Journal of Associated Medical Sciences 2023; 56 (1): 49-53

JAMS
Journal of Associated Medical Sciences

Scopus indexed & Thai-Journal Citation Index Centre (TCI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

In vitro resistance pattern of Citrobacter infections: A retrospective study

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ARTICLE INFO

Article history:
Received 6 July 2022
Accepted as revised 12 September 2022
Available online 21 September 2022

Keywords:
Citrobacter spp, Citrobacter fruendii, Citrobacter koseri, extended spectrum beta lactamase positive, carbapenem resistance

ABSTRACT

Background: Citrobacter infections are associated with a high mortality rate of about 33-48% if infected patients develop bacteremia. This is partly due to high prevalence of intrinsic resistance, extended spectrum beta lactamases and inducible chromosomal Amp C beta lactamases in *Citrobacter* spp. thus limiting the therapeutic options. We undertook this study to throw light on the current scenario of infection with this organism.

Materials and methods: This retrospective study was conducted over a period of 1 year in Microbiology laboratory of a tertiary care teaching hospital in Eastern part of India. From various samples, 146 clinically significant *Citrobacter* spp. identified by standard biochemical tests and susceptibility testing performed by Kirby Bauer's disc diffusion method were included in this study.

Results: Majority of patients age ranged of 41-50 year. The highest number (70/146, 47.9%) of *Citrobacter* spp. was isolated from pus and wound swabs followed by urine (46/146, 31.5%) and out of these, 51.4% strains were of *Citrobacter koseri* whereas 48.6% were of *Citrobacter fruendii*. Of *Citrobacter* isolates 36.6% were ESBL producers. They showed 54.1%, 37.9%, 47.1%, 39.8% and 58.5% resistance to imipenem, netilmycin, piperacillin tazobactam, minocycline and levofloxacin respectively. We also found 9.6% and 28.4% strains of *Citrobacter* spp. being resistant to colistin and tigecycline respectively.

Conclusion: *Citrobacter* spp. showed high degree of resistance to carbapenem and there were colistin resistance strains as well. This study reiterates the emerging resistance in these supposedly low virulence microbes which may pose future challenge in infection control activities.

Introduction

Genus *Citrobacter*, a member of family Enterobacterale has about 11 species; *Citrobacter fruendii* and *Citrobacter koseri* being the organisms of paramount clinical significance. *Citrobacter* spp. which was previously regarded as a contaminant or colonizer, is presently being associated in many infections particularly in neonates, immunocompromised adults as well as serious nosocomial outbreaks.[1,2] Various infections like urinary tract infections, wound infections, respiratory tract infections, bone infections, peritonitis, endocarditis, meningitis and bacteremia are associated with this organism.[3] Different studies attribute a 6.8% mortality in case of various Citrobacter infections patients, which can significantly increase to 17.8-56% when there is associated bacteremia.[4] The higher mortality rate may also be due to inappropriate empiric therapy of the infections caused by them as this organism is often resistant to many routinely used antibiotics

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doi: 10.12982/JAMS.2023.007
E-ISSN: 2539-6056

like ampicillin-sulbactam, amoxicillin-clavulanic acid, 1st and 2nd generation cephalosporins and cephamycins.[5] These strains are potent carriers of Amp-C β-lactamase, broad-spectrum β-lactamase, extended-spectrum β-lactamase (ESBL), plasmid-mediated quinolone resistance determinants and even carbapenemase.[6] Recent emergence of multidrug-resistant strains of *Citrobacter* has resulted in longer hospital stays and higher antibiotic costs.[6] So this study was undertaken to enlighten us regarding on the current scenario of infection with this understated organism especially their pattern of drug resistance.

Materials and methods

This retrospective study was conducted in a 1000 bedded tertiary care teaching hospital in Odisha, Eastern part of India. *Citrobacter* spp. grown on aerobic cultures from various samples received in the central laboratory over a period of 1 year (March 2021 to February 2022) was considered. Various specimens like blood, bile, cerebrospinal fluid, urine, pus, wound swabs, high vaginal swabs, and body fluids were collected from patients and transported with utmost aseptic precautions in accordance with standard microbiological protocol.[7] All samples on receipt in the lab were inoculated on blood agar and Mac Conkey agar plates while urine sample was put on cystine lactose electrolyte deficient (CLED) agar. Blood and bile culture was done in BacT Alert, Biomerieux automated system which was plated on blood and Mac Conkey agar plates after being flagged positive by the instrument. Colonies received on the agar plate was examined following overnight incubation and interpreted as per clinical and Gram staining findings. Central line tip culture was considered when the same organism was isolated from blood culture as well with similar antibiotic sensitivity pattern. For respiratory samples the culture was considered when associated with correlating findings on Gram stain along with clinical evidence of infection. Only significant colony counts received on semi-quantitative in urine and respiratory culture (colony count >10^5 CFU/mL) were processes further.

Identification

Clinically significant bacteria isolated from different samples were identified by Gram staining and conventional biochemical tests as per the standard protocol.[7] The non-lactose or late lactose fermenting citrate utilizing Gram negative motile bacilli isolates with catalase, methyl red, and ortho nitro phenyl pyruvic acid test positive and oxidase, voges proskauer, phenyl pyruvic acid and lysine tests negative, were identified as genus *Citrobacter*. *Citrobacter koseri* (*C. koseri*) and *Citrobacter fruendii* (*C. freundii*) were distinguished by indole, sugar fermentation and reaction on triple sugar iron agar media. *C. koseri* is indole positive, utilizes malonate and adonitol and is K/A +G and without H_2S on TSI slant.[7]

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was performed by the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar and interpreted as per Clinical Laboratory Standard Institute (CLSI) guidelines.[8] The antibiotics tested were as follows (potency in µg/disc): amoxycillin-clavulanic acid (30/10), ceftriaxone (30), cefoperazone-sulbactam (75/30), ceftazidime (30), cefepime (30), piperacillin-tazobactam (100/10), imipenem (10), meropenem (10), amikacin (30), netilmycin (30), levofloxacin (5), and ofloxacin (5) minocycline (30), tigecycline(15).(Hi-Media Labs). tigecycline zone diameter was interpreted as per US FDA approved method.[9]

Quality control

Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *Klebsiella pneumoniae* ATCC 700603 were used as quality control strains for all the procedures. Antibiotic sensitivity of colistin was done using colistin broth disk elution method and interpreted as CLSI guidelines for Enterobacterales.[8]

ESBL detection

Extended spectrum β-lactamase (ESBL) screening was done for all strains by double disk potentiation test using ceftazidime (CAZ) (30 µg) and combination of ceftazidime and clavulanic acid (30/10 µg) (CAC) disk (Himedia Labs) according to the CLSI guidelines.[8]

The data was retrieved for all the samples from laboratory register. The patient's age, gender, diagnosis, site of sample collection and antibiotic sensitivity pattern were noted. The data thus retrieved was entered in MS Excel and analyzed by basic statistical methods. Probability value (*p* value) was calculated by Graph pad prism and was considered significant at $p<0.05$.

Results

During the study period out of 29,894 clinical samples processed in laboratory, 3221 (10.8%) were culture positive of which, 2024 were *Enterobactarales* alone. Among the Enterobactarale, 146 (7.2%) were clinically significant *Citrobacter* spp. These samples were from 146 patients of whom 98 were males and 48 were females. Majority of patients, 34 and 31 cases respectively, belonged to the age group of 41-50 and 51-60.

The highest number 70/146 (47.95%) of *Citrobacter* spp. was isolated from pus and wound swabs followed by 46/146 (31.5%) from urine. In the present study, 75 (51.4%) were *C. koseri* strains, whereas 71 (48.6%) were *C. fruendii* strains. There was no significant difference in isolation rate of both the species in any of the samples except for bile where all the isolates were *C. fruendii* (Table 1), 36.6% of *Citrobacter* spp. were ESBL positive. Most of ESBL positive *Citrobacter* spp. (50/51 98%) were isolated from pus and wound swabs, respiratory samples, and urine specimen. A negligible percentage was contributed by bile and other sterile body fluids (Table 1).

Among the betalactam (BL) antibiotics, *Citrobacter* spp. showed very low level of sensitivity to 3rd and 4th generation drugs (37.9% and 26.2% to ceftriaxone and cefepime respectively). Cefoperazone-sulbactam and piperacillin- tazobactam were effective in around 52% of Citrobacter infections. These organisms were rather more sensitive to aminoglycosides (69.8% sensitivity to amikacin and 62.1% sensitivity to netilmycin) than the beta lactam antibiotics. Of the quinolones, these were more sensitive to ofloxacin (57%) than levofloxacin (41.5%). But in this study, the strains of *Citrobacter* spp. were resistant to carbapenems in more than 50% cases

with susceptibility to meropenem is slightly better than that for imipenem. The strains showed maximum sensitivity to the reserve drugs, tigecycline and colistin. But cases of colistin resistant *Citrobacter* were also encountered in this study. *C. koseri* strains were more sensitive than *C. fruendii* to most of the tested antibiotics. This difference in pattern of sensitivity is significant with $p<0.05$ for netilmycin, cefepime, tigecycline and colistin (Table 2).

Table 1 Distribution of Citrobacter spp. and their ESBL pattern.

Sample	Total Number (%)	ESBL positive No (%)	*C. fruendii* (%)	*C. Koseri* (%)
Respiratory samples*	23 (15.75)	13 (25.49)	11 (15.49)	12 (0.16)
Pus and wound swabs	70 (47.95)	21 (41.18)	34 (47.89)	36 (48)
Urine	46 (31.5)	16 (31.37)	21 (29.58)	25 (33.33)
Bile	4 (2.74)	0 (0)	4 (5.63)	0 (0)
Lacrimal discharge swab	1 (0.69)	0 (0)	0 (0)	1 (1.33)
Central line tip	1 (0.69)	0 (0)	1 (1.4)	0 0 (0)
Pancreatic fluid	1 (0.69)	1 (1.96)	00 (0)	1 (1.33)
Total	146	51 (36.6)	71(48.6)	75 (51.4)

Note: **Respiratory samples include tracheal aspirate and bronchoalveolar lavage fluid.*

Table 2 Susceptibility pattern (in %) of the different *Citrobacter* spp. to antibiotics.

	AMC	AK	NET	CTR	CPM	CFS	PIT	OF	LE	MRP	IPM	MI	TGC	CL
Citrobacter spp	57.1	69.8	62.1	37.9	26.2	53.2	52.9	57	41.5	50	45.9	60.2	71.6	90.4
Citrobacter fruendii	-*	74.2	50	29.8	2.8	47.5	49.2	54.5	36.4	45.8	45.0	50.8	52.9	84.4
Citrobacter koseri	57.1	65.6	72.6	44.6	57.7	58.9	56.7	58.9	46.9	53.7	47.1	73.3	87.9	96.5
p value (at <0.05)	.-	0.29	0.012	0.12	.00001	0.31	0.412	0.66	0.39	0.37	0.83	0.018	0.00005	0.029

Note: **Intrinsic resistance to these antibiotics, AMC: amoxycillinclavulanic acid, AK: amikacin, NET: netilmycin, CTR: ceftriaxone, CPM: cefepime, CFS: cefoperazone tazobactam, PIT: piperacillin tazobactam, OF: ofloxacin, LE: levofloxacin, MRP: meropenem, IPM: imipenem, MI: minocycline, TGC: tigecycline, CL: colistin.*

Discussion

Citrobacter spp., one of the members of family Enterobacterale is a facultative anaerobic, motile, gram-negative bacillus. Only 6% of the infections attributed to family Enterobacterale is caused by this genus.[10] In the present study *Citrobacter* spp. accounted for 7.2% of the infection among various Enterobacterale.

It can cause infections like Urinary tract infection, bacteremia, meningitis, pneumonia, osteomyelitis, peritonitis, and endocarditis when the host defenses are breached.[11] The bacteria in the present study were most isolated from pus and urine. This is similar to findings by Mohanty *et al*[12] but in contrast other studies by Khanna *et al*.[11] and Samonis *et al*.[13] report pus as the commonest sample.

Citrobacter bacteremia is associated with a high mortality rate between 33% and 48%.[5,14] But in the present study, no case of bacteremia was detected as supported from studies by other workers.[12,15] This may be due to the fact that in this study only a tiny proportion of samples belonged to extreme age groups where the bacteremia is classically seen.

Citrobacter spp. causes of infections in neonates particularly in NICU.[16] In a study by Lipsky *et al*.[9] most of the infected patients were elderly, and nearly all had significant underlying illnesses. But in the present study 41-50 year was the most common age group of isolation of this organism. Male predominance was seen in this study which had been reported by other studies also.[12] *C. freundii* and *C. koseri* are the two most common pathogens and infections can be acquired from exogenous as well as endogenous sources, being ubiquitous in nature as a saprophyte in soil and sewage and as a commensal in human gastrointestinal tract. In the present study although *C.koseri* (51.4%) outnumbered *C. fruendii* (48.6%), there was no significant difference in rate of isolation of both the species. In the study done by Metri *et al*.[17] from Southern India, of the 563 isolates of *Citrobacter* spp., *C. koseri* was in 70% of samples. Similarly, in another study[12] from Northern India, *C. koseri* (90.2%) far exceeded *C. freundii* (9.8%) cases. But contrasting to these Mohan *et al*.[18] found *C. fruendii* as the predominant species and majority being isolated from pus samples. Mohan *et al*.[18] also isolated rarer species like *C. farmeri* (8.2%), *C. braakii* (5.4%), *C. werkmanii* (5.4%) and *C. gilleni* (3.4%) which were not reported in other studies. In a previous study, these fewer common species have also been proved as potential pathogens.[19]

Prevalence of ESBL in *Citrobacter* spp. worldwide was reported to be 0.5-36%.[20,21] In this study, 36.6% of *Citrobacter* isolates were ESBL producers. But this is strikingly different from few studies from India where the prevalence of ESBL is much higher; 61.6% in study by Khanna *et al*. and

80.9% in study by Praharaj *et al*.[11,22] It is generally recognized that patients infected with ESBL-producing organisms are at risk for poor outcome.

These organisms are intrinsically resistant to multiple antibiotics thus narrowing their treatment options. Both the *Citrobacter* spp. are resistant to ampicillin while *C. fruendii* is resistant to ampicillin-sulbactam, amoxicillin-clavulanic acid, 1st and 2nd generation cephalosporins and cephamycins. In this study, these antibiotics were reported as resistant upon confirming identification and excluded from further analysis. Among the other tested antibiotics, sensitivity to aminoglycosides was around 60%, with 69.8% for amikacin and 57.1% for netilmycin. Sensitivity for third and fourth generation cephalosporins (37.9% for ceftriaxone and 26.2% for cefepime) had a very dismal performance. Among the flouroquinolones tested, ofloxacin was more sensitive (57%) than levofloxacin (41.5%). Similar findings have also been noted by other studies.[12,23]

Other previous studies have stated carbapenems and beta-lactam/beta-lactamase inhibitor combinations appear to be promising alternatives.[24,25,26] But present study negates this finding as in this tertiary care set up, carbapenem resistance was seen in about 50% cases. A similar lower degree of susceptibility to carbapenems was noted in about 80% cases in another study.[12] Among beta lactam combination agents cefoperazone-sulbactam and piperacillin-tazobactam were effective with sensitivity in 53.2% and 52.9% strains respectively. In this study, there were strains where resistance to the known reserve drugs tigecycline and colistin was seen. Colistin resistance in this species is not yet reported in literature. Further analysis of the colistin resistant strains was not possible because of the retrospective nature of this work which may be considered as a limitation of the study.

Citrobacter is thought of as a commensal flora of intestine but presence of this low virulence yet resistant bacteria in hospitalized patients may complicate surveillance and infection control efforts.[5] Present study illustrates the high degree of drug resistance in this organism. There is a high prevalence of carbapenem resistance in *Citrobacter* spp., which is a cause of concern. Although other Gram negative Enterobacterales, *E. coli* and *Klebsiella* spp. have been major multi drug resistant pathogens, but probably this genus is not far behind. Colistin resistance is being noted in strains of *Klebsiella* spp. but further molecular studies are needed to characterize the colistin resistance in this genus. As *Citrobacter* is also a part of fecal flora further surveillance of the resistance genes to block dissemination of the organism in hospital environment is the need of the hour.

Acknowledgments

The Authors are highly grateful to Chairman of Siksha O Anusandhan (deemed to be) University Prof. M.R Nayak for providing all the support during the work. The authors are also thankful to the students of the IMS & SUM Hospital for participating in this study.

Conflicts of interest

The authors declare there is no conflict of interest among them.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or notfor- profit sectors.

Ethics Statement

No ethical approval required in this Study.

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