**Prevalence and drug resistance pattern of Citrobacter sps – A retrospective study**

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**Abstract**

**Introduction:** Citrobacter are motile, straight, gram negative bacilli and are facultative anaerobes. Nosocomial infections by Citrobacter account for about 3-6% of all infections caused by Enterobacteriaceae. Urinary tract infections are the most common infection caused by Citrobacter sps. Followed by infections of GI tract, skin and soft tissues and pneumonia. The present study was conducted in the microbiology department of our diagnostic centre between July to December 2018 with the aim of studying the prevalence of Citrobacter infections in our clinical setting and also to study its antibiotic sensitivity pattern.

**Materials and Methods:** This was a prospective study carried out in Microbiology department of our diagnostic centre between July to December 2018. A total of 3758 patients of both sexes registered for culture and sensitivity testing were included in the study. Samples were collected by following thorough aseptic techniques in sterile containers/swabs and were plated on 5% sheep blood agar and Macokey agar and incubated at 37°C for 18-24 hours. Isolated organism was identified by Gram’s stain and colony morphology and further by biochemical tests. Antibiotic sensitivity was done on Vitec II (Biomerieux).

**Results:** Out of 3758 clinical specimens received in the laboratory for culture and sensitivity, bacterial growth was observed in 1226(32.6%) specimens. Growth of Citrobacter sps was observed in 1.22% (15/1226) of all positive cultures. Citrobacter was isolated in 12(2.19%) urine cultures, and one each in blood pus and vaginal swab. In urinary isolates, Nalidixic acid was resistant and intermediate sensitivity to Ciproflaxacin. In blood and vaginal swab, Citrobacter showed sensitivity to Piperacillin/ Tazobactum, Cefoprazone/ Sulbactum, Ertapenem, Meropenem and Imipenem, Amikacin, Gentamycin, Tigecycline, Nitrofurantoin and Colistin. In pus, sensitivity was observed to Piperacillin/Tazobactumand Colistin only while all other antibiotics were resistant.

**Conclusion:** Citrobacter sps is emerging as an opportunistic pathogen especially in immunocompromised patients and in hospital settings with resistance to multiple drugs. Thorough aseptic precautions by hospital staff and proper surveillance measures will help in preventing emergence of multidrug resistant strains of Citrobacter sps.

**Keywords:** Citrobacter, Multi drug resistance, Nosocomial infection.

**Introduction**

Citrobacter species are a part of Enterobacteriaceae family. They are motile, straight, gram negative bacilli and are facultative anaerobes. They are motile by peritrichous flagella. Werkman and Gillen in 1932 proposed the genus Citrobacter.¹ Citrobacter sps are found in water, soil and as commensals in the gastrointestinal tract of animals and humans. Citrobacter infections are usually nosocomial but can be community acquired. In a large surveillance study by Jones RN et al in 1991, it was found that nearly 0.8% of all gram negative bacteria were due to Citrobacter sps.² Nosocomial infections by Citrobacter account for about 3-6% of all infections caused by Enterobacteriaceae.³,⁴ Citrobacter infections may be transmitted vertically from mother to baby or by horizontal route through carriers or other nosocomial sources.⁵ Citrobacter infections are common in neonates presenting as sepsis or meningitis or even brain abscess. Elderly debilitated, immunocompromised patients are also at risk of developing Citrobacter infections.⁶,⁷ Urinary tract infections are the most common infection caused by Citrobacter sps. Followed by infections of GI tract, skin and soft tissues and pneumonia.⁸ Citrobacter sps are commensals of oral cavity, lower GI tract and respiratory tract. It can cause outbreaks of nosocomial infections through hands of carriers in hospital staff.⁹,¹⁰ Urinary tract infections are one of the commonest nosocomial infections accounting for about 40% of all hospital acquired infections and result in a great deal of morbidity and mortality.¹¹,¹² Citrobacter accounts for about 5-12% of all urinary isolates in adults.¹³ The present study was conducted in the microbiology department of our diagnostic centre between July to December 2018 with the aim of studying the prevalence of Citrobacter infections in our clinical setting and also to study its antibiotic sensitivity pattern.

**Materials and Methods**

This was a prospective study carried out in Microbiology department of our diagnostic centre between July to December 2018. A total of 3758 patients of both sexes registered for culture and sensitivity testing were included in the study. The patients were divided into 0-20, 21-40, 41-60, 61-80 and more than 80 years age group in both the sexes. All clinical specimens like urine, stool, pus, CSF, vaginal swab etc were included in the study. Samples were collected by following thorough aseptic techniques in sterile containers/swabs and were plated on 5% sheep blood agar and Macokey agar and incubated at 37°C for 18-24 hours. Isolated organism was identified by Gram’s stain and colony morphology and further by biochemical tests. Antibiotic sensitivity was done on Vitec II (Biomerieux).

The criteria for identification of Citrobacter was:

**Gram Stain:** Gram negative, straight rods, singly or in pairs, about 1 micrometer in diameter.
Motility: Motile by peritrichous flagella.
Colony Morphology: 2-4 millimeters in diameter, smooth, low, convex and moist, translucent or opaque and gray with a shiny surface and an entire edge. Facultatively anaerobic.
Biochemical Reactions: Citrate utilized as the sole carbon source. Catalase-positive. Oxidase-negative. Nitrate reduced to nitrite.
Lysine: Decarboxylase-negative.
Methyl: Red-positive.
Voges: Proskauer-negative.

Results
This was a retrospective study carried out in the microbiology department of our diagnostic centre between July to December 2018. There were 3758 patients out of which 1769(47.1%) were males and 1989(52.9%) were females with a male to female ratio of 0.88:1.
Maximum number of patients were below 20 years of age(33.7%), followed by 31.1% in 21-40 years age group, 40.5% in 40 years age group and only 2.2% patients were above 80 years of age. (Table 1)
In patients below 20 years, there was a male preponderance (40.5%) as compared to females (27.6%) while in 21-40 years age group there were 38.9% females and 22.4% males. A male preponderance was also observed in 61-80 years age group with 17.9% males and 11.9% females.

Out of 3758 clinical specimens received in the laboratory for culture and sensitivity, bacterial growth was observed in 1226(32.6%) specimens while there was no growth in 2532(67.37%) patients. Growth of Citrobacter sps was observed in 1.22% (15/1226) of all positive cultures. Citrobacter was isolated in 12(2.19%) urine cultures, and one each in blood pus and vaginal swab. Out of 15 Citrobacter isolates, urine was the most common clinical specimen (80%) (Table 2)
In patients below 20 years, there was a male preponderance (40.5%) as compared to females (27.6%) while in 21-40 years age group there were 38.9% females and 22.4% males. A male preponderance was also observed in 61-80 years age group with 17.9% males and 11.9% females.

Table 1: Demographic data of patients

| S. No. | Age (years) | Male | % Male | Female | % Female | Total |
|--------|-------------|------|--------|--------|----------|-------|
| 1      | 0 - 20      | 717  | 56.6%  | 549    | 43.4%    | 1266  |
| 2      | 21 - 40     | 396  | 33.8%  | 773    | 66.2%    | 1169  |
| 3      | 41 - 60     | 295  | 42.9%  | 392    | 51.1%    | 687   |
| 4      | 61 - 80     | 317  | 57.3%  | 236    | 42.7%    | 553   |
| 5      | >80         | 44   | 53.00% | 39     | 47.0%    | 83    |
| Total  |             | 1769 | 47.1%  | 1989   | 52.9%    | 3758  |

Table 2: Incidence of citrobacter in different clinical specimens

| S. No. | Specimen          | Overall Total Patients | No Growth Total Patients | Growth Total Patients | % Total Growth | Citrobacter Growth Total Patients | % Citrobacter Growth |
|--------|-------------------|------------------------|--------------------------|-----------------------|---------------|-----------------------------------|----------------------|
| 1      | Urine             | 1904                   | 1357                     | 547                   | 28.7%         | 12                                | 2.19%                |
| 2      | Blood             | 966                    | 703                      | 263                   | 27.2%         | 1                                 | 0.38%                |
| 3      | Pus               | 355                    | 128                      | 227                   | 63.9%         | 1                                 | 0.44%                |
| 4      | Sputum            | 108                    | 66                       | 42                    | 38.9%         | 0                                 | 0.00%                |
| 5      | Stool             | 54                     | 45                       | 9                     | 16.7%         | 0                                 | 0.00%                |
| 6      | Vaginal Swab      | 152                    | 106                      | 46                    | 30.3%         | 1                                 | 2.17%                |
| 7      | Throat Swab       | 122                    | 65                       | 57                    | 46.7%         | 0                                 | 0.00%                |
| 8      | CSF               | 19                     | 18                       | 1                     | 5.26%         | 0                                 | 0.00%                |
| 9      | ET Secretion      | 17                     | 16                       | 16                    | 94.12%        | 0                                 | 0.00%                |
| 10     | Body Fluid        | 45                     | 34                       | 11                    | 24.44%        | 0                                 | 0.00%                |
| 11     | Semen             | 14                     | 9                        | 5                     | 35.71%        | 0                                 | 0.00%                |
| 12     | Breast Abscess    | 2                      | 0                        | 2                     | 100.00%       | 0                                 | 0.00%                |
| Total  |                   | 3758                   | 2532                     | 1226                  | 32.62%        | 15                                | 1.22%                |
Table 3: Demographic data of citrobacter isolates in urine

| S. No. | Age (years) | Urine C/S | Male | Female |
|--------|-------------|-----------|------|--------|
| 1      | 0 - 20      |           | 2    | 0      |
| 2      | 21 - 40     |           |      |        |
| 3      | 41 - 60     |           | 1    | 2      |
| 4      | 61 - 80     |           | 0    | 0      |
| 5      | >80         |           | 0    | 0      |

Table 4: MIC of citrobacter in different clinical specimens

| Specimen Antibiotics | Urine | Blood | Pus | Vaginal Swab |
|----------------------|-------|-------|-----|--------------|
| Amoxicillin/Clavulanic Acid | <=0.25 | <=0.25 | <=0.25 | <=0.25 |
| Piperacillin/Tazobactam | <=0.5 | <=0.5 | <=0.25 | <=0.5 |
| Cefuroxime | <=4 | <=4 | <=4 | <=4 |
| Cefuroxime Axetil | <=4 | <=4 | <=4 | <=4 |
| Ceftriaxone | <=1 | <=1 | <=1 | <=1 |
| Cefoperazone/Sulbactam | <=8 | <=8 | <=8 | <=8 |
| Cefepime | <=1 | <=1 | <=1 | <=1 |
| Ertapenem | <=0.25 | <=0.25 | <=0.25 | <=0.25 |
| Imipenem | <=0.25 | <=0.25 | <=0.25 | <=0.25 |
| Meropenem | <=0.25 | <=0.25 | <=0.25 | <=0.25 |
| Amikacin | <=2 | <=2 | <=2 | <=2 |
| Gentamicin | <=1 | <=1 | <=1 | <=1 |
| Nalidixic Acid | >16 | >16 | >16 | >16 |
| Cefoproxilne | >16 | >16 | >16 | >16 |
| Tigecycline | <=0.5 | <=0.5 | <=0.5 | <=0.5 |
| Nitrofurantoin | <=16 | <=16 | <=16 | <=16 |
| Colistine | <=0.5 | <=0.5 | <=0.5 | <=0.5 |
| Trimethoprim/Sulfamethoxazole | <=20 | <=20 | <=20 | <=20 |

Discussion

Citrobacter sps are normal inhabitants of the intestinal tract and are found in the human and animal faecal matter, in soil, sewage and food. It is an important cause of nosocomial infections as epidemics are known to occur due to carriage of Citrobacter on the hands and in the GI tract of hospital staff. The prevalence of urinary infections by Citrobacter sps is on the rise. Invasive procedures like catheterization help the bacteria to colonise the urinary bladder and when the immunity is compromised it can cause severe bacteremia. The situation is further aggravated by emergence of multidrug resistant strains of Citrobacter sps. In our study, we had a slight female preponderance with an overall incidence of 1.22% for isolation of Citrobacter sps in various clinical specimens. Maximum patients were below 20 years of age. Out of the total 15 Citrobacter isolates, 12 were isolated from urine specimens, mostly in 21-40 years age group (58.5%) with 75% females. Maximum sensitivity was observed to piperacillin Tazobactum, Cefoperazone sulbactum, colistine, Tigecycline, Ertapenem, meropenem, imipenem. Citrobacter demonstrated Resistance to amoxycyavinic acid, cefuroxime, cefuroxime axetil and nalidixic acid and 100% resistance to ciprofloxacin. The multi drug resistance pattern of Citrobacter may be attributed to the fact that both clinical and environmental strains may be a reservoir of antimicrobial resistance determinants. Hiba Sami et al in their study observed a prevalence rate of 3.46% for Citrobacter sps. With a female preponderance. In their study Amikacin was susceptible in 85.2% isolates while there was poor activity of fluoroquinolones against Citrobacter. The most effective antibiotic was Imipenem. Okonko et al also had similar findings in their study. Maripandi et al reported Citrobacter prevalence rate of 1.3% in their study which is similar to our study. Metri Basavraj et al observed an isolation rate of 15.7% in urinary infections and these isolates were found to be resistant to Cefalaxin, Norfloxacine, Ciprofloxacine and aminoglycosides. Shih et al also had similar findings in their study. Sneha Mohan et al in their study had maximum Citrobacter isolates in pus specimens with sensitivity to Carbapenems and Penicillin group of antibiotics.

Conclusion

Citrobacter sps is emerging as an opportunistic pathogen especially in immunocompromised patients and in hospital settings with resistance to multiple drugs. This is a cause of great concern. Early diagnosis and timely and judicious initiation of antibiotic therapy is key factor in eliminating the pathogen and preventing further nosocomial spread and
indiscriminate use of antibiotics. Thorough aseptic precautions by hospital staff and proper surveillance measures will help in preventing emergence of multidrug resistant strains of Citrobacter spp.

Conflict of Interest: None.

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