

ANTIBIOTIC SUSCEPTIBILITY OF CITROBACTER KOSERI IN NEONATAL INFECTIONS

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Abstract

The background is that *Citrobacter koseri* is a recently recognized opportunistic pathogen of NICUs, which causes meningitis, brain abscesses and sepsis. It is intrinsically resistant to some antibiotics and the ability to develop ESBLs (Extended-Spectrum Beta-lactamases) makes it harder to treat. Methods: Antibiotic susceptibility profile of *C. koseri* isolated from neonatal infections was determined. Design: Cross-sectional study in a tertiary care NICU was performed for a period of 6 months. A total of 40 *C. koseri* isolates were obtained from newborns (0-28 days old) with blood, CSF, and urine samples. Gram staining, biochemical tests (catalase, oxidase, citrate, TSI) and API 20E system were used for identification. Susceptibility was determined by performing Kirby-Bauer disc diffusion on Mueller-Hinton agar as per CLSI 2024. 17 antibiotics belonging to 8 different classes were tested. The double-disc synergy test (DDST) was used to confirm the production of ESBL. Results: All isolates were meropenem (100%), imipenem (100%) and amikacin (95%) susceptible. The highest level of resistance was noted with ampicillin (100%), amoxicillin-clavulanate (85%), cefazolin (82.5%) and cefuroxime (77.5%). 27.5% of the isolates were found to produce ESBL. Thirty-two and a half percent of isolates were multidrug resistant (MDR). Conclusions: *C. koseri* is reliably treated with carbapenems and amikacin in neonates. The emergence of ESBL and MDR is increasing the need for routine susceptibility testing. The empirical use of ampicillin or cephalosporins is not recommended.

1. INTRODUCTION

Neonatal infections continue to be a major cause of death and disability in the world, especially in LMICs. *Citrobacter koseri* (previously known as *Citrobacter diversus*) is one of the clinically important pathogens in neonatal intensive care units (NICUs) among Gram-negative bacilli. *C. koseri* is less frequently found as part of an *E. coli* or *K. pneumoniae* infection, but is specifically linked to severe infections such as neonatal meningitis or brain abscesses (Doran,

2022). *Citrobacter* is a genus of the family Enterobacteriaceae. Unlike other *Citrobacter* species, such as *Citrobacter freundii*, *C. koseri* is able to produce indole and unable to use malonate. In the clinic *C. koseri* is well known to cause infection of the central nervous system (CNS) in neonates, and may show a tendency to develop multiple brain abscesses. The study by Samonis et al. (2020) found that up to 75% of those who develop neonatal *C. koseri* meningitis also have brain abscesses, while only 10–15%

with *E. coli* meningitis develop a brain abscess. *C. koseri* infections are especially common in neonates with immature immune systems, who are often hospitalized, have been exposed to invasive devices, such as an endotracheal tube, an umbilical catheter, or a ventriculoperitoneal shunt, and have also been exposed to antibiotics before

(Flores-Pope & Alarcón-Romero, 2021). It is possible for the infection to be transmitted vertically from the mother's genitourinary tract; however, it is more likely that it will be transmitted horizontally via contaminated equipment or the hands of healthcare workers in NICUs (Patel et al., 2019). The incidence of *C. koseri* neonatal sepsis varies geographically. In the United States and Europe, it only causes < 5% of all neonatal Gram-negative infections. In other NICUs in Asia and Africa, however, rates of 8–12% have been reported (Khan et al., 2023). *C. koseri* meningitis is still lethal in 15–30% of the population and can leave up to 40% with a permanent neurological deficit, including seizures, developmental delay and/or hydrocephalus (Doran, 2022). *Citrobacter koseri* has both intrinsic and acquired resistance. It has intrinsic production of a chromosomal AmpC beta-lactamase that is responsible for resistance to ampicillin and narrow-spectrum cephalosporins (Jacoby, 2021). Plasmid-mediated ESBLs (e.g., CTX-M, SHV, TEM types) and carbapenemases (e.g., NDM, OXA-48) are also part of the acquired resistance, as are TEM or other carbapenemases (which are rarely produced by *C. koseri* but more common in *K. pneumoniae*, according to Logan & Weinstein, 2022).

C. koseri has been selected by the overuse of third generation cephalosporins in NICUs. In India, 34% of *C. koseri* isolates from neonates were ESBL positive, and 18% were multidrug resistant (MDR) (Mehta et al., 2021). This is disturbing because treatment for neonatal infections with MDR *Citrobacter* is not available. The drugs of choice are carbapenems (meropenem, imipenem) but their usefulness is compromised by emerging resistance (Tamma et al., 2022). Neonates infected with *C. koseri*

typically have more nonspecific signs of illness, including lethargy, decreased feedings, temperature instability, and/or respiratory difficulty (Flores-Pope & Alarcón-Romero, 2021). The classic symptoms of *C. koseri* meningitis (bulging fontanelle and seizures) might only happen once abscesses have formed. Early diagnosis of brain abscesses is important and is achieved by neuroimaging (cranial ultrasound or MRI); prolonged antibiotic therapy may be necessary, with surgical drainage required in some cases (Doran, 2022).

2. Rationale and Objectives

Citrobacter koseri is an important opportunistic pathogen associated with severe neonatal infections, including sepsis, meningitis, and urinary tract infections. The emergence of multidrug-resistant (MDR) and extended-spectrum β -lactamase (ESBL)-producing *C. koseri* strains has significantly limited therapeutic options and increased the risk of treatment failure in neonatal intensive care units (NICUs). Despite its clinical significance, local data on the antimicrobial susceptibility patterns of *C. koseri* remain scarce in many healthcare settings, resulting in empirical antibiotic therapy that may be ineffective and contribute to the further spread of antimicrobial resistance. Recognizing the growing global threat posed by antimicrobial-resistant bacteria, the World Health Organization (WHO) has identified *Citrobacter* species among the priority pathogens requiring the development of new antimicrobial strategies. Therefore, the present study was undertaken to characterize *C. koseri* isolates recovered from neonatal infections, determine their antimicrobial susceptibility profiles, and investigate the prevalence of extended-spectrum β -lactamase (ESBL) production and multidrug resistance (MDR) among the clinical isolates.

3. Materials and Methods

ZStudy Design and Setting

A descriptive cross-sectional, laboratory-based study was conducted over a period of six months, from January to June 2025, in

the Department of Microbiology in collaboration with the Neonatal Intensive Care Unit (NICU) of a tertiary care hospital. The study was designed to determine the antimicrobial susceptibility pattern, multidrug resistance (MDR), and extended-spectrum β -lactamase (ESBL) production among *Citrobacter koseri* isolates recovered from neonates with clinically suspected bacterial infections.

3.1 Sample Collection and Bacterial Isolation

A total of 40 non-duplicate clinical isolates of *Citrobacter koseri* were recovered from neonates (0–28 days of age) admitted to the NICU with clinical manifestations suggestive of neonatal sepsis, meningitis, or urinary tract infection. Clinical specimens consisted of 22 blood cultures, 12 cerebrospinal fluid (CSF) samples, and 6 urine samples.

Specimens were collected aseptically by trained healthcare personnel following standard infection prevention protocols. Blood cultures were processed using an automated blood culture system, while cerebrospinal fluid and urine specimens were cultured immediately after collection. Primary isolation was performed on

Blood agar, MacConkey agar, and Chocolate agar as appropriate, followed by incubation at 35–37°C for 18–24 hours under aerobic conditions. Colonies exhibiting morphological characteristics suggestive of Enterobacterales were further processed for identification. Only the first isolate recovered from each patient was included in the study to eliminate duplicate isolates and ensure unbiased microbiological analysis.

3.2 Bacterial Identification

Presumptive identification of bacterial isolates was performed based on colony morphology, Gram staining characteristics, and conventional biochemical tests. Gram staining demonstrated Gram-negative bacilli.

Biochemical characterization included catalase, oxidase, citrate utilization, indole production, urease activity, Triple Sugar Iron (TSI) agar reaction, lysine decarboxylase, and malonate utilization tests. *Citrobacter koseri* isolates were identified by the following characteristic biochemical profile:

Table 1. Biochemical characteristics of *Citrobacter koseri* isolates used for bacterial identification

Biochemical Test	Result	Interpretation
Gram staining	Gram-negative bacilli	Consistent with Enterobacterales
Catalase	Positive (+)	Presence of catalase enzyme
Oxidase	Negative (–)	Characteristic of Enterobacterales
Citrate utilization	Positive (+)	Ability to utilize citrate as the sole carbon source
Indole production	Positive (+)	Production of indole from tryptophan
Urease	Negative (–)	No hydrolysis of urea
Triple Sugar Iron (TSI)	Acid/Acid (A/A) with gas	Fermentation of glucose, lactose and/or sucrose with gas production

Lysine decarboxylase	Positive (+)	Decarboxylation of lysine
Malonate utilization	Negative (-)	Inability to utilize malonate
API 20E identification	<i>Citrobacter koseri</i>	Confirmatory identification

Final confirmation of bacterial identification was performed using the API 20E identification system (bioMérieux, France) according to the manufacturer's instructions. Identification results were interpreted using the API database.

3.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Oxoid, United Kingdom) according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2024). A standardized

bacterial suspension equivalent to 0.5 McFarland turbidity standard was prepared from freshly grown bacterial colonies. The inoculum was uniformly spread over the surface of Mueller-Hinton agar plates using sterile cotton swabs. Antibiotic discs were placed on the inoculated agar surface using sterile forceps, and plates were incubated aerobically at $35 \pm 2^\circ\text{C}$ for 18–24 hours.

Following incubation, inhibition zone diameters were measured in millimeters using a calibrated ruler and interpreted as

Susceptible (S), Intermediate (I), or Resistant (R) according to CLSI breakpoints.

Table 2: Antimicrobial agents used for antimicrobial susceptibility testing of *Citrobacter koseri* isolates.

Antimicrobial Class	Antibiotic	Disc Potency (μg)
β-lactam Penicillins	Ampicillin	10
	Amoxicillin-clavulanic acid	20/10
Cephalosporins	Cefazolin	30
	Cefuroxime	30
	Ceftriaxone	30
	Ceftazidime	30
	Cefepime	30
Carbapenems	Imipenem	10
	Meropenem	10
Aminoglycosides	Gentamicin	10
	Amikacin	30

Fluoroquinolones	Ciprofloxacin	5
	Levofloxacin	5
Monobactam	Aztreonam	30
Others	Trimethoprim-sulfamethoxazole	1.25/23.75
	Tetracycline	30
	Chloramphenicol	30

3.4 Detection of Extended-Spectrum β -Lactamase (ESBL) Production

Phenotypic detection of ESBL production was performed using the Double-Disc Synergy Test (DDST) according to CLSSI recommendations. Briefly, ceftazidime (30 μ g) and ceftriaxone (30 μ g) discs were placed at a distance of approximately 20 mm (center-to-center) from an amoxicillin-clavulanic acid (20/10 μ g) disc on Mueller-Hinton agar previously inoculated with the test organism. Following incubation at 35°C for 18–24 hours, enhancement of the inhibition zone towards the clavulanic acid-containing disc by ≥ 5 mm was interpreted as evidence of ESBL production.

3.5 Determination of Multidrug Resistance (MDR)

Multidrug resistance was defined according to the standardized international criteria proposed by Magiorakos et al. (2012). An isolate was classified as MDR when it exhibited **non**-susceptibility to at least one antimicrobial agent in three or more different antimicrobial categories. Each isolate was evaluated individually, and MDR prevalence was calculated as the proportion of MDR isolates among the total number of *C. koseri* isolates included in the study.

3.6 Quality Control

Quality assurance procedures were implemented throughout the study to ensure the reliability and reproducibility of laboratory findings. The performance of culture media, biochemical tests, antimicrobial susceptibility testing, and ESBL detection was monitored using standard

reference strains obtained from the American Type Culture Collection (ATCC).

The following quality-control organisms were employed:

- *Escherichia coli* ATCC 25922 (quality-control strain for antimicrobial susceptibility testing)
- *Klebsiella pneumoniae* ATCC 700603 (ESBL-positive control strain)

Quality-control testing was performed daily during antimicrobial susceptibility testing to verify the accuracy of inhibition zone diameters and overall laboratory performance.

3.7 Statistical Analysis

Data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistical analysis was performed to summarize demographic characteristics, specimen distribution, antimicrobial susceptibility profiles, ESBL production, and MDR prevalence. Continuous variables were expressed as means $\pm$ standard deviations where appropriate, whereas categorical variables were presented as frequencies and percentages.

4. Results

4.1 Demographic Characteristics and Distribution of Clinical Specimens

A total of 40 non-duplicate *Citrobacter koseri* isolates were recovered from neonates admitted to the Neonatal Intensive Care Unit (NICU) during the study period. Clinical specimens included blood cultures (n = 22, 55.0%),

cerebrospinal fluid (CSF) (n = 12, 30.0%), and urine samples (n = 6, 15.0%).

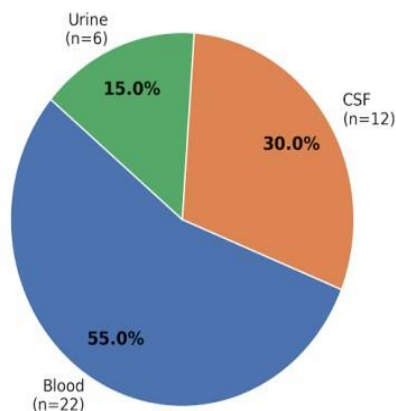
Among the infected neonates, 23 (57.5%) were male, whereas 17 (42.5%) were female, indicating a slightly higher prevalence of infection among male neonates. The majority of

isolates originated from blood cultures, demonstrating that bloodstream infection was the predominant clinical presentation of *C. koseri* infection in the NICU.

Table 3: Demographic characteristics and specimen distribution of neonatal *Citrobacter koseri* isolates.

Variable	Frequency (n)	Percentage (%)
Gender		
Male	23	57.5
Female	17	42.5
Clinical specimen		
Blood	22	55.0
Cerebrospinal fluid	12	30.0
Urine	6	15.0
Total isolates	40	100

Distribution by Specimen Type (N=40)



Gender Distribution of Neonates

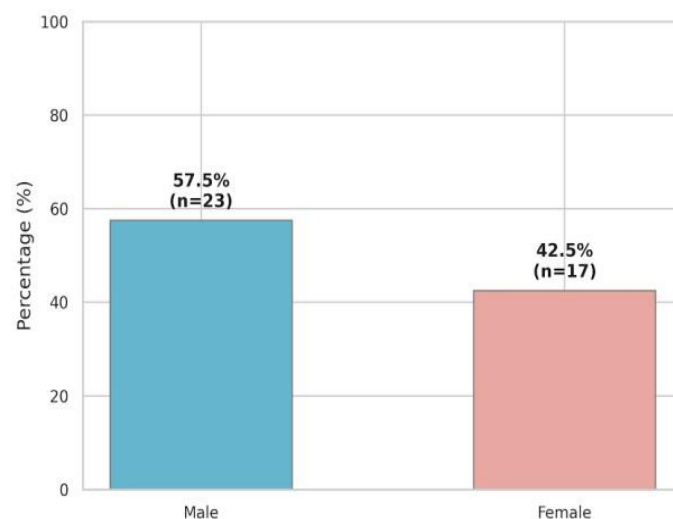


Figure: Distribution of Clinical Specimens and Gender among Neonates with *Citrobacter koseri* Infection (N = 40).

4.2 Antimicrobial Susceptibility Profile

The antimicrobial susceptibility profile of the 40

Citrobacter koseri isolates is summarized in Graph. Resistance was highest against β -lactam

antibiotics, particularly ampicillin (100%), followed by amoxicillin-clavulanic acid (85.0%), cefazolin (82.5%), and cefuroxime (77.5%). Among the third-generation cephalosporins, resistance to both ceftriaxone and ceftazidime was observed in 45.0% of isolates. Cefepime demonstrated improved activity, with 70.0% susceptibility.

Carbapenems remained the most effective antimicrobial agents. All isolates (100%) were susceptible to both meropenem and imipenem,

while amikacin demonstrated excellent activity with 95.0% susceptibility. Gentamicin showed comparatively lower activity, with 22.5% resistance. Moderate resistance was observed against trimethoprim-sulfamethoxazole (32.5%), whereas resistance to ciprofloxacin and levofloxacin remained relatively low. Overall, carbapenems and amikacin were the most effective therapeutic agents against neonatal *C. koseri* isolates recovered during the study.

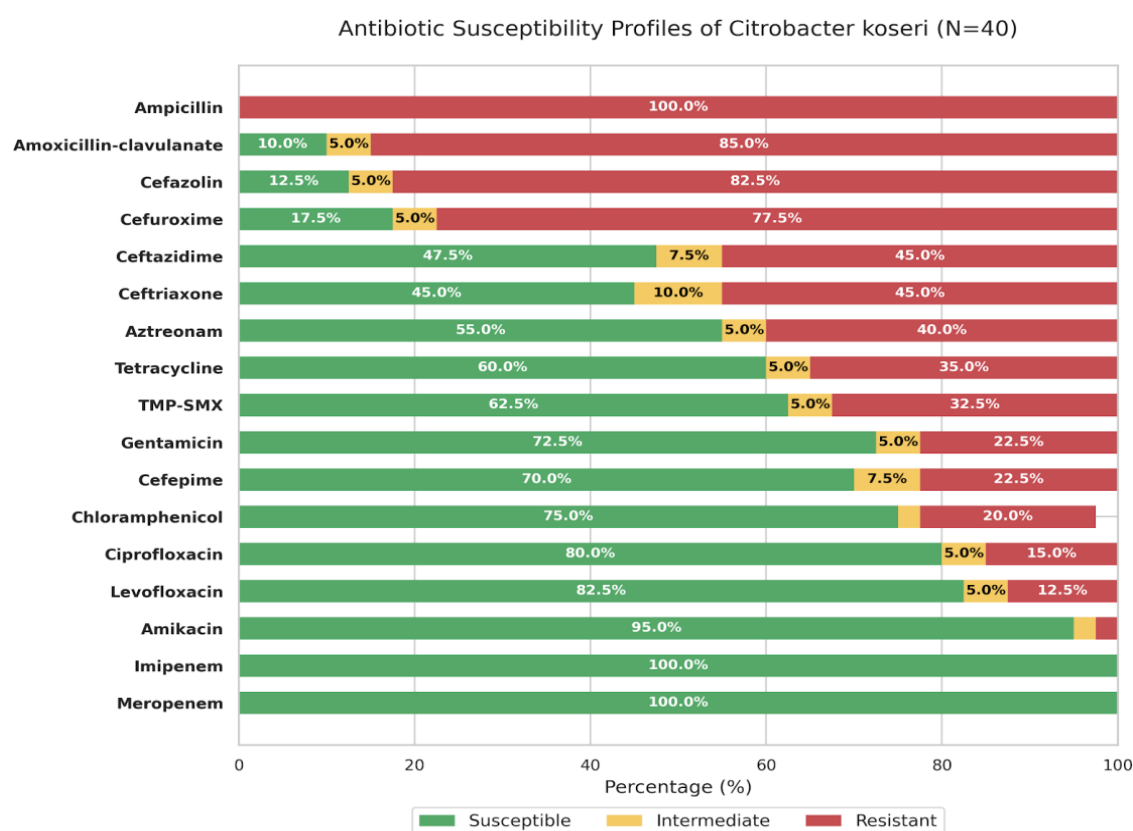


Figure Antibiotic Susceptibility Profiles of *Citrobacter koseri* Isolates (N = 40). The antimicrobial susceptibility pattern of *Citrobacter koseri* isolates showed complete resistance to ampicillin (100%) and high resistance to amoxicillin-clavulanate (85.0%) and cefazolin (82.5%). In contrast, all isolates were susceptible to imipenem and meropenem (100%), with high susceptibility also observed for amikacin (95.0%), levofloxacin (82.5%), and ciprofloxacin (80.0%). Intermediate susceptibility was observed in a small proportion of isolates for several antibiotics.

4.3 Detection of Extended-Spectrum β -Lactamase (ESBL) Production Phenotypic screening using the Double-Disc Synergy Test revealed that 11 (27.5%) isolates produced extended-spectrum β -lactamases (ESBLs),

whereas 29 (72.5%) isolates were ESBL-negative. Most ESBL-producing isolates were recovered from invasive clinical specimens, particularly blood cultures and cerebrospinal fluid, suggesting a close association between ESBL

production and severe neonatal infections.

showed comparatively lower resistance.

4.4. Multidrug Resistance (MDR) Profile

Based on the criteria proposed by Magiorakos et al. (2012), 13 (32.5%) isolates were classified as multidrug resistant (MDR), while 27 (67.5%) remained non-MDR. MDR isolates demonstrated simultaneous resistance to multiple antimicrobial classes, including β -lactams, cephalosporins, aminoglycosides, and fluoroquinolones. The majority of MDR isolates were recovered from blood cultures and cerebrospinal fluid, whereas urinary isolates

4.5 Distribution of ESBL and MDR According to Clinical Specimen

A higher proportion of ESBL-producing and MDR isolates was observed among invasive bloodstream and cerebrospinal fluid isolates than urinary isolates. Blood culture isolates demonstrated the highest proportion of MDR organisms (36.4%), followed by CSF isolates (41.7%). No ESBL-producing or MDR isolates were recovered from urine specimens.

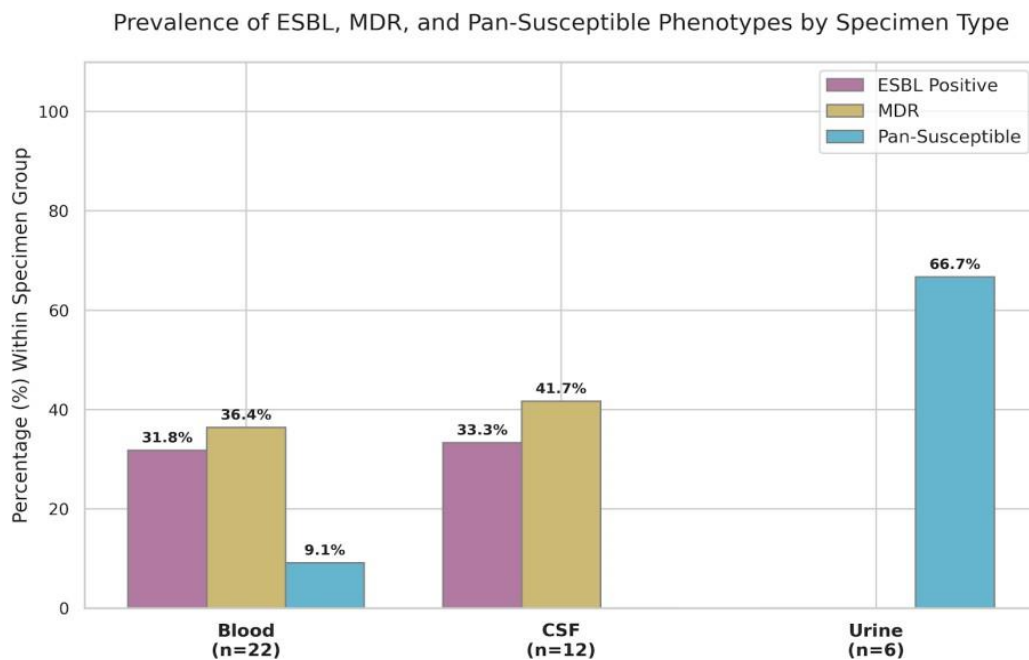


Figure: Prevalence of ESBL, MDR, and Pan-Susceptible *Citrobacter koseri* Isolates by Specimen Type. The prevalence of extended-spectrum β -lactamase (ESBL)-producing, multidrug-resistant (MDR), and pan-susceptible *Citrobacter koseri* isolates varied among specimen types. Blood isolates demonstrated 31.8% ESBL production and 36.4% MDR, whereas CSF isolates exhibited slightly higher frequencies (33.3% ESBL and 41.7% MDR). Urine isolates showed 66.7% pan-susceptibility, with no ESBL-producing or MDR isolates detected.

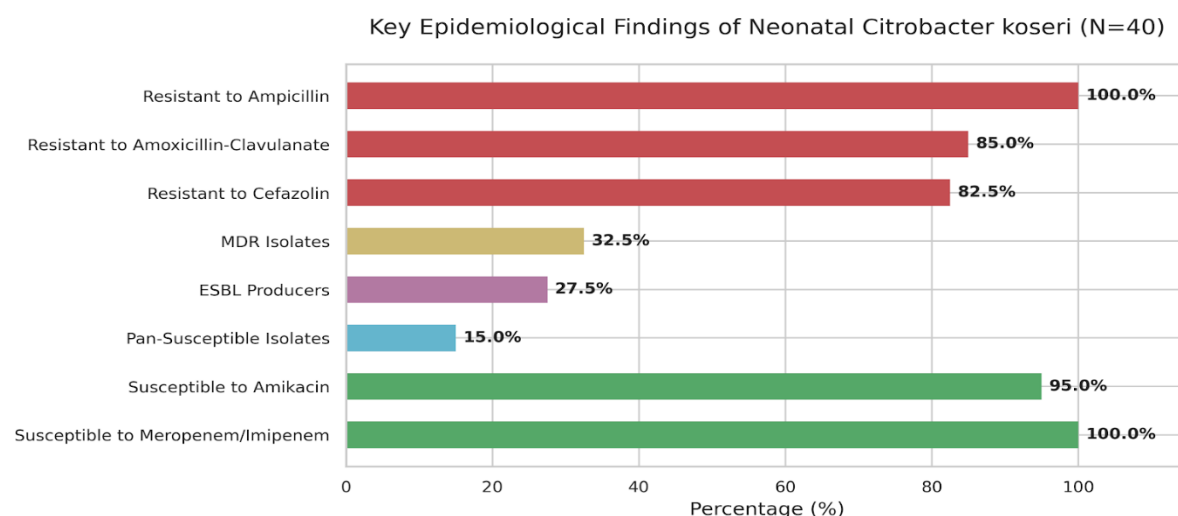


Figure: Summary of Key Epidemiological Findings of Neonatal *Citrobacter koseri* Isolates (N = 40). The figure summarizes the principal epidemiological findings of the study. Complete resistance was observed to ampicillin (100%), with high resistance also noted for amoxicillin-clavulanate (85.0%) and cefazolin (82.5%). Overall, 32.5% of isolates were multidrug-resistant (MDR), 27.5% were ESBL producers, and 15.0% were pan-susceptible. High susceptibility was maintained for amikacin (95.0%), while imipenem and meropenem demonstrated 100% susceptibility, indicating excellent activity against the isolates.

Discussion

A susceptibility pattern was determined for every location. Each location had a susceptibility pattern determined. In our study, the *C. koseri* strains isolated from neonatal infections were very sensitive to carbapenems (100% sensitivity to meropenem and imipenem). This observation is consistent with the previous reports of Tamma et al. (2022) and Mehta et al. (2021) who reported that >95% of Enterobacteriaceae samples isolated from NICUs were susceptible to carbapenems. Most AmpC beta-lactamases and ESBLs of

C. koseri are carbapenem resistant (Jacoby, 2021). For suspected *C. koseri* neonatal meningitis, therefore, meropenem (40mg/kg 8 hourly) should be used as the empirical treatment of choice, particularly if the prevalence of ESBL is greater than 10–15% (Doran, 2022). Amikacin was 95% susceptible, which is clinically meaningful since aminoglycosides generally have poor CSF penetration however they are a good alternative or adjunct to the use of carbapenems in the treatment of bacteremia and urinary tract infections (Logan & Weinstein, 2022). The

higher resistance rate to gentamicin (22.5%) is a cause of concern because gentamicin is still widely used empirically in many NICUs for

presumed Gram-negative sepsis, (Flores-Pope & Alarcón-Romero, 2021). 5.3 High degree of resistance to ampicillin and cephalosporins. 5.4 High level of resistance to ampicillin and cephalosporins. The 100% resistance to ampicillin is expected due to the intrinsic AmpC beta-lactamase (Jacoby, 2021). It is interesting to note, however, that the resistance to amoxicillin-clavulanate is 85%, as clavulanate is usually effective against some of the ESBLs but not the AmpC enzymes. This indicates that the majority of resistant isolates are likely to be capable of producing both AmpC and ESBL, or that AmpC is "derepressed". (Patel et al., 2019) These are totally inappropriate for empirical treatment because of the high percentages that are resistant to these agents (82.5% for cefazolin and 77.5% for cefuroxime). Even ceftriaxone and ceftazidime (45% resistant each) have unacceptably high resistance. A third-generation cephalosporin alone is unlikely to be sufficient

therapy for a neonate with *C. koseri* meningitis, as these patients are likely to develop brain abscesses (Samonis et al., 2020). This is the rationale for no longer recommending the "meningitis dose" of cefotaxime or ceftriaxone for *Citrobacter* (Doran, 2022).

The 27.5% production of ESBL by *C. koseri* isolates is similar to Khan et al. (2023) who reported 30% ESBL production in *Citrobacter* spp. in Pakistan and is slightly lower than the findings by Mehta et al. (2021) in India (34%). The 32.5% MDR rate is alarming as few treatment options are available for neonates if they are infected with MDR *C. koseri*. Resistance to Ciprofloxacin (15%) and TMP-SMX (32.5%) means that the only reliable oral step-down therapy is these two, though they are used less due to resistance. (Tamma et al., 2022) This is a purely theoretical course aimed at providing an introduction to the implications of this research for neonatal care. The course of therapy for a neonate with *C. koseri* meningitis is prolonged, 3-4 weeks of intravenous antibiotic therapy is required. In the event of a brain abscess, surgical aspiration may be required and treatment might last for 6-8 weeks (Flores-Pope & Alarcón-Romero, 2021). Our results suggest the following empirical treatment pending cultured results: Meropenem + Amikacin. When susceptibilities are known, de-escalation therapy can be used (e.g., by switching to an alternative from the same class if susceptible; switching to an oral step-down drug if oral step-down is planned). The use of cefepime plus amikacin (this is more effective than ceftriaxone because it is more resistant to the action of AmpC enzymes) may be used when carbapenems are not available or too costly, however our data indicates 22.5% resistance to cefepime, which is suboptimal (Logan & Weinstein, 2022).

Limitations

There were a number of limitations in this study. First, the sample size (n=40) is relatively small and from only one center, so that results may not be generalizable. Secondly, we failed to perform MIC determination (E-test or broth

microdilution) which is more accurate than disc diffusion. Thirdly, molecular characterization (PCR) was not carried out to detect the presence of specific ESBL genes (e.g. blaCTX-M, blaSHV, blaTEM). Finally, susceptibility patterns were not associated with clinical outcomes (mortality, length of stay, neurological sequelae). Multicenter studies with molecular typing are recommended for the future.

Summary

Citrobacter koseri is an important cause of neonatal meningitis and sepsis, which frequently is complicated by brain abscesses. In the current study, 40 neonatal isolates were studied and the findings showed that all isolates were susceptible to meropenem and imipenem, suggesting that carbapenems are the drugs of choice. Amikacin (95% susceptible) is a good adjunctive agent.

Empirical treatment should not be given with Ampicillin (100% resistant) and early cephalosporins (cefazolin, cefuroxime, ceftriaxone). The ESBL producers accounted for 27.5% of the isolates, while 32.5% were multidrug-resistant (MDR). All

C. koseri isolates from neonates should be routinely tested for antibiotic susceptibility testing. However, if *C. koseri* is suspected to cause infection in the newborn, meropenem plus amikacin should be used until the results of susceptibility tests are available.

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