns revealed the isolates to be resistant to commonly used antibiotics. Susceptibility testing of Citrobacter isolates demonstrated resistance to a broad range of antibiotics. Ampicillin resistance was seen in 98% of the strains and 100% of the strains showed susceptibility to Imipenem. The sensitivity to other antibiotics is given in **Table V**. The susceptibility of all the isolates to Imipenem may be due to the reason that Imipenem is mainly used as a reserve drug. Most were resistant to cephalosporins as well. Combination drugs such as Cefoperazone / Sulbactam and Piperacillin / Tazobactam have shown better sensitivity pattern than individual drugs. Though they are more effective than single β- lactam drugs, resistance has already been observed and is likely to

Graph I: Age-Sex distribution of patients with Citrobacter infections

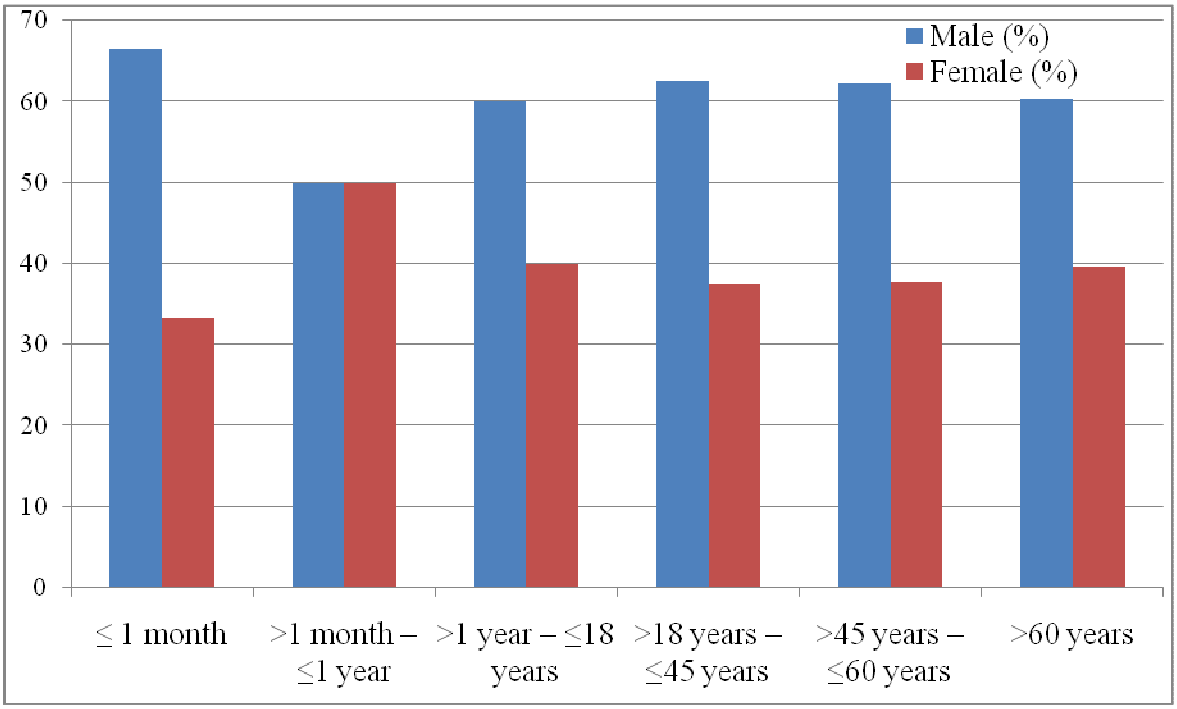

Table IV: Monomicrobial and polymicrobial infections

Association	Male (%)	Female (%)	Total
Monomicrobial	86 (57.33)	52 (34.66)	138 (92)
Polymicrobial	7 (4.66)	5 (3.33)	12 (8)
Total	93	57	150

increase rapidly because of injudicious use of these antibiotics.

In case of aminoglycosides, sensitivity to Amikacin (52%) was better than Gentamicin (32%). The sensitivity pattern of fluoroquinolones is as follows– Ciprofloxacin 40.66 %, Nalidixic acid 28.2 %, Norfloxacin 43.59% and Ofloxacin 52%. Excessive use or misuse of these drugs in hospital area might have resulted in decreased effectiveness of fluoroquinolones.

High level resistance to β-lactam agents has also been noted by various studies across the world which is consistent with our study.[23, 24, 25]

The study by Pepperell et al has found that antibiotic-resistant, low virulence *Citrobacter species* are common colonizers of immunosuppressed patients exposed to multiple antimicrobial agents.[3] These bacteria demonstrate broad drug resistance encoded by a diverse array of genetic mechanisms. It is possible that

Table V: Antibiotic sensitivity patterns of *Citrobacter species*

Antibiotics	Sensitive (%)	Resistant (%)
Ampicillin	3 (2)	147 (98)
Amikacin	78 (52)	72 (48)
Ampicillin/Sulbactam	30 (20)	120 (80)
Cefipime	82 (54.66)	68 (45.33)
Amoxicillin/Clavulanic acid	75 (50)	75 (50)
Cefoperazone/Sulbactam	122 (81.33)	28 (18.66)
Ceftazidime	58 (38.66)	92 (61.33)
Ceftriaxone	55 (36.66)	95 (63.33)
Cefuroxime	53 (35.33)	97 (64.66)
Cefotaxime	60 (40)	90 (60)
Chloramphenicol	88 (58.66)	62 (41.33)
Ciprofloxacin	61 (40.66)	89 (59.33)
Co-trimoxazole	75 (50)	75 (50)
Gentamicin	48 (32)	102 (68)
Imipenem	150 (100)	0
Meropenem	147 (98)	3 (2)
Nalidixic acid	11 (28.2)	28 (71.79)
Norfloxacin	17 (43.589)	22 (56.41)
Ofloxacin	78 (52)	72 (48)
Aztreonam	120 (80)	30 (20)
Piperacillin/Tazobactam	108 (72)	42 (28)
Cefoxitin	90 (60)	60 (40)
Cefixime	91 (60.66)	59 (39.33)

Citrobacter and similar organisms may contribute to the evolution of bacterial pathogens by acting as persistent sources of resistance genes. The presence of low virulence, resistant bacteria in hospitalized patients may complicate surveillance and infection control efforts.[3]

In recent years, extended-spectrum beta - lactamases (ESBLs) have become more and more prevalent in species

characterized by inducible class C cephalosporinase (AmpC) such as *C. freundii* which frequently segregate mutants with high-level constitutive production of AmpC enzymes.[10]

In our study, only 6 (4%) isolates out of 150 were ESBL producers and only 8 (5.33%) isolates were AmpC producers. The study done by Shetty J et al has shown inducible AmpC β-lactamase activity in only 1.97% of the Citrobacter isolates (14/ 709) but a higher proportion of ESBL producing isolates which comprised about 53.31%.[26] Another study done by Shobha KL et al to determine the prevalence of ESBLs in urinary isolates, showed that out of the 300 gram negative bacilli isolates, 10 *Citrobacter species* were obtained and the screening test showed 2 of the 10 *Citrobacter species* (20%) to be ESBL positive.[27]

It is generally recognised that patients infected with ESBL-producing organisms are at risk for poor outcome if they are treated with antibacterials to which the organism exhibits high level resistance. Most commonly, the transient carriage of organisms on the hands of healthcare workers are implicated in patient to patient spread. Risk factors for acquisition of ESBL-producing Enterobacteriaceae generally are indicators of severity of illness and medical intervention.

The stability of carbapenems against a wide variety of β-lactamases is potentially of increasing importance as the incidence of clinical strains expressing ESBLs appears to be increasing.[28] The emerging resistance to Meropenem shows that when the selective pressure of antibiotics is changed, the resistance pattern of *Citrobacter* strains may also change.

Conclusions:

Therefore, there is a greater need for informed antibiotic treatment guided by not only routine antimicrobial susceptibility but also by knowledge of the ESBL status of the isolate. Phenotypic detection of these resistance mechanisms though not confirmatory, are faster, far more cost effective, less labour intensive, not requiring a high level of technical expertise and thus easier to perform on a daily basis, the outcome of which undoubtedly will be better patient care.

References:

1. Wang JT, Chang SC, Chen YC and Luh KT. Comparison of antimicrobial susceptibility of *Citrobacter freundii* isolates in two different time periods. J Microbiol Immunol Infect China 2000; 33: 258-262.

2. Thapa B, Adhikari P, Mahat K, Chhetri MR, Joshi LN. Multidrug-resistant nosocomial *Citrobacter* in a hospital in Kathmandu. Nepal Med Coll J 2009; 11(3): 195-199

3. Pepperell C, Kus JV, Gardam MA, Humar A and Burrows LL. Low virulence *Citrobacter species* encode resistance to multiple antimicrobials. Antimicrob Agents Chemother 2002; 46(11): 3555-3560.

4. Menon T, Bindu D, Kumar CPG, Nalini S, Thirunarayan MA. Comparison of double disc and three dimensional methods to screen for ESBL producers in tertiary care hospital. Indian J Med Microbiol 2006; 24:117-20.

5. Howard BJ. Clinical and Pathologic Microbiology 2nd ed. St. Louis: Mosby Washington 1994.

6. O'Hara CM, Roman SB, and Miller JM. Ability of commercial identification systems to identify newly recognised species of *Citrobacter*. J Clin Microbiol 1995; 33(1): 242 - 245.

7. Brenner DJ, O'Hara CM, Grimont PA, Janda JM, Falsen E, Aldova E et al. Biochemical identification of *Citrobacter species* defined by DNA hybridization and description of *C. gillenii* sp. nov. (Formerly *Citrobacter* genomospeices 10) and *C. muriliniae* sp. nov. (Formerly *Citrobacter* genomospecies 11) J Clin Microbiol 1999; 37: 2619 - 2624.

8. Bauer AW, Kirby W, Sherris J, Turck M. Antibiotic susceptibility testing by a standard single disc method. Am J Clin Pathol 1996; 45: 493– 496.
9. Jarlier V, Nicholas MH, Fournier G. and Philippon A. Extended broad spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae : hospital prevalence and susceptibility patterns. Rev Infect Dis 1988; 10: 867-78.
10. Livermore DM and Brown DFJ. Detection of β - lactamase mediated resistance. JAC 2001; 48: 59-64.
11. Mackie and MacCartney. Practical Medical Microbiology; 14th Ed. New York; Churchill Livingstone Inc. 1996.
12. Khanna A, Singh N, Aggarwal A, Khanna M. Antibiotic Resistance Pattern in *Citrobacter* Species : An Emerging Nosocomial Pathogen in a Tertiary Care Hospital. JCDR 2012; 6(4):642–644.
13. Samonis G, Karageorgopoulous DE, Kofteridis DP, Matthaion DK, Sidiropoulou V, Maraki S, et al. *Citrobacter* infection in a general hospital: characteristics and outcomes. Eur J Infect Dis 2009; 28: 61-8.
14. Manganello S, Tayara A, Perazzi B, Neira L, Famiglietti A, Pugliese L et al. Characterisation and distribution of *Citrobacter* species in a university hospital. Enferm Infecc Microbiol Clin 2001; 19:11-4.
15. Arens and Verbist L. Differentiation and susceptibility of *Citrobacter* isolates from patients in a university hospital. Clin Microbiol Infect 1997; 3:53-57.
16. Carlini A, Mattei R, Mazzotta L. *C. braakii*, an unusual organism as cause of acute peritonitis in peritoneal dialysis patients. Peritoneal Dialysis International 2005; 25: 405-406.
17. Gupta R, Rauf SJ, Singh S, Smith J and Agraharkar ML. Sepsis in a Renal Transplant recipient due to *C. braakii*. Southern Med J 2003; 96:796-798.
18. Morgan MG, Stuart C Leanord AT, Enright M and Cole GF. *C . diversus* brain abscess: case reports and molecular epidemiology. J Med Microbiol 1992; 36: 273 - 278.
19. Drelichman V, Band JD. Bacteremias due to *C. diversus and C. freundii*: Incidence, Risk Factors and Clinical Outcome. Arch Intern Med 1985; 145(10): 1808 - 1810.
20. Jones SR, Ragsdale AR, Kutscher E and Sanford JP. Clinical and bacteriologic observations on a recently recognised species of Enterobacteriaceae, *C. diversus*. J Infect Dis 1973; 128:563-565.
21. Williams RD and Simmons RL. *Citrobacter* perinephric abscess presenting as pneumoscrotum in transplant recipient. Urology 1974; 3:478-480.
22. Kim BN, Woo JH, Ryu J and Kim YS. Resistance to extended spectrum cephalosporins and mortality in patients with *C. freundii* bacteremia. Infection 2003; 31: 202-207.
23. Jones ME, Avison MB, Damdinsuren E, Macgowan AP and Bennett PM. Heterogeneity at the beta - lactamase structural gene AmpC amongst *Citrobacter* Spp. assessed by PCR analysis: potential for typing at a molecular level. J Med Microbiol 1994; 41: 209-214.
24. Gupta N, Yadav A, Choudhary U, Arora DR. *Citrobacter* bacteremia in a tertiary care hospital. Scand J Infect 2003; 35:765-8.
25. Ash RJ, Mauck B and Morgan M. Antibiotic resistance of Gram Negative Bacteria in Rivers. United States. Emerg Infect Dis 2002; 8(7).
26. Shetty J, Kotigadde S. Antibiotic sensitivity pattern of *Citrobacter* isolated from various clinical specimens in a tertiary care hospital. Indian J Pathol Microbiol 2007; 50: 666-668.
27. Shobha KL, Rao G, Rao S, Sreeja CK. Prevalence of extended spectrum β lactamases in urinary isolates of *Escherichia coli, Klebsiella* and *Citrobacter* species and their antimicrobial susceptibility pattern in a tertiary care hospital. IndMedica 2007; 3:1-2.

28. Shah PM and Isaacs RD. Ertapenem, the first of a new group of carbapenems. J Antimicrob Chemother 2003; 52: 538-542.

****

Conflicts of interest- Nil
Acknowledgements- Nil

Date of submission: 03-03-2015
Date of acceptance: 02-04-2015

Authors details:

1- Assistant Professor, Department of Microbiology, Government Medical College, Idukki, Kerala

2- **Corresponding author:** Professor and Head, Department of Microbiology, Kannur Medical College, Anjarakandy, Kannur. 670612. E-mail: drdoms@gmail.com

3- Associate Professor, Department of Microbiology, Kasturba Medical College, (Manipal University)Mangalore- 575001