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Dr. Gulsiv Nair
Assistant Professor, Department of Microbiology, Al Azhar Medical College and Superspecialty Hospital, Thodupuzha, Kerala, India

Dr. M Lakshminarayana
Statistician Cum Assistant Professor, Department of Community Medicine, Hassan Institute of Medical Sciences, Hassan, Karnataka, India

Dr. Lavanya Nagaraj
Professor and Head, Department of Pharmacology, Sree Narayana Institute of Medical Science, Chalakka, Ernakulam, Kerala, India

Dr. Naveen Kumar M
Professor and Head, Department of Pharmacology Al Azhar Medical College and Superspecialty Hospital, Thodupuzha, Kerala, India

Dr. Thilak SA
Professor, Department of Community Medicine, Al Azhar Medical College and Superspecialty Hospital, Thodupuzha, Kerala, India

Corresponding Author:
Dr. Naveen Kumar M
Professor and Head, Department of Pharmacology, Al Azhar Medical College and Superspecialty Hospital, Thodupuzha, Kerala, India

Urinary infections and Citrobacter: An unpleasant scenario

Gulsiv Nair, M Lakshminarayana, Lavanya Nagaraj, Naveen Kumar M and Thilak SA

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Abstract
Urinary infections due to Citrobacter is very common, and rising antibiotic resistance complicates the situation.
Urine samples received in the microbiology laboratory at Al Azhar Medical College from October 1st, 2018 to September 30th, 2019, were processed. Isolates identified as Citrobacter were studied.
3207 urine samples were processed, of which 2481 showed significant growth. 124 Citrobacter isolates were identified, of which 72 were *C. koseri* and 52 were *C. freundii*.
For *C. koseri*, susceptibility to co-amoxyclav, 3rd generation cephalosporins and co-trimoxazole ranged from 60-70%. Susceptibilities to fluoroquinolones and nitrofurantoin was 76-77%.
For *C. freundii*, susceptibility to 3rd generation cephalosporins was around 50%. Susceptibilities to co-trimoxazole and fluoroquinolones was around 60% and for nitrofurantoin, it was 67.31%.
Susceptibility for both was good for aminoglycosides and piperacillin-tazobactam. Nearly 45% isolates were resistant to 3 or more antibiotic classes.
Our data shows that upto a third of urinary infections due to Citrobacter can end up with treatment failure with the usual empirical antibiotics, and the management must be tailored based on evidence from culture and sensitivity reports.

Keywords: citrobacter, urinary infection, antibiotic resistance, cephalosporins, fluoroquinolones

Introduction
Citrobacter species are Gram-negative bacilli belonging to the order Enterobacterales [1]. They are facultative anaerobic and are typically motile. They are cosmopolitan and are commonly found in water, soil, food, and the intestinal tracts of animals and humans [2]. There are eleven genomospecies included in the tribe Citrobactereae [1].
Many Citrobacter infections are nosocomially acquired; but can also be community acquired. The infection may occur as sporadic cases or nosocomial outbreaks [2]. Citrobacter can cause a wide spectrum of infections in humans, such as infections in the urinary tract (most commonly), respiratory tract, wounds, bone, peritoneum, endocardium, meninges, and bloodstream etc [1].
Citrobacter species accounted for 3.31% of all GNB isolates in a 3 year study in Europe [3].
Urinary tract infection (UTI) continues to be the most common infection diagnosed in outpatients as well as in hospitalized patients [4].
Hospital-acquired urinary tract infection (UTI) is the commonest health care associated infection (nosocomial infection) accounting for 35-40% of the total health care infections, thus posing a grave medical problem [5].
Citrobacter is well documented as a frequent urinary pathogen in hospitalised patients [6].
In fact, Metri *et al.* found Citrobacter spp. as the third most common urinary pathogen accounting for 9.4% of the total isolates [7].
The isolation of this organism was associated with catheterization, genitourinary instrumentation, or obstructive uropathy. Also, the age group most affected was that of elderly hospitalized patients, especially males [8].
A study found that prevalence of UTI caused by Citrobacter spp. was 12% patients in 1961, and since then has been increasing [9].
Citrobacter koseri (previously *Citrobacter diversus*) and *Citrobacter freundii* are two of the most commonly isolated genomospecies [10, 11, 12, 13].

Other species include *C. braakii*, *C. amalonaticus*, *C. youngae*, *C. sedlakii*, *C. farmeri* (8.2%), *C. werkmanii* and *C. gilleni* [14, 15, 16].

Both *C. koseri* and *C. freundii* have been implicated in causing a variety of infections, including hospital acquired infections, and both can act as opportunistic pathogens. To complicate matters further, both are able to acquire multiple drug resistance rather easily [3, 17].

Drug resistance was observed to be relatively more in *C. freundii* as compared to *C. koseri* in many studies [14, 18, 19] while the reverse was found to be true in by Mohanty *et al.* [20]. Lee *et al.*, however, didn't find much of a difference in the resistance patterns of the two species [21].

Lee *et al.*, in a study on Citrobacter bacteremia, didn't find much change in the rate of susceptibility of Citrobacter over a 11-year period [21]. However, Wang *et al.* did find a marked reduction in the susceptibility to fluoroquinolones and aminoglycosides over a similar time period [22].

This study aims to assess the magnitude of the health problem due to Citrobacter UTI, and determine the efficacy of commonly used antibiotics therein.

Methodology

A retrospective analysis was carried out in patients with urine culture positive for Citrobacter species from patients coming to Al Azhar Medical College and Superspeciality Hospital, Thodupuzha, Kerala, which is a tertiary care centre, from October 1st, 2018 to September 30th, 2019.

The present study was cleared by the Dissertation Review Committee without any ethical, financial or scientific concerns being raised.

The samples were cultured on blood and MacConkey agar and incubated at 35 °C -37 °C for 18 hours. The samples which showed significant growth i.e., satisfied criteria for significant bacteriuria, were processed further. Growth on the culture plates were carefully examined for colony morphology, and identification was done with standard microbiological techniques like Gram staining, catalase and oxidase tests, standard biochemical reactions. *Staphylococcus aureus* (ATCC® 25923), *E. coli* (ATCC® 25922) and *Pseudomonas aeruginosa* (ATCC® 27853) (ATCC, Virginia, U.S) were used as controls.

Antibiotic susceptibility testing by Kirby-Bauer disc diffusion method was done according to CLSI recommendations on Mueller Hinton Agar [23].

Antibiotic discs [HiMedia, Mumbai, India] of the following strengths were used –Amoxycillin-clavulanate (20/10 µg), Cefuroxime (30 µg), Cefixime (5 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Co-trimoxazole (1.25/23.75 µg), Nitrofurantoin (300 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Gentamicin (10 µg), Amikacin (30 µg), Piperacillin-Tazobactam (100/10 µg).

Results and Discussion

The current study was done on urine samples received in the microbiology laboratory at Al Azhar Medical College over a period of one year. A total of 3207 samples of urine were processed between October 1st, 2018 to September 30th, 2019.

Of these, 480 had either no growth, or had growth deemed not significant.

Cultures from 207 samples grew more than 3 types of colonies and were reported as such. These were not processed further.

591 samples grew Gram positive cocci.

1890 samples grew Gram negative bacilli, of which 124 isolates were identified as Citrobacter species.

39 samples grew Candida species.

The identification and susceptibility pattern of isolates other than Citrobacter is beyond the scope of this study.

Table 1: Distribution of isolates

Organism	No. of isolates
No growth	480
Mixed growth	207
Gram positive cocci	591
Gram negative bacilli	1890
Candida species	39

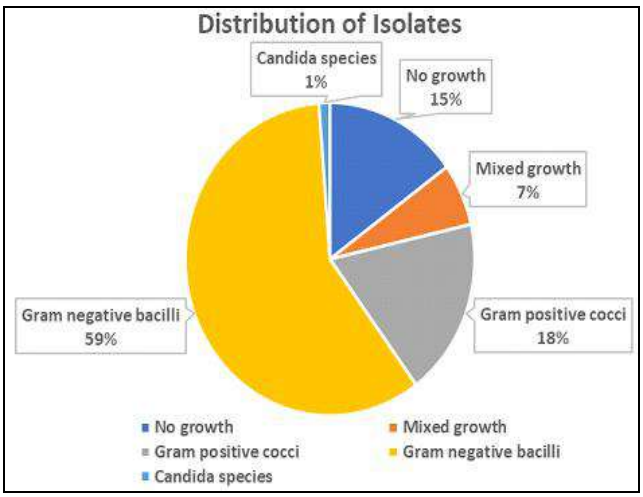


Fig 1: Distribution of isolates

A total of 124 isolates of Citrobacter were obtained. The prevalence of UTI due to Citrobacter spp in our study was 4.998%, which is similar to the findings of Sami *et al.* [4], but about half that found by Metri *et al.* [7].

Of these, 72 (about 58%) were identified as *Citrobacter koseri*, and 52 (nearly 42%) as *Citrobacter freundii*. This is similar to the results of Metri *et al.* [7], even though the difference is not as marked

Two of the samples from which *Citrobacter freundii* was isolated, also yielded other organisms in significant counts-namely *E. coli* and Enterococcus species.

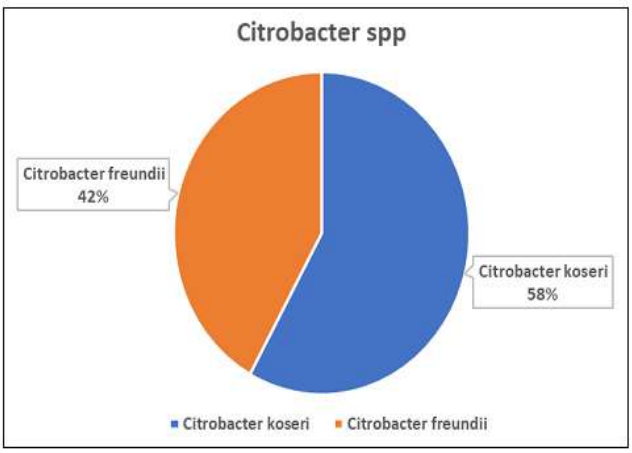


Fig 2: Distribution of Citrobacter species

Table 2: Age wise distribution

Age	0-20	21-40	41-60	61-80	>80
Nos	30	42	22	21	09

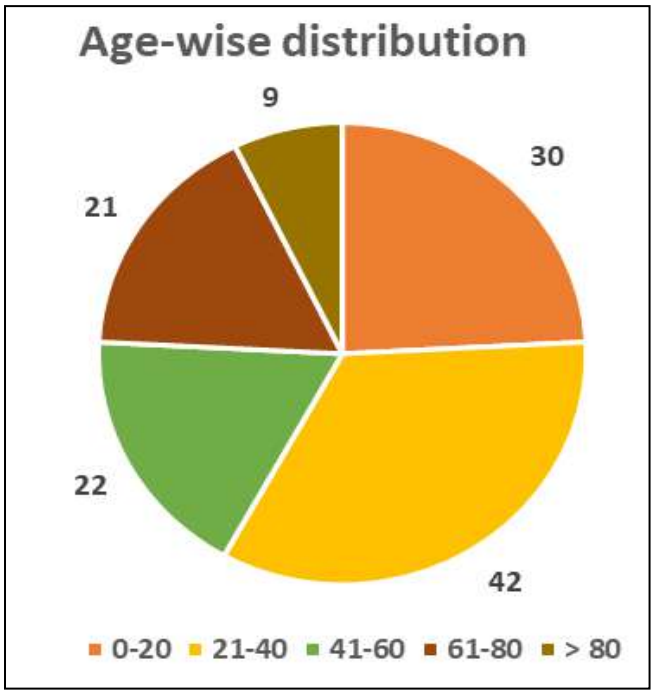


Fig 3: Age wise distribution

The maximum number of patients were from the young adult age group (21-40). This is similar to the findings of Sami *et al*. [4], although it may be more a reflection of the prevalence of UTI in this age group, rather than a characteristic of the organism per se. This same fact may also apply to the fact that we found that the majority were isolated from females (as mentioned below), again reflecting the higher rate of UTI in females.

Of the 124 Citrobacter isolates, samples from male patients accounted for 29.84% (37 of 124), while those from female patients accounted for 70.16% (87 of 124) (Figure 4).

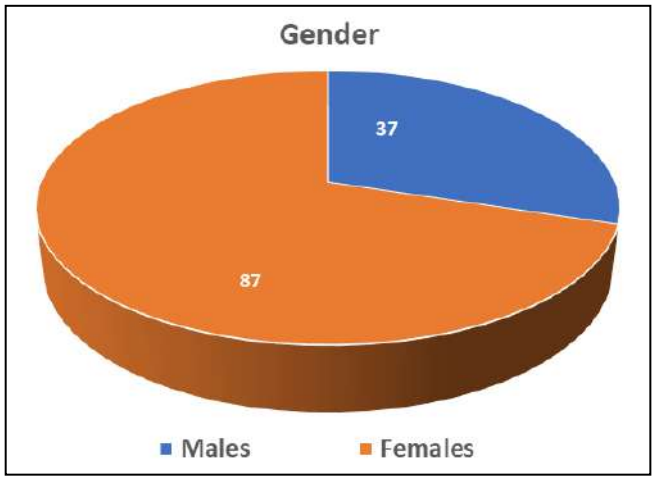


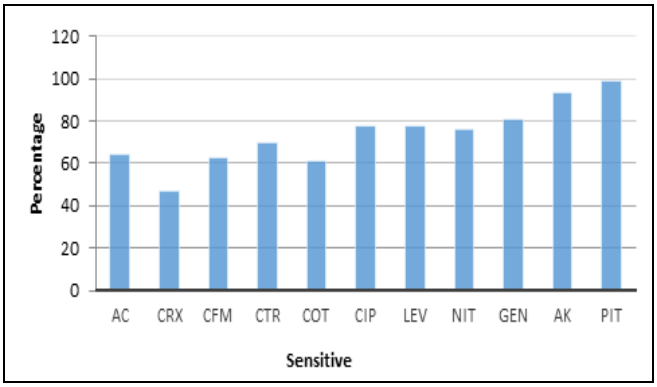
Fig 4: Gender distribution of Patients (Females- 87; Males- 37)

Antimicrobial Susceptibility Pattern

The susceptibility patterns of *C. koseri* and *C. freundii* have been considered separately. Furthermore, Citrobacter are known to have intrinsic resistance to various antibiotics. For example, *Citrobacter koseri* is resistant to Ampicillin and Ticarcillin, while *Citrobacter freundii* is resistant to Ampicillin, Amoxicillin-clavulanate, Ampicillin-sulbactam, cephamycins, and first and second generation cephalosporins [23]. These have, therefore, not been considered here.

Table 3: *Citrobacter koseri* susceptibility

Antibiotic	Susceptibility
Amoxicillin- Clavulanate	46/72 (63.89%)
Cefuroxime	34/72 (47.22%)
Cefixime	45/72 (62.5%)
Ceftriaxone	50/72 (69.44%)
Co-trimoxazole	44/72 (61.11%)
Ciprofloxacin	56/72 (77.78%)
Norfloxacin	56/72 (77.78%)
Nitrofurantoin	55/72 (76.39%)
Gentamicin	58/72 (80.56%)
Amikacin	67/72 (93.06%)
Piperacillin-Tazobactam	71/72 (98.61%)



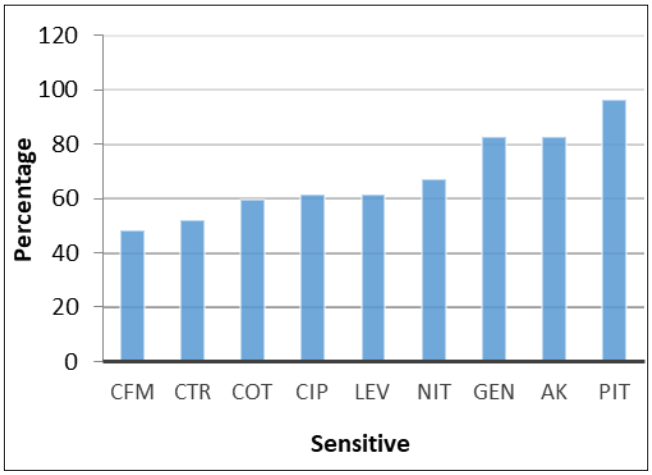
*AC- Amoxicillin-Clavulanic acid, CRX- Cefuroxime, CFM- Cefixime, CTR- Ceftriaxone, COT- Co-trimoxazole, CIP- Ciprofloxacin, LEVO- Levofloxacin, NIT- Nitrofurantoin, GEN- Gentamicin, AK- Amikacin, PIT- Piperacillin- Tazobactam

Fig 5: *Citrobacter koseri* susceptibility

The isolates of *Citrobacter koseri* from urine samples in this study shows a poor picture with respect to treatment with penicillins, cephalosporins and co-trimoxazole. The beta lactam-beta lactamase inhibitor (BL-BLI) combinations, as also the cephalosporins showed sensitivities of less than 70%. In other words, a third of the UTIs due to *C. koseri* with these antibiotics will lead to treatment failure. Three fourths of the isolates were susceptible to the oral fluoroquinolones and also nitrofurantoin. The isolates were fairly susceptible to the aminoglycosides. Only one isolate was resistant to Piperacillin-tazobactam. It was found to be sensitive to carbapenems on further processing. 23 of 72 (31.94%) were resistant to three or more classes of antibiotics.

Table 4: *Citrobacter freundii* susceptibility

Antibiotic	Susceptibility
Cefixime	25/52 (48.08%)
Ceftriaxone	27/52 (51.92%)
Co-trimoxazole	31/52 (59.61%)
Ciprofloxacin	32/52 (61.54%)
Norfloxacin	32/52 (61.54%)
Nitrofurantoin	35/52 (67.31%)
Gentamicin	43/52 (82.69%)
Amikacin	43/52 (82.69%)
Piperacillin-Tazobactam	50/52 (96.15%)



* CFM- Cefixime, CTR- Ceftriaxone, COT- Co-trimoxazole, CIP- Ciprofloxacin, LEVO- Levofloxacin, NIT- Nitrofurantoin, GEN- Gentamicin, AK- Amikacin, PIT- Piperacillin- Tazobactam

Fig 6: *Citrobacter freundii* susceptibility

The picture which emerges on examining the susceptibility pattern of the *C. freundii* isolates appears to be even more dismal. As mentioned earlier, *Citrobacter freundii* is inherently resistant to few of the antibiotics used commonly for UTIs like penicillins, BL-BLIs, and first and second generation cephalosporins. We found that only around half of the isolates were susceptible to the cephalosporins. More than a third of the isolates were resistant to co-trimoxazole, the oral fluoroquinolones, and nitrofurantoin. To put it in perspective, none of the oral antibiotics tested here would have been effective for a third of the patients with *C. freundii* UTI.

Only the parenteral antibiotics showed fairly good susceptibility.

Only two isolates were resistant to Piperacillin-tazobactam. As with *C.koseri*, these were also found to be susceptible to carbapenems on further processing.

32 of 52 (61.54%) were resistant to three or more classes of antibiotics.

Overall, the Citrobacter isolates showed good susceptibility to the aminoglycosides and piperacillin-tazobactam, similar to the findings in quite a few studies [4, 14, 21, 24].

Susceptibility to penicillins and cephalosporins was poor, similar to the findings of Mohan *et al.* [16] and Shetty *et al.* [25]. Poor susceptibility was also seen for co-trimoxazole. This was very unlike the study by Maraki *et al.* [26] in Greece as recently as 2017, who found quite high susceptibility to cephalosporins and co-trimoxazole. This is an example showing that data from Western countries may not entirely applicable to the Indian subcontinent.

55 of 124 (44.35%) were resistant to three or more classes of antibiotics, similar to findings by Gross *et al.* [17], but thankfully lesser than what was found by Liu *et al.* [15], Mohan *et al.* [16] and Gupta *et al.* [18].

Conclusion

Citrobacter is quite a common uropathogen in the hospital setting. Our findings are additional evidence demonstrating the rise in antimicrobial resistance in Citrobacter.

As most of the cases of UTI are treated with oral antibiotics, it is of critical importance to tailor the management according to laboratory evidence by culture and sensitivity, in order to avoid treatment failure, and to *prevent* contributing to further increase in antibiotic resistance as far as possible.

Conflict of Interest

The authors declare no conflict of interest.

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