CHARACTERIZATION AND ANTIBIOTIC RESISTANCE PATTERN OF GENUS CITROBACTER AMONG VARIOUS CLINICAL ISOLATES

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ABSTRACT   *Citrobacter spp. are emerging as an important cause of nosocomial infections. This study was conducted to identify Citrobacter upto species level to quantitate and assess the real magnitude of these infections, and to study the pattern of antibiotic resistance among them.*
MATERIALS AND METHODS : The Citrobacter spp. were identified till species level using O'Hara scheme of identification. Antibiotic susceptibility testing was done as per CLSI guidelines.
RESULTS AND OBSERVATIONS : Out of 14555 samples received, 228 were identified as Citrobacter spp. The overall prevalence was 1.57 %. Maximum Citrobacter isolates were from urine (46.05%) followed by pus (35.53%) and blood (14.03%). C. koseri (45.61%) was the most predominant species followed by C. freundii (34.65%) while other species constituted 19.74%. Among all the Citrobacter isolates in our study, 76.32% were from indoor patients while 23.68% were from outdoor patients.
CONCLUSION: The antimicrobial sensitivity patterns of common Citrobacter isolates will provide guidelines to the clinicians to start appropriate empirical antibiotic therapy depending upon the clinical scenario. This can prevent indiscriminate use of antibiotics.

INTRODUCTION

Citrobacter, a member of Enterobacteriaceae family, is a normal inhabitant of the gut but when host defenses are weak or other factors favour their establishment in other tissues, serious infections may result. [1] Although *Citrobacter* species were once considered as less frequent nosocomial pathogen, they are posing a major challenge for the microbiologist & clinician because of their multidrug resistance. Recent worldwide reports show an upsurge of *Citrobacter* infection in hospital settings and emergence of resistant strains.

In India, there are very few literatures which have dealt with *Citrobacter* and its antibiotic susceptibility pattern. Keeping this fact in mind, this study was conducted to identify and isolate *Citrobacter*, to know their prevalence amongst gram negative bacilli and to find its resistance pattern in this part of the country.

MATERIALS AND METHODS

A prospective study was conducted on the various clinical samples received in the Department of Microbiology, Government Medical College & Hospital, Amritsar over a period of one and a half year (Dec 2014- June 2016). Various clinical specimens such as urine, pus, cerebrospinal fluid, blood, body fluids including peritoneal, pleural and bile fluids, respiratory tract specimens like bronchial aspirates, tracheal aspirates, bronchoalveolar lavage fluid, ear and nasal swabs, catheter tips and drain tips were analyzed. The genus *Citrobacter* was established if the isolate was motile, catalase positive, oxidase negative, nonlactose fermenter or late lactose fermenter, ONPG positive, Methyl Red positive, VP negative, PPA negative, Citrate positive Gram-negative rod.[1] Further identification upto species level was done as per O' Hara scheme of classification.[2]

The anti-microbial sensitivity testing of the isolates of *Citrobacter* spp. was performed on Mueller Hinton agar by the Kirby Bauer method by using the disc diffusion technique and the results were interpreted as per the Clinical Laboratory Standard Institute (CLSI) guidelines.[3] Screening test for ESBL production was done as part of routine susceptibility testing. Suspected isolates were confirmed by Combination Disc Test

(CDT). The test was considered positive if the inhibition zone diameter was ≥ 5 mm larger with clavulanic acid than without clavulanic acid. MBL detection was done by using imipenem and imipenem-EDTA discs.
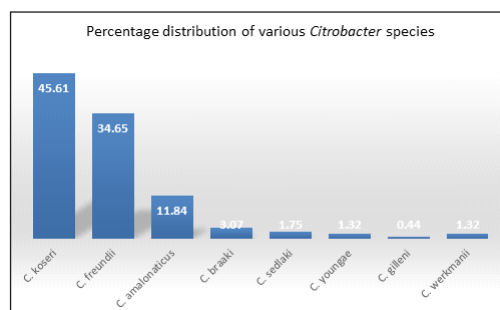
RESULTS AND OBSERVATIONS:

During the study period, the overall prevalence rate of *Citrobacter* spp. was 1.57 %. *Citrobacter* spp. accounted for 2.66% (228 / 8580) of culture positive isolates. Of the total gram negative isolates among culture positive, 4.5% were of *Citrobacter* spp.

TABLE 1: Distribution of *Citrobacter* Isolates in Various Specimens

Specimen	No. of isolates	% age of isolates
Urine	105	46.05
Pus	81	35.53
Blood	33	14.47
Others	9	3.95
Total	228	100

As shown above, maximum Citrobacter isolates were from urine followed by pus specimens.

Percentage distribution of various *Citrobacter* species

C. koseri 45.61; C. freundii 34.65; C. amalonaticus 11.84; C. braakii 3.07; C. sedlakii 1.75; C. youngae 1.32; C. gilleni 0.44; C. werkmanii 1.32

FIGURE 1: Percentage Distribution of *Citrobacter species*

Out of 228 *Citrobacter* isolates, maximum were *C. koseri*

(45.61%), followed by *C. freundii* (34.65%) while other species constituted 19.74%.

TABLE 2: Antimicrobial Resistance Pattern of *Citrobacter* Isolates

Antibiotic	Resistant(no.)
Amikacin	84
Gentamicin	143
Ciprofloxacin	164
Ceftriaxone	151
Ceftazidime	143
Ceftazidime-clavulanic acid	86
Piperacillin-tazobactam	28
Imipenem	8

In our study, Citrobacter isolates showed maximum resistance to Ciprofloxacin (71.93%), followed by Ceftriaxone (66.23%), Gentamicin (62.72%) and Ceftazidime (62.28%). Least resistance was seen to Imipenem (3.51%) and Piperacillin –Tazobactam (12.28%).

In the present study, risk factors observed in majority of cases were prior antibiotics (26.09%), immunosuppressed state (22.74%), in-situ medical devices (19.4%), trauma (17.39%), abdominal surgery (14.05%), diabetes mellitus (11.37%), chronic renal failure (9.36%), hypertension (8.03%) and malignancy (1.34%)

DISCUSSION:
Citrobacter spp. are ubiquitous in nature (food, soil, water) and a colonizer of human gastrointestinal tract.
It can cause. difficult-to-treat nosocomial wound and other infections. *Citrobacter* share similar morphological, cultural and biochemical characteristics with other organisms of family Enterobacteriaceae so they are often misidentified. We used O' Hara scheme for identification of *Citrobacter* upto species level as it was most practical in our set up.[2]

In our study, 228 isolates of *Citrobacter* were identified to species level and prevalence rate was 1.57 % with a p- value of < 0.05, justifying *Citrobacter* as an emerging health concern.

Our study emphasizes that urinary tract infections, wound infections and bacteremia are the commonest infections caused by *Citrobacter*. Similar reports of their higher isolation from urine than pus have been reported in other studies.[4] In our study, 46.05 % of isolates of *Citrobacter* were from urine sample in patients with urinary tract infections (UTI) and having indwelling catheters. Catheterization helps the bacteria to colonize urinary bladder. This may explain *Citrobacter* as an emerging cause of UTI in admitted patients in our setting.

Majority of infections in the current study were from indoor patients (76.32%) consistent with reports of other previous studies. Several well-documented nosocomial outbreaks caused by *Citrobacter* spp. resulting in colonization or disease have been described in the literature.[4,5,6]

The overall resistance rate to third generation cephalosporins was 64.47% and was significantly associated with prolonged stay in hospital, previous antibiotic therapy and invasive interventions. In the current study multidrug resistance was observed in 65/228 isolates i.e. 28.51% Citrobacter isolates. Ours being a tertiary care hospital, the principle factor in the emergence of such high level of antimicrobial resistance might be widespread use of broad spectrum antibiotics leading to selective pressure exerted on the microorganisms.

CONCLUSION:
The antimicrobial sensitivity patterns of common *Citrobacter* isolates will provide guidelines to the clinicians to start appropriate empirical antibiotic therapy depending upon the clinical scenario. This can prevent indiscriminate use of antibiotics. The importance of strict asepsis and appropriate patient care, with an extra care of i.v. lines, catheters etc. should be reinforced. Considering the correlation between the use of antibiotics, prolonged hospital stay and resistance development, the *Citrobacter* infections and their resistance pattern are likely to be decreased with the reasonable use of antibiotics and better health care.

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