



CITROBACTER- AN EMERGING HEALTHCARE ASSOCIATED PATHOGEN IN CLINICAL SAMPLES ISOLATED FROM A TERTIARY CARE CENTRE, HYDERABAD

S.Pavani¹, Shaik Meherunnisa², N.Sai Phani Vikas³

¹Associate Professor, Department of Microbiology, Osmania Medical College,

²Assistant Professor, Department of Microbiology,Osmania Medical College;

³Intern, Gandhi medical college; Dr.B.M.Shanker Venkatesh, Professor & HOD,Department of Microbiology,Osmania Medical College.

Address for Correspondence

Dr.Badike Usha

1st Year Postgraduate, Department of Microbiology,Osmania Medical College.

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ABSTRACT

The genus *Citrobacter* is distinct group of aerobic Gram negative bacilli from Enterobacteriaceae family. There is emergence of *Citrobacter* as a nosocomial pathogen in hospitalized patients. A prospective study was carried out in various samples(pus,urine,bodyfluids,blood,sputum) received to microbiology lab from September2023 to January2024. Identification of isolates was done using standard microbiological techniques. All isolates were tested for antimicrobial susceptibility on Mueller-Hinton agar by standard disc diffusion method(CLSI) guidelines. Out of 1702 samples processed in microbiology lab for aerobic culture 313 samples were culture positive for *Citrobacter*. The predominant species isolated was *Citrobacter koseri* 267(85.3%) followed by *Citrobacter freundii* 46(14.7%). The majority of isolates were from pus 148(47.2%) followed by urine 91(29%), bodyfluids 23(7.3%), blood 20(6.38%),implants 16(5.1%),sputum15(4.7%). Antibigram suggests that *Citrobacter* species are sensitive to Imipenem, Cefepime, Piptaz and resistant to Ceftazidime,Ceftriaxone,Ampicillin,CefoperazoneSulbactam,Ciprofloxacin,Cotrimoxazole,Nitrofuranto in,Gentamicin,Amoxyclav,Amikacin.

KEY WORDS: *Citrobacter*, Nosocomial pathogen.

INTRODUCTION

Nosocomial infection is a major public health concern these days and a cause of substantial mortality and morbidity for hospitalized patients. *Citrobacter* species are an important cause of health care associated infections and serious nosocomial outbreaks due to *Citrobacter* species have been reported(1,2).They can cause a wide variety of infections including urinary tract, respiratory, intra-abdominal, CNS, & bloodstream infections(3,4). Most human cases of *Citrobacter* infection are caused by *Citrobacter koseri* & *Citrobacter freundii* causing infections in immunocompromised patients. *Citrobacter koseri* is

responsible for meningitis & brain abscess in the neonatal period. *Citrobacter* species are facultative anaerobes, oxidase negative Gram negative bacilli belonging to the family Enterobacteriaceae. They are ubiquitous in nature (food, soil & water) and colonizer of human gastrointestinal tract. The genus *Citrobacter* comprises of 11 different species. Among these, *C.koseri* (previously known as *C.diversus*) & *C.freundii* are the commonest species implicated in infections. Recently the isolation of this pathogen in hospital settings is increasing & multidrug resistant strains present a challenge for clinician & clinical microbiologist.

MATERIALS & METHODS

The prospective study was conducted in the Department of Microbiology, Osmania General Hospital, Telangana during September 2023 to January 2024.

. Inclusion criteria : 1) patients with culture negative during the time of admission 2) any type of infections developed after 48 hours of admission (nosocomial) 3) patients with specimen culture positive later during the course of stay in a hospital. Specimens like blood, sputum, urine, wound swabs, pus, peritoneal fluid and swab from suction tube were collected and bacterial culture was carried out along with biochemical tests with standard procedures. Antibiotic susceptibility test was performed on Muller Hinton agar using the Kirby-Bauer disk diffusion test following manufacturer's guidelines (HiMedia Pvt. Ltd, Mumbai, India). The antibiotic disks and the concentration used were as follows; Cefoperazone Sulbactam (75/10 µg), Ceftriaxone (30 µg), Ampicillin (10 µg), Cefixime (5 µg), Ciprofloxacin (5 µg), Ceftazidime (30 µg), Co-trimoxazole (trimethoprim, 1.25/sulphamethoxazole, 22.75 µg), Nitrofurantoin (300 µg), Gentamycin (10 µg), Amoxiclav (30 µg), Amikacin (30 µg), Imipenem (10 µg), Piptaz (100/10 µg), Cefepime (30 µg). Zone of inhibition (mm) was interpreted as resistant and susceptible following the manufacturer's guidelines.

Statistical analysis

Categorical data was analyzed using Chi-Square test. Two different hypothesis were set up for examining

- 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.

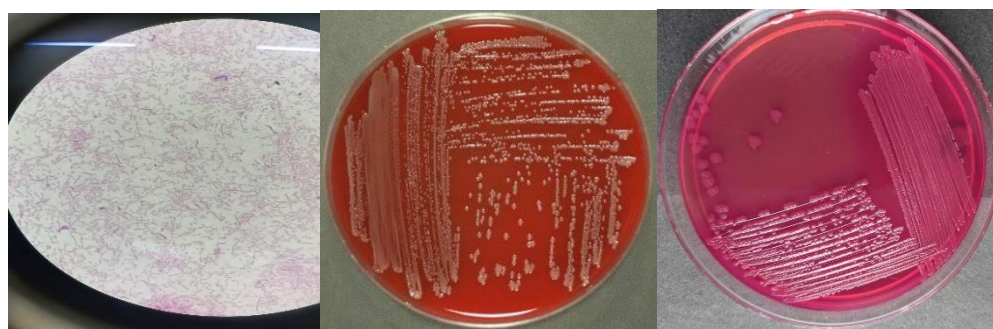


FIG 1: Microscopy and culture characteristics of *Citrobacter*.

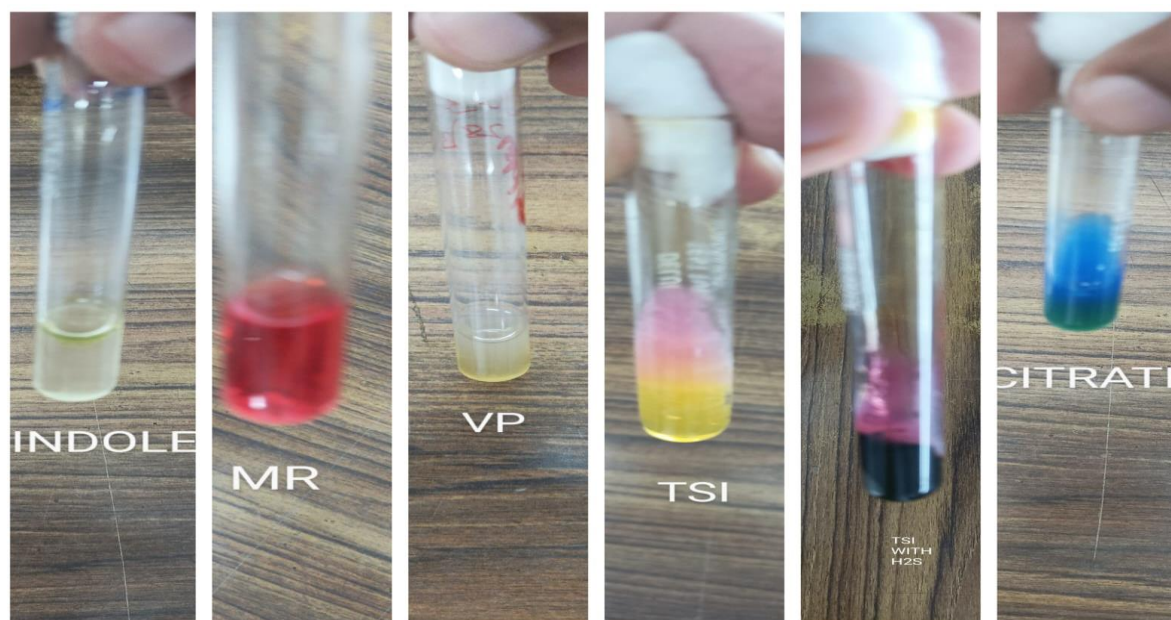


FIG 2: Biochemical Reactions of Citrobacter

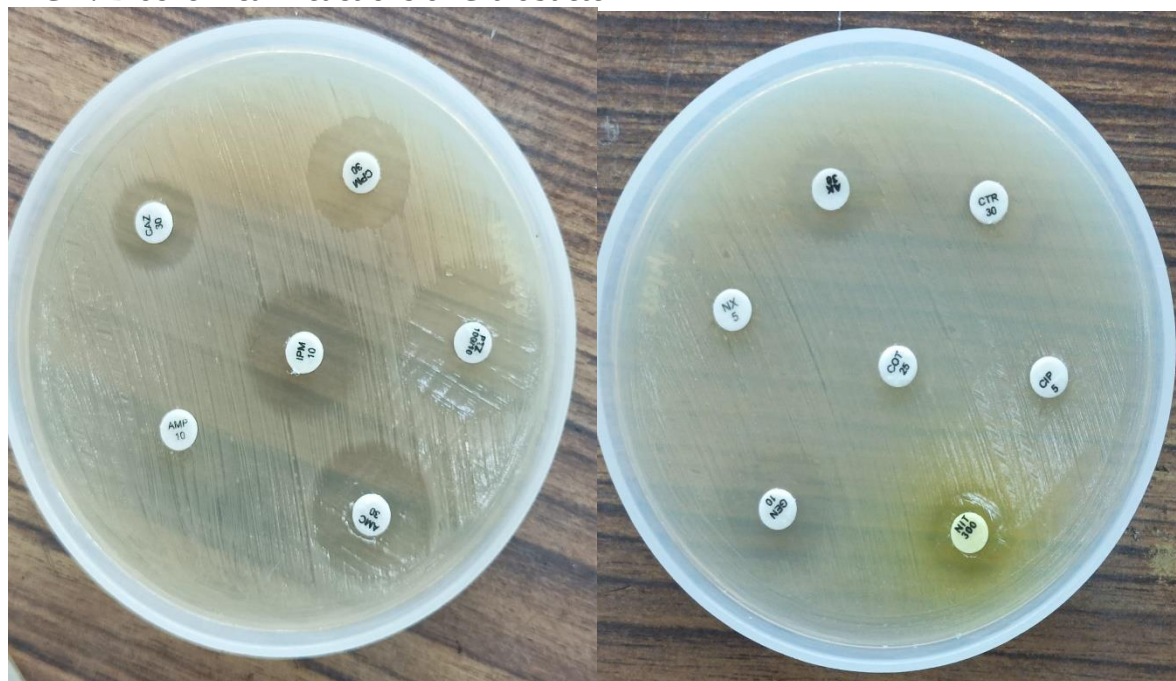


FIG 3: Antimicrobial Susceptibility Testing of Citrobacter.

RESULTS

A total of 313 isolates of *Citrobacter* species were isolated from 1702 samples. Isolates are from pus, urine, sputum, body fluids, blood and implants [Table 1]. Out of 313 *Citrobacter* species isolated, 267(85.3%) were *C. koseri* and 46 (14.7%) were *C. freundii*. Out of total 313 patients, infection was nosocomially-acquired in 255 (81.4%) patients and 58(18.5%) were community-acquired. The mean age of the patients affected were 48 years. Of all samples with *Citrobacter*, majority were received from surgery department followed by medicine department [Table 2]. Anti-biograms of *Citrobacter* isolates to 14 anti-microbial agents including imipenem, cephalosporins, aminoglycosides, and fluoroquinolones are presented in Table 4. The most effective agent against *Citrobacter* isolates was imipenem (95.8% sensitive), followed by cefepime(92%), piperacillin/tazobactam (82.7%) and amikacin (81.4%).

Amikacin was observed to be more effective than gentamicin. More than 70% of all isolates of Citrobacter were resistant to ampicillin, cefexime, amoxycylav, and ceftazidime (100%, 81%, 80.8%, and 71.8%, respectively).

TABLE 1: DISTRIBUTION OF CITROBACTER ISOLATES IN VARIOUS CLINICAL SPECIMENS

SAMPLE	NO.OF CITROBACTER ISOLATES	%
Pus	148	18.2
Urine	91	12.1
Body fluids	23	5.2
Blood	20	10.2
Implants	16	3.4
Sputum	15	3.2
Total	313	-

TABLE 2: DISTRIBUTION OF CITROBACTER ISOLATES IN VARIOUS CLINICAL DEPARTMENTS

DEPARTMENT	NO. OF CITROBACTER ISOLATES	%
Medicine	42	15.2
Surgery	183	32.2
Orthopaedic	18	3.5
Out-patient	58	11.2
Urology	9	1.5
ICU	3	0.9
Total	313	-

TABLE 3: AGE DISTRIBUTION

AGE IN YEARS	FREQUENCY	%
Below 15	22	7.02
15-25	50	15.9
26-35	40	12.7
36-55	85	27
55-75	116	37
Total	313	100

TABLE 4: ANTIBIOTIC SUSCEPTIBILITY PATTERN OF CITROBACTER ISOLATES

ANTIBIOTIC DISCS(μ g)	SUSCEPTIBLE(S)		RESISTANT(R)	
	NO.	%	NO.	%
AMPICILLIN(10 μ g)	-	-	313	100
AMOXYCLAV(30 μ g)	60	19	253	80.8
AMIKACIN(30 μ g)	255	81.4	58	18.5
COTRIMOXAZOLE(25 μ g)	163	52	150	48
CIPROFLOXACIN(5 μ g)	126	40.2	187	59.7
CEFIXIME(5 μ g)	59	18.8	254	81
CEFTAZIDIME(30 μ g)	88	28	225	71.8
CEFTRIAZONE(30 μ g)	131	42	182	58
CEFOPERAZONE SULBACTAM(75/10 μ g)	267	85	46	14.6
CEFEPIME(30 μ g)	290	92	23	7.3
PIPERACILLIN TAZOBACTAM(100/10 μ g)	259	82.7	54	17.2
IMIPENEM(10 μ g)	300	95.8	13	4.1
GENTAMYCIN(10 μ g)	84	26.8	229	73.1
NITROFURANTOIN(300 μ g)	40	43.9	51	56.04

DISCUSSION

In this study, *Citrobacter* infections were observed to be more common among elderly people. Similar results were seen in the study conducted by Shih et al. [6]. Out of 313 isolates, 267(85.3%) were *C. koseri* and 46 (14.7%) were *C. freundii*. In evaluating the serotypes of the *Citrobacter*, *C. koseri* has been reported as most predominant isolate in most of the studies[7,8,9]. Similarly, we found *C. koseri* in 85% of our patients; however, Gill, et al.,[10] have reported *C. freundii* to be the commonest species isolated. In majority of the patients, infections were hospital-acquired (81.4%), which is consistent with other studies[7,11,12]. 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 (47.2%), followed by urine (29%), body fluids (7.3%), blood (6.38%),implants(5.1%),sputum(4.7%). Similarly, in the study conducted by Shetty, et al., [13] majority of the isolates were recovered from pus. However, in the study conducted by Mohanty, et al., [14] majority of the isolates were from urine, followed by sputum and pus.

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 313 isolates of *Citrobacter*, maximum numbers (100%) were resistant to ampicillin and lowest to imipenem (4.1%). Resistance was observed to commonly used antibiotics such as ampicillin, cefexime,amoxiclav,ceftazidime, gentamicin,ciprofloxacin & co-trimoxazole. The greater prevalence of resistance to commonly used antibiotics has also been reported by other studies.[7,8,9] 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 (cefexime, ceftazidime) and piperacillin-tazobactam were 81%,71.8%,17.2% respectively.

CONCLUSION

The emergence of *Citrobacter* species as nosocomial pathogen which is resistant to routine antibiotics is alarming. Therefore selective and judicious usage of drugs and continuous evaluation of antimicrobial susceptibility patterns helps in preventing further emergence of drug resistance & nosocomial infections.

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