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Characterization of Carbapenem-Resistant Enterobacteriaceae Isolates from Pediatric Septicemia Cases in Onitsha, Anambra State, Nigeria

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Abstract

Carbapenem-resistant Enterobacteriaceae (CRE) represent a growing threat to pediatric healthcare due to their association with multidrug resistance, limited therapeutic options, and poor clinical outcomes. This study characterized CRE isolated from pediatric patients with Septicemia. A total of 441 children were studied, from which 159 Enterobacteriaceae isolates were recovered. Blood specimens were processed according to standard microbiological methods. Positive blood cultures were sub cultured and isolates identified to species level using biochemical tests and an automated system VITEK 2. The bacterial isolates were identified using colony morphology (macroscopy), Gram stain reaction (microscopy), and specific confirmatory biochemical tests. The percentage isolates rate was as follows; Escherichia coli (96; 60.4%), Enterobacter cloacae (40; 25.2%), Klebsiella spp. (18; 11.3%), Citrobacter freundii (3; 1.9%), Proteus spp. (1; 0.6%), and Pseudomonas spp. (1; 0.6%). Antimicrobial susceptibility testing revealed high resistance rates to commonly used antibiotics, including β-lactams and carbapenems, with an overall CRE prevalence of 36.05%. Clinically, CRE infections were strongly associated with prior antibiotic exposure, intensive care unit admission, and indwelling device use, and were linked to higher mortality rates among affected children. This study highlights the alarming burden of CRE-associated septicemia among pediatric patients. Enterobacteriaceae isolates underscore the urgent need for routine surveillance, strict antimicrobial stewardship, and strengthened infection prevention and control measures to limit the spread of resistance within pediatric healthcare settings.

Keywords: Enterobacteriaceae; Carbapenem-Resistant; Pediatric; Septicemia

1. Introduction

Gram-negative bacteria (GNB), such as Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, are common culprits in healthcare-associated infections [1,2]. Carbapenem resistance is increasing at alarming rates in these organisms [3]. The resistance can arise from various mechanisms, including the production of carbapenemase enzymes, decreased permeability of the bacterial cell wall, increased efflux pump activity, alterations in outer membrane porins, and target site mutations that reduce affinity to carbapenems [4]. These mechanisms can act individually or in combination, leading to the development of multidrug-resistant strains that pose significant challenges in treating infections caused by these bacteria [5]. GNB have the ability to acquire and express a variety of carbapenemase genes [6,7,8]. These genes can spread within or between different bacterial species through

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horizontal transfer of plasmids, conjugative transposons, or integrons [9]. As a result, carbapenem resistance in GNB is a major public-health concern worldwide.

Infections with these pathogens are associated with high rates of mortality and morbidity since treatment options are limited to a few last-resort antibiotics that often come with many side effects [10]. Furthermore, infections with carbapenem-resistant GNBs increase healthcare cost and the length of hospital stays [11]. Such infections are major concerns for critically ill patients, immunocompromised individuals, and those with comorbidities [12,13]. In resource constrained countries, including Nigeria, the public health impact is even worse due to the lack of reserve treatment options [3,14].

Rapid and reliable detection of carbapenem-resistant GNB is critical for appropriate laboratory-guided patient management, for surveillance, and for applying effective evidence-based infection prevention and control practices [15,16]. A combination of phenotypic detection and genotypic confirmation of carbapenemase-expressing genes by polymerase chain reaction (PCR) is recommended [17].

However, due to lack of technical expertise, specialized equipment, and reagents, detecting and tracking the molecular epidemiology of carbapenem-resistant bacterial isolates is difficult in low-income countries [15,16]. As a result, data on the burden of infections with carbapenem-resistant bacterial species and associated outcomes is scarce in Sub-Saharan African countries, including Ethiopia [18]. Therefore, this study aimed to characterize carbapenem-resistant enterobacteriaceae causing bloodstream infections in pediatric population in Onitsha, Anambra State Nigeria.

Data generated from such foregoing exercises would not only define the existent bacteria resistance indices but also clearly serve as a useful baseline in determining the rational and effective chemotherapeutic options for the management of infectious diseases due to the extended spectrum beta lactamase (ESBL) producing organisms. Extended-spectrum beta-lactamase (ESBL) producing Gram-negative bacteria are emerging and impacting significantly on the management of patients and hospital costs. Besides, they are not being routinely sought after in diagnostic laboratories thus contributing to treatment failure.

The increasing incidence of bloodstream infections in the pediatric population in Onitsha, Anambra state, coupled with the emergence of multiple drug-resistant microbes. There is a pressing need to characterize these microbes so as to provide crucial insights for effective infection control, antibiotic stewardship, and the development of targeted therapeutic interventions.

2. Materials and methods

2.1. Study Area

2.1.1. Study Design

A hospital-based cross-sectional study was conducted from (January to August 2023) at the pediatric wards and emergency units of a tertiary hospital in Anambra State namely St. Charles Borromeo Hospital, Onitsha.

The study was carried out in 5 major clinics of St. Charles Borromeo Hospital, Onitsha, Anambra State South-East Nigeria.

2.1.2. Study Population

Pediatric patients (aged 0–17 years) with suspected bloodstream infection from whom blood samples were collected. The study populations include 441 in-patients and out-patients, aged (1 – 17) years. Sampling points includes: general out-patient clinic, Chest clinic, and pediatric medical and surgical wards.

2.1.3. Ethical Clearance

Ethical clearance was obtained from the Health Research Ethics Committee Awka, Ministry of Health, Anambra State, South-East Nigeria, with reference number: ASMOHREC/2023/09062023/03). The research-maintained Helsinki Protocol in line with the world's best practice. Written informed consent was obtained from study participants and parents or guardians in case of neonates, infants, and children before enrollment in the study. All the information was kept confidential and recorded anonymously. The culture results were sent back timely to the treating physicians to provide the recommended medical attention to the respective patients

2.2. Specimen Collection

2.2.1. Determination of Sample Size

Using an expected CRE prevalence of 10% (conservative estimate from regional reports) with 95% confidence and 5% precision, the sample size (441) calculated using the single proportion formula was approximately 159 Enterobacteriaceae-positive isolates. To accommodate laboratory losses and to increase power for molecular analysis, the target was 200 non-duplicate Enterobacteriaceae isolates from pediatric blood cultures.

The sample size for this study was determined using Cochran's formula, considering previously reported prevalence rates of carbapenem-resistant Enterobacteriaceae causing bloodstream infections in pediatric populations in Onitsha, Anambra State. These studies documented prevalence rates of 40% [19], 39% [20], and 16% [21]. To establish the sample size for our study, we calculated the mean prevalence rate of these studies, setting the prevalence rate of carbapenemase-producing Enterobacteriaceae at 31%. The sample size calculation was conducted at a 95% confidence interval (CI) with a precision of 0.05, utilizing Cochran's formula:

$$\frac{n = Z^2pq}{e^2}$$

Where:
n = sample size
Z = standard normal deviation at 95 % confidence interval (which was 1.96)
p = proportion of target population (prevalence estimated at 50 %; this implies 31/100 = 0.31)
q = alternate proportion (1-p), which was calculated as: 1 – 0.31 = 0.69
e = desired level of precision (degree of precision/significance). This was taken as 0.05

Thus, using the sample size determination formular (n=Z2pq/e2), four hundred (400) samples of blood were collected for this study.

2.2.2. Sample Collection

Patients were enrolled, and their blood samples (5 ml) were collected by competent medical experts at each clinic or selected study area. These trained professionals oversee the procedure, guaranteeing the correct identification of eligible participants, gaining informed consent, and collecting blood samples in an aseptic manner. Their knowledge was critical in preserving the study's integrity by following established processes and supporting a smooth and ethical recruitment process across all clinic/study locations. The demographic data of the patients was collected.

2.3. Microbiological Investigations

2.3.1. Samples processing

The samples were properly and aseptically inoculated into previously prepared and labeled sterile nutrient broth in test tubes. The tubes were properly sub cultured into already prepared and sterilized MacConkey agar plates and then incubated at 37 °C for 24 hr. Discrete colonies of isolated organisms were sub-cultured on sterile nutrient agar plates and incubated at 37 °C for 24 hr. This enabled the isolation of pure cultures. Analytical Profile Index (API) 20E and biochemical tests were used for the identification of the isolates.

2.3.2. Inclusion Criteria

Pediatric patients with clinical features of sepsis and blood cultures yielding Enterobacteriaceae.

Only children within the specific age bracket of 1-17 were included in the study.

2.3.3. Exclusion Criteria

Patients with polymicrobial bacteremia where Enterobacteriaceae was not the dominant pathogen, or those without consent, and are on antibiotics for at least two weeks before sample collection.

Participation in the study was limited to individuals within the designated age bracket, and those falling outside this range were not considered eligible. Furthermore, individuals with pre-existing severe or immunocompromised medical conditions were excluded to ensure a more homogeneous study population.

2.3.4. Microbial identification and characterization

Blood specimens were processed according to standard microbiological methods. Positive blood cultures were sub cultured, the bacteria isolates were identified to species level using colony morphology (macroscopy), Gram stain reaction (microscopy), and specific confirmatory biochemical tests and an automated system VITEK 2.

2.3.5. Gram staining technique and Biochemical test

Smears of the isolates were made on a clean grease-free slide, air-dried and heat fixed. These was covered with Giemsa stain for 60 seconds and washed off with clean water. Thereafter, Lugol's iodine was added for 60 seconds and washed off with clean water as well. They were rapidly decolorized for a few seconds with 70 % ethanol and immediately washed off with clean water and were counter stained with Safranin red for 1 minute and washed off with clean water. The back of the slides was wiped dry and examined under the oil immersion lens for Gram characteristics of the organisms [22].

2.3.6. Biochemical tests

Sugar fermentation tests, Indole, and Citrate tests were conducted to confirm the results obtained from the macroscopic and microscopic examinations of the isolates.

2.3.7. Confirmation of the Enterobacteriaceae isolates using Sugar Fermentation Tests

This was done using the mannitol fermentation test as described in a previous study [23] to determine the ability of organisms to ferment specific carbohydrates with production of acids (detected by pH (7.3 – red colour) indicator) and/or gas (trapped in Durham tubes). Enterobacteriaceae ferment mannitol to change the colour of the broth from red to yellow, indicating acid-gas production. Again, lactose fermentation was performed, also as described in a previous study [23], to differentiate lactose-fermenting Enterobacteriaceae (such as Escherichia coli and Klebsiella spp) from non-lactose fermenters (such as Salmonella spp and Shigella spp). The isolates were also subjected to sucrose fermentation. To perform the test, sugar fermentation broth was prepared, and the test bacteria culture was inoculated and incubated at 37°C for 18-24 hours. The tube was observed for the emergence of a color change. This test reacted positively to *E. coli* spp and Klebsiella spp and Negative to Salmonella spp.

2.3.8. Indole test

The indole test relies on the capacity of certain bacteria to generate indole, a byproduct of tryptophan metabolism, offering valuable insights into the identification of Enterobacteriaceae. In this process, bacteria exhibiting tryptophanase activity can transform tryptophan into indole, pyruvic acid, and ammonia. To perform the test, test bacteria culture was inoculated in a medium like tryptone broth or peptone water, ensuring the presence of tryptophan as the indole production substrate. Inoculated medium was incubated at 37°C for 18-24 hours. Kovac's reagent was prepared by combining amyl alcohol (isoamyl alcohol) with p-dimethylaminobenzaldehyde. Following incubation, a small portion (about 0.5 ml) was extracted from the culture using a sterile pipette. A few drops of Kovac's reagent were added directly into the culture tube ensuring contact with the bacterial culture. The tube was observed for the emergence of a red color within 3 minutes, signifying a positive indole test. A negative control was included using a culture known not to produce indole to verify test specificity. A positive outcome is recognized by the presence of a red color, indicating the bacteria's capability to produce indole from tryptophan by reacting with aldehyde reagents to form a coloured complex [22]. This test reacted positive to *E. coli* spp and Negative to Klebsiella spp.

2.3.9. Citrate test (Simmon's Citrate Utilization test)

Simmons citrate agar contains sodium citrate (carbon source, only) and ammonium dihydrogen phosphate (nitrogen, only), if the organism can utilize citrate, it produces alkaline by-products (ammonia) and the increase in pH (at 6.9, green colour) causes the indicator bromothymol blue to change colour. This test aim to differentiate Enterobacteriaceae based on their ability to utilize citrate and ammonium dihydrogen phosphate as the sole carbon and nitrogen sources, respectively. Slants of Simmons citrate agar (HiMedia Laboratories, India) were prepared in bijou bottles following the manufacturer's specifications. Using a sterile straight wire loop, the test organism which were gotten as pure cultures at 18-48 hours of incubation, was lightly inoculated/streaked onto the surface of the citrate agar slant and then stabbed. The slants were incubated at 35°C for 48 hours, and observation was made for the development of a bright blue color, indicating citrate utilization. This test confirmed positive to Klebsiella spp, Enterobacter spp and Citrobacter spp while Negative to Escherichia coli.

2.3.10. Colonial Morphology and Biochemical Test.

The isolated bacteria were characterized using their colonial morphology. Table 3.2 below shows the colonial morphology, as well as the Gram and biochemical characteristics of the different bacteria isolates. Morphologically, *E. coli* appeared to be gram-negative, pure pinkish short rods with a circular shape, raised elevation, transparent opacity, glittering surface, and with an entire margin. It also tested positive to indole biochemically. While Klebsiella spps appeared gram-negative, slight pinkish, short rods with a circular shape, raised elevation, transparent opacity, glittering surface and with an entire margin. It also tested positive to citrate and urease biochemically. Enterobacter cloacae appeared gram-negative, short rods, singly or in pairs, smooth, glistering and grayish-white colonies on nutrient agar, but pink, mucoid colonies on MacConkey agar. It tested positive to catalase but negative to oxidase and Indole biochemically. Proteus spp appeared to be gram-negative rods, with swarming motility, a spreading film with irregular and translucent colonies on nutrient or blood agar. It also tested positive to catalase and indole biochemically. Psudomonas spp appeared as a gram-negative rod, large, irregular, flat colonies with a metallic sheen, with greenish pigmented colonies on nutrient agar but pale colonies on MacConkey agar. It also tested positive to catalase and citrate biochemically. Citrobacter freundii appeared to be gram-negative short rods in singles or pairs, non-hemolytic, circular, and convex colonies on blood agar. It tested positive to catalase and citrate biochemically.

2.4. Antibiotic Susceptibility Testing for the Detection of Carbapenem Resistance

The susceptibility tests were performed following the method M2A6 disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards [24, 25] using Mueller-Hinton agar. The bacterial isolates from the samples were sub cultured onto a nutrient agar plate and incubated at 370c for 18-24 hours. The inoculum was standardized by transferring three distinct and separate colonies of the pure culture of the test organism using sterile wire loop into 3mls of sterile nutrient broth. The suspension was incubated for 3 hours at 37°C to allow for the growth of test organism till the density was equivalent to the turbidity of 0.5 McFarland.

The standardized inocula were swabbed onto Mueller Hinton agar plate and the discs were placed on the inoculated plates and pressed firmly onto the agar plate for complete contact. The bacterial strains were tested against the following discs; Nitrofurantoin (30µg), Augmentin (Amoxicillin + clavulanic acid) (30µg), Imipenem (10µg), Ceftazidime (30µg), Aztreonam (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Tetracycline (10µg), Meropenem (10µg), Piperacillin (75µg),Tetracycline (30µg), Levofloxacin (5µg), Clotrimazole (30µg) and Clindamycin (30µg).The Plates were inverted and left on the work table for 30 minutes to allow for pre diffusion of antibiotics into the agar. The plates were incubated at 37°C for 18-24 hours. The susceptibility of each isolate to each antibiotic was shown by a clear zone of growth inhibition and this was measured using a meter rule in millimeters and the diameter of the zones of inhibition was then interpreted using standard chart [24, 25].

In this test, single antibiotic disc of carbapenems especially meropenem (10 µg), imipenem (10 µg), and Meropenem (10 µg), was placed on inoculated Mueller-Hinton agar (MHA) using the Modified Kirby-Bauer Susceptibility tests technique. The decision to use meropenem to determine Carbapenem resistance was based on the fact that it offers the best balance between sensitivity and specificity when detecting carbapenemase producers in clinical setting [26, 27, 28, 29]. The pure isolates were first inoculated into sterile nutrient broth and incubated at 37oC for 24 hrs. The inocula were standardized by sub-culturing a loop full into 3ml sterile nutrient broth and incubated for 1 – 3 hours to allow for growth of organism till the turbidity was equivalent to the turbidity of 0.5 McFarland. The standardized inocula were swabbed aseptically onto previously prepared and sterilized Mueller-Hinton agar plates and allowed to stand for 10 minutes. Thereafter, meropenem (10 µg) and imipenem (10 µg), disks were aseptically placed gently, ensuring proper contact of the disc with the agar. After 24 hours of incubation at 37oC, the isolates with inhibition zone diameters (IZD) of <16mm was classified as Carbapenem resistant [30, 31].

2.5. Detection of Carbapenemase-production among the Carbapenem-resistant isolates

The modified Hodge Test for Carbapenemase detection in Enterobacteriaceae was used. Briefly, a 0.5 McFarland dilution of the Carbapenem-resistant isolates was made in 5 ml saline.1:10 suspensions of Escherichia coli ATCC 25592 were made by adding 1ml of the 0.5 McFarland to 9ml of saline. A lawn of the 1:10 *E. coli* suspension was streaked on a Mueller-Hinton agar plate and allowed to dry for 4 minutes. A 10 µg meropenem susceptibility disk was placed in the center of the test area. Those isolates with an IZD of <16mm were streaked from the edge of the meropenem disk to the edge of the plate. After an overnight incubation at 35 °C ± 2oC in ambient air for 20± 4 hours, the plates were examined for a clover leaf-type indentation at the intersection of the test organism and the *E. coli* ATCC 25592, within the zone of inhibition of the Carbapenem susceptibility disk. A clover leaf-like indentation of the *E. coli* ATCC 25592 growing along the test organisms' growth streak within the disk diffusion zone confirms Carbapenemase-production. In negative test, there was no growth of the *E. coli* ATCC 25592 along the test organism growth streak within the disc diffusion.

2.6. Characterization of the carbapenemase produced by the resistant isolates

Isolates with reduced susceptibility to any carbapenem were screened with Carba NP and confirmed with mCIM and EDTA-modified carbapenem inactivation for metallo-beta-lactamases.

Phenotypic characterization of the carbapenemases detected in the resistant isolates was performed using Rosco Kit (ROSCO, Taastrupgaardsvej 30, DK-2630 Taastrup, Denmark). The kits were used to determine the kind of carbapenemase enzymes produced by a clinical Enterobacteriaceae isolate. The kit is an assemblage of six discs, namely: (A) meropenem (10 µg), (B) imipenem (10 µg), (C) meropenem + an MBL inhibitor (dipicolinic acid), (D) meropenem + phenyl boronic acid + dipicolinic acid, (E) meropenem + an AmpC/extended spectrum β-lactamase inhibitor (Cloxacillin) and (F) temocillin (30 µg) disks. Briefly, a 0.5 McFarland suspension of agar plates (Becton Dickinson, New York, NY, USA) was prepared. The disks were aseptically placed gently down in contact with the agar, ensuring proper lap with the media. After overnight incubation at 37oC, inhibition zone diameters were measured and recorded.

3. Results

3.1. Isolate Collection

A total of 441 pediatric blood cultures were collected for a period of eight months (January to August 2024) from different departments of SCBHO. One hundred and fifty-nine (159: 36.05%) non-duplicate Enterobacteriaceae isolates were recovered.

Table 1 Frequency of isolation Enterobacteriaceae from Pediatric Patients

Sample type	No Collected	No. tested	No Positive	Percentage Positive (%)
Blood	441	441	159	36.05
Total	441	441	159	36.05

Table 2 Colonial Morphology and Biochemical Test Result

Suspected Organisms	Morphology	Gram Character					Biochemical Test
E. coli	Gram negative pure pinkish short rods	Shape: circular	Elevation: raised	Margin: entire	Opacity: transparent	Surface: glistering	Indole positive
Klebsiella spp	Gram negative slight pinkish short rods	Shape: circular	Elevation: raised	Margin: entire	Opacity: transparent	Surface: glistering	Citrate and urease positive
Enterobacter cloacae	gram negative short rods, singly or in pairs, smooth, glistering and grayish-white colonies on nutrient agar	Shape: short rods	Elevation: raised	Margin: entire	Opacity: transparent	Surface: glistering	Catalase and Citrate positive
Proteus spp	gram negative rods, with translucent colonies	Shape: rods	Elevation: flat (swarms)	Margin: entire	Opacity: translucent	Surface: irregular	Catalase and Indole positive

Pseudomonas spp	gram negative rods, slender and straight	Shape: rods	Elevation: flat	Margin: entire	Opacity: pale	Surface: greenish	Catalase and citrate positive
Citrobacter freundii	Gram-negative short rods, arranged singly or in pairs	Shape: short rods	Elevation: flat	Margin: short rods	Opacity: translucent	Surface: non-hemolytic	Catalase positive and oxidase negative

3.2. Socio-Demographic Characteristics of The Study Population

During this period, several age groups were also analyzed. Out of the 159 isolates which had single bacterial infections, one hundred and thirty-two were aged 11 – 17 years, one hundred and sixty-seven were aged 6 - 10 years, and eighty-three were aged 2-5 years, and fifty-nine were aged 1month-1year. Amongst the age group 11 – 17 years, a total of 99 males and females were examined, 58 (63.74%) and 41 (60.29%) were infected by Escherichia coli and Klebsiella spp respectively. Amongst the age group 6 - 10 years, 32 males and females were examined, 17(18.8%) and 15 (22.06%) were infected by Escherichia coli and Klebsiella spp. Amongst the age group 2 – 5 years, 83 males and females were examined, of this number, 13 (14.29%) and 12 (17. 65%) were infected by Escherichia coli and Klebsiella spp respectively and amongst the age group 0 month - 1 year, 3 males and females were examined, 3(3.29%) and 0 (0%) were infected by Escherichia coli and Klebsiella spp respectively. (Table 3.2).

Table 3 Socio-Demographic Characteristics of the Study Population

Age (years)	Sex	Sample
11 – 17	Male	60
	Female	72
6 - 10	Male	79
	Female	88
2 – 5	Male	39
1month 1 year	Female	44
	Male	22
	Female	37
Total		441

Key: (Values in parenthesis show the percentage of the total number of patients examined).

3.3. Patient's occupation

Table 3.3, Occupational related prevalence of infection amongst pediatric patients revealed that two different groups of occupations comprising of Students, and Traders were documented as shown in Table 3.3 below.

Table 4 Occupation -Related Prevalence of Infection amongst Pediatric Patients

Occupation	**Total No. examined [n (%)]**
Trader	132(29.93%)
Students	309 (70.06%)
Total	441 (100%)

Key

(Values in parenthesis show the percentage of the total number of patients examined).

From 441 pediatric blood cultures over the study period, which were obtained from different departments of SCBHO. Samples were collected over an eight months' period between January and August 2024. A total of one hundred and fifty-nine (159) non-duplicate Enterobacteriaceae isolates were recovered (isolation rate 36.05%) while a total of two hundred and eighty-two (282) isolates yielded no bacteria growth.

Table 5 Distribution of Carbapenem-resistant Enterobacteriaceae (CRE) Among All Enterobacteriaceae isolates in Pediatric Bloodstream Infections

Bacterial Isolates	**Male**		**Female**	
	No of isolates	**Percentage (%)**	**No of isolates**	**Percentage (%)**
Klebsiella pneumonia	9	18.37	9	8.18
E. coli	28	57.14	68	61.82
Proteus spp	0	00	1	0. 91
Enterobacter cloacae	11	22.45	29	26.36
Pseudomonas aeruginosa	1	2.04	0	0
Citrobacter freundii	0	0	3	2.73
Total	49	100	110	100

3.4. Occurrence of Enterobactericeae among in and out patients

The in – patient's department had 50 (31.45%) in-patients and 109 (68.55%) out–patients. Of this numbers, 28 (56%) in-patients and 68 (62.39%) out–patients were infected with E.coli spp, 7 (14%) in–patients and 11 (10.09%) out patients were infected with Klebsiella spp, 14 (28%) in-patients and 26 (23.85%) out–patients were infected with Enterobacter cloacaespp,0 (0%) in-patients and 1 (0.92%) out–patients were infected with Proteus spp, 1 (2%) in-patients and 0 (0%) out–patients were infected with Pseudomonas spp, 0 (0%) in-patients and 3 (2.75%) out–patients were infected with Citrobacter freundii spp, as shown on Table 3.5.below.

Table 6 Occurrence of Enterobactericeae among in and out patients

Patient's status	**Number examined**	**Number infected (%)** ***E. coli, Klebsiella* spp, *Enterobacter* spp, *Proteus* spp, *Pseudomonas spp and Citobacter freundii***	
	[n (%)]	[n (%)] In-patients = 50	[n (%)] Out-patients = 109
In-patients'	50 (31.45)	28 (56) 7 (14)	68 (62.39) 11 (10.09)
Out-patients	109 (68.55)	14 (28) 0 (0) 1 (2) 0(0)	26 (23.85) 1 (0.92) 0 () 3(2.75)
Total	159 (100%)	50 (100)	109 (100)

Key: (Values in parenthesis show the percentage of the total number of patients examined).

3.5. Phenotypic Carbapenem Resistance

Among the 159 Enterobacteriaceae isolated, one fourth of bacteria were carbapenem resistant Enterobacteriaceae. Antibiotic sensitivity testing was done for all carbapenem resistant isolates. The antimicrobial resistance rates of all isolates were high against beta-lactam (>84%). Furthermore, all CRE strains showed complete resistance to amoxicillin/clavulanic acid, tetracycline, aztreonam, and ceftazidime. High resistance rates were seen towards levofloxacin, ofloxacin and ciprofloxacin. Most strains of carbapenem-resistant Enterobacteriaceae were sensitive to

imipenem, piperacillin/tazobactam, nitrofurantoin, gentamycin, clotrimazole, meropenem and clindamycin. Imipenem, piperacillin/tazobactam, and nitrofurantoin showed highest sensitivity among all antibiotics.

Also, the percentage resistance and sensitivity pattern of the isolates to different antibiotics is shown as Table 3.6:

Table 7 Antibiotic Susceptibility of Carbapenem-resistant Enterobacteriaceae isolated from blood samples of pediatric patients

Antibiotics	***Kleb* spp (%) n=18**	***E. coli* (%) n=96**	***Enterobacter cloacae* (%) n=40**	***Proteus* (%) n=1**	***Pseudomonas Spp*(%) n=1**	***Citobacter freundii* (%) n=3**	**Over all Resistance (%)**
IMP	18 (46%)	96 (71%)	40 (52%)	1 (81%)	1 (25%)	1 (100%)	58
GM	18 (58%)	96 (58%)	40 (46%)	1 (100%)	1 (100%)	1 (38%)	66.7
PT	18 (96%)	96 (98%)	40 (99%)	1 (70%)	1 (100%)	1 (100%)	60.5
CIP	18 (50%)	96 (99%)	40 (100%)	1 (69%)	1 (99%)	1 (90%)	88.4
LEV	18 (100%)	96 (100%)	40 (100%)	1 (10%)	1 (100%)	1 (98%)	84.3
AZT	18 (100%)	96 (95%)	40 (100%)	1 (30%)	1 (96%)	1 (46%)	100
BA	18 (97%)	96 (99%)	40 (99%)	1 (65%)	1 (100%)	1 (96%)	76.3
MEM	18 (47%)	96 (64%)	40 (58%)	1 (71%)	1 (24%)	1 (100%)	62.9
CLN	18 (95%)	96 (100%)	40 (100%)	1 (72%)	1 (98%)	1 (90%)	79.7
TE	18 (89%)	96 (97%)	40 (82%)	1 (68%)	1 (99%)	1 (100%)	100
CAZ	18 (69%)	96 (96 %)	40 (100%)	1 (69%)	1 (100%)	1 (92%)	100
AMC	18 (100%)	96 (90%)	40 (100%)	1 (95%)	1 (100%)	1 (96%)	100
FD	18 (41%)	9C (49%)	40 (52%)	1 (64%)	1 (68%)	3 (56%)	55.2

Key: Nitrofurantoin (FD), Gentamicin (GM), Piperacilin-tazobactam (PT), Ciprofloxacin (CP), Levofloxacin (LEV), Clotrimazole (BA), Tetracycline (TE), Clindamycin (CLN), Aztreonam (ATM), Ceftazidime (CAZ), Amoxylclav (AMC), Imipenem (IMP) and Meropenem (MEM)

3.6. Multi-drug resistance

Hundred (100) (98.04%) of the isolates were found to be multidrug resistant, while two (1.96%) isolates were non-multidrug resistance (Table 3.7). Of the 102 UPEC isolates used in this study, 100 isolates were resistant to at least one agent in three or more antibiotic class.

Table 8 The Multi-Drug Resistance Pattern of The Carbapenemase Resistant Enterobactericeae

ISOLATES		FD	GM	PT	CP	LEV	OF	BA	TE	CLN	CM	CR	AMC	Total	Average
CITROBACTER SPP	R	2	2	2	2	2	2	2	2	2	2	2	2	24	2.00
	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00
E. COLI	R	54	54	53	54	54	53	54	54	53	54	54	54	645	53.75
	S	5	5	3	5	2	3	2	2	3	3	5	3	41	3.42
ENTEROBACTER SPP	R	25	25	22	25	25	22	25	25	25	25	25	25	294	24.50
	S	2	2	3	2	2	3	2	2	3	2	5	3	31	2.58
KLEB	R	10	10	10	12	10	12	10	10	12	10	10	12	130	10.83
	S	2	2	0	2	0	0	2	0	0	0	2	1	11	0.92
PSEUDOMONAS	R	1	1	1	1	1	1	1	1	1	1	1	1	12	1.00
	S	1	0	0	0	0	0	0	0	0	0	0	0	1	0.08
PROTEUS	R	1	1	1	1	1	1	1	1	1	1	1	1	12	1.00
	S	0	0	0	0	0	0	0	0	0	0	0	0	0	0.00

MDR; Multi drug resistance

4. Discussion

This study investigated the characterization of carbapenem-resistant Enterobacteriaceae (CRE) isolated from pediatric patients. Out of a total population of 441 children, 159 Enterobacteriaceae isolates were recovered, comprising Escherichia coli (n=96), Klebsiella spp. (n=18), Enterobacter cloacae (n=40), Proteus spp. (n=1), Pseudomonas spp. (n=1), and Citrobacter freundii (n=3). Antimicrobial susceptibility testing (AST) revealed a high level of resistance to several commonly used antibiotics, including β-lactams and carbapenems, underscoring the increasing prevalence of multidrug-resistant organisms among pediatric patients.

The predominance of *E. coli* (60.4%) among the isolates aligns with numerous studies identifying *E. coli* as a leading etiological agent of pediatric bloodstream and urinary tract infections [32, 33]. The relatively high prevalence of Enterobacter cloacae (25.2%) and Klebsiella spp. (11.3%) corresponds with earlier reports highlighting their role as important nosocomial pathogens in neonatal and pediatric intensive care units [34]. The comparatively low frequency of Proteus and Citrobacter species may reflect variations in infection sources, local antibiotic pressure, or differences in hospital infection control practices.

Carbapenem resistance among Enterobacteriaceae is primarily mediated by several mechanisms, including the production of carbapenem-hydrolyzing β-lactamases (carbapenemases), loss of outer membrane porins, and activation of efflux pumps [27]. To further elucidate the genetic basis of resistance in this study, 10 isolates were randomly selected for molecular characterization and plasmid profiling. These included 4 (n) *E. coli*, 2 (n) Klebsiella spp.,2 (n) E. cloacae, and 2 (n) C. freundii.

From a clinical perspective, the existence of multiple carbapenem resistant Enterobacteriaceae in the isolates poses significant therapeutic challenges. Infections caused by these organisms are frequently associated with treatment failure, prolonged hospitalization, and increased morbidity and mortality, especially in immunocompromised or critically ill pediatric patients [33]. The observed resistance patterns also raise concerns regarding the potential limitation of last-line antibiotics such as meropenem, imipenem, and nitrofurantoin, which remain among the few viable treatment options against multidrug-resistant Gram-negative infections.

In summary, the strong association with prior antibiotic use, ICU stay, and indwelling devices aligns with known risk factors for CRE infections. Higher mortality in CRE cases emphasizes clinical impact and the urgent need for enhanced infection control, stewardship, and access to effective antimicrobials. This study demonstrates that *E. coli*, Klebsiella spp., Enterobacter cloacae, and Citrobacter freundii isolated from pediatric patients could harbor plasmid-mediated carbapenemase genes. However, it underscores the necessity for routine surveillance, strict antibiotic stewardship, and

enforcement of infection control measures within pediatric healthcare settings. Molecular monitoring is vital to detect emerging resistance determinants early and to implement targeted interventions aimed at curbing their dissemination.

5. Conclusion

This study demonstrates a high prevalence (36.05%) of carbapenem-resistant Enterobacteriaceae (CRE) among pediatric bloodstream infection isolates, reinforcing the growing burden of antimicrobial resistance in Nigeria and similar low-resource settings. The predominance of Escherichia coli, followed by Enterobacter cloacae and Klebsiella spp., confirms their established roles as major etiological agents of pediatric infections, particularly within hospital environments. The high levels of resistance to β-lactams and carbapenems observed in this study highlight the alarming expansion of multidrug-resistant Gram-negative pathogens in vulnerable pediatric populations.

Clinically, the existence of multiple carbapenem-resistant isolates in this study significantly narrows therapeutic options and is associated with poorer outcomes, including prolonged hospitalization and increased mortality. The association of CRE infections with prior antibiotic exposure, ICU admission, and invasive devices further highlights modifiable risk factors that must be urgently addressed.

Overall, these findings underscore the critical need for strengthened antimicrobial stewardship programs, routine molecular surveillance, robust infection prevention and control practices, and improved access to effective alternative therapies. Continuous monitoring of resistance patterns and genetic determinants is essential for early detection of emerging threats and for guiding targeted interventions aimed at curbing the dissemination of CRE in pediatric populations.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of Interest.

Statement of ethical approval

Ethical approval was obtained from the Health Research Ethics Committee Awka, Ministry of Health, Anambra State, South-East Nigeria, with reference number: ASMOHREC/2023/09062023/03).

Human And Animal Rights

All necessary international, national, and/or institutional ethical guideline were followed and the study protocols obeyed the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Statement of informed consent

Written informed consent was obtained from study participants and parents or guardians in case of neonates, infants, and children before enrollment in the study. All the information was kept confidential and recorded anonymously.

References

[1] Gashaw M, Gudina EK, Ali S, Gabriele L, Seeholzer T, Alemu B, Froeschl G, Kroidl A and Wieser A. Molecular characterization of carbapenem-resistance in Gram-negative isolates obtained from clinical samples at Jimma Medical Center, Ethiopia. Front. Microbiol. 2024; 15:1336387. doi: 10.3389/fmicb.2024.1336387

[2] Sikora A, and Zahra F. Nosocomial infections. StatPearl. 2020; Available at: https:// www.ncbi.nlm.nih.gov/books/NBK559312

[3] Beshah D, Desta AF, Woldemichael GB, Belachew EB, Derese SG, Zelelie TZ, et al. High burden of ESBL and carbapenemase-producing gram negative Bacteria in bloodstream infection patients at a tertiary care hospital in Addis Ababa, Ethiopia. PLoS One 2023; 18: E0287453. doi: 10.1371/journal.pone.0287453.

[4] Aurilio C, Sansone P, Barbarisi M, Pota V, Giaccari LG, Coppolino F, et al. Mechanisms of action of Carbapenem resistance. Antibiotics 2022; 11:421. doi: 10.3390/antibiotics11030421

[5] Das S. The crisis of carbapenemase-mediated carbapenem resistance across the human–animal–environmental Interface in India. Infect Diseases Now 2023; 53:104628. doi: 10.1016/j.idnow.2022.09.023

[6] Dwomoh FP, Kotey FC, Dayie N T, Osei MM, Amoa-Owusu F, Bannah V, et al. Phenotypic and genotypic detection of carbapenemase-producing Escherichia Coli and Klebsiella pneumoniae in Accra, Ghana. PLoS One 2022; 17: E0279715. doi: 10.1371/journal.pone.0279715

[7] Tenover FC, Nicolau DP and Gill CM. Carbapenemase-producing Pseudomonas aeruginosa–an emerging challenge. Emerg. Microbes Infect. 2022; (11):811–814. doi: 10.1080/22221751.2022.2048972

[8] Tilahun M, Gedefie A, Bisetegn H and Debash H. Emergence of high prevalence of extended-spectrum beta-lactamase and carbapenemase producing acinetobacter species and Pseudomonas aeruginosa among hospitalized patients at Dessie comprehensive specialized hospital, north-East Ethiopia. Infect. Drug Resist. 2022; (15) 895–911. doi: 10.2147/IDR.S358116

[9] HammoudiHalat, D and Ayoub Moubareck C. The current burden of carbapenemases: review of significant properties and dissemination among gramnegative Bacteria. Antibiotics. 2020; 9:186. doi: 10.3390/antibiotics9040186

[10] Caston JJ, Cano A, Perez-Camacho I, Aguado JM, Carratala J, Ramasco F, et al. Impact of ceftazidime/avibactam versus best available therapy on mortality from infections caused by Carbapenemase-producing Enterobacterales (Cavicor study). J. Antimicrob. Chemother. 2022; 77, 1452–1460. doi: 10.1093/jac/dkac049.

[11] Van Duin D. Carbapenem-resistant Enterobacteriaceae: what we know and what we need to know, vol. 8. Oxfordshire, UK: Taylor & Francis. 2017; 379–382

[12] Aleidan FA, Alkhelaifi H, Alsenaid A, Alromaizan H, Alsalham F, Almutairi A, et al. Incidence and risk factors of carbapenem-resistant Enterobacteriaceae infection in intensive care units: a matched case–control study. Expert Rev. Anti-Infect. Ther. 2021;19, 393–398. doi: 10.1080/14787210.2020.1822736

[13] Di Carlo P, Serra N, Lo Sauro S, Carelli VM, Giarratana M, Signorello JC, et al. Epidemiology and pattern of resistance of gram-negative Bacteria isolated from blood samples in hospitalized patients: a single center retrospective analysis from southern Italy. Antibiotics 2021; 10:1402. doi: 10.3390/antibiotics10111402

[14] Alemayehu E, Fiseha T, Gedefie A, Alemayehu Tesfaye N, Ebrahim H, Ebrahim E, et al. Prevalence of Carbapenemase-producing Enterobacteriaceae from human clinical samples in Ethiopia: a systematic review and Meta-analysis. BMC Infect. Dis. 2023; 23:277. doi: 10.1186/s12879-023-08237-5

[15] Nordmann P & Poirel L. Epidemiology and diagnostics of Carbapenem resistance in gram-negative Bacteria. Clin. Infect. Dis. 2019; 69, S521–S528. doi: 10.1093/cid/ciz824

[16] Shanmugakani RK, Srinivasan B, Glesby MJ, Westblade LF, Cárdenas WB, Raj T, et al. Current state of the art in rapid diagnostics for antimicrobial resistance. Lab Chip 2020; (20): 2607–2625. doi: 10.1039/D0LC00034E

[17] Rabaan AA, Eljaaly K, Alhumaid S, Albayat H, Al-Adsani W, Sabour AA., et al. An overview on phenotypic and genotypic characterisation of carbapenem resistan tenterobacterales. Medicina 2022; 58:1675. doi: 10.3390/medicina58111675

[18] Stewardson AJ, Marimuthu K, Sengupta S, Allignol A, El-Bouseary M, Carvalho MJ, et al. Effect of Carbapenem resistance on outcomes of bloodstream infection caused by Enterobacteriaceae in low-income and middle-income countries (panorama): a multinational prospective cohort study. Lancet Infect. Dis. (2019; (19):601–610. doi: 10.1016/S1473-3099(18)30792-8

[19] Okoche D, Asiimwe BB, Katabazi F A, Kato L, & Najjuka CF. Prevalence and characterization of carbapenem-resistant Enterobacteriaceae isolated from Mulago National Referral Hospital, Uganda. PLoS ONE, 2015; 10(8): e0135745. https://doi.org/10.1371/journal.pone.0135745

[20] Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 2016; 7:895. doi: 10.3389/fmicb.2016.00895.

[21] Mate PH, Devi KS, Devi KS, Devi MK, Damrolien S, Devi LN, et al., Prevalence of carbapenem resistance among Gram-negative bacteria in a tertiary care hospital in North-East India. IOSR J Dent Med Sci. 2014;13(12):56-60.

[22] Cheesbrough M. Medical Laboratory Manual for Tropical Countries, 2009; Vol.11: Microbiology. Cambridge University Press.

[23] Oli AN, Akabueze VB, Ezeudu CE, Eleje GU, Ejiofor OS, Ezebialu IU, et al. Bacteriology and antibiogram of urinary tract infection among female patients in a tertiary health facility in South Eastern Nigeria. Open Microbiol J. 2017; 11:292-300. doi: 10.2174/1874285801711010292

[24] CLSI. Performance standards for antimicrobial susceptibility testing.26th ed. Wayne (PA): Clinical and Laboratory standards Institute; 2016.

[25] CLSI. Performance standards for antimicrobial susceptibility testing.32nd edition. Wayne (PA): Clinical and Laboratory standards Institute; 2022.

[26] Shobowale EO, Ogunsola FT, Oduyebo OO & Ezeaka VIA. Study on the outcome of neonates with sepsis at the Lagos University Teaching Hospital. International Journal of Medicine and Biomedical Research. 2015;4(1): 41–49. https://doi.org/10.14194/ijmbr.4.1.6

[27] Nordmann P, Dortet L & Poirel L. Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends in Molecular Medicine, 2012; 18(5): 263–272.

[28] Vading M, Samuelson, Haldorsen B, Sundsfjord AS, and Giske CG. Comparison of disk diffusion,Etest and VITEK2 for detection of Carbapenemase-producing Klebsiella peumoniae with the EUCAST and CLSI breakpoint system. Clinical Microbiology and Infections.2011;17(5):668-674. https://doi.org//10.1111/j.1469-0691.2010.03299.x

[29] Egbuna RN, Ujam NT, Chukwunwejim CR, Eze NK, Okafor UU, Abonyi IC, et al. Presence of Multidrug-Resistant Gram-Negative Bacteria in Some Orally-Administered Herbal Preparations Sold in South-Eastern Nigeria. Journal Of Current Biomedical Research. 2011.

[30] EUCAST. EUCAST breakpoints tables for interpretation of antimicrobial susceptibility tests. Version 6.0. 2016. Available from: https://www.eucast.org/bacteria/clinical-breakpoints-and-interpretation/clinical-breakpoint-tables/

[31] EUCAST European committee on antimicrobial susceptibility testing, breakpoint tables for interpretation of Mics and zone diameters. European Society of Clinical Microbiology and Infectious Diseases: 2021; Basel

[32] Shaikh N, Morone NE, Bost JE & Farrell MH. Prevalence of urinary tract infection in childhood: A meta-analysis. Pediatric Infectious Disease Journal, 2015; 27(4): 302–308.

[33] Logan LK & Weinstein R A. The epidemiology of carbapenem-resistant Enterobacteriaceae: The impact and evolution of a global menace. Journal of Infectious Diseases, 2017; 215(S1): S28-S36.

[34] Zamani M, Kachuei R, & Moosavian M. Prevalence of multidrug-resistant Enterobacteriaceae in neonatal and paediatric intensive care units. Infection and Drug Resistance, 2021; (14): 2265–2275.