Anti-Infective Agents, 2025, *23*, e22113525363630

CROSS SECTIONAL STUDY

Analysis of Carbapenemase and Extended-spectrum Beta-lactamases-producing Cephalosporin-resistant Strain of *Escherichia coli* and *Klebsiella pneumoniae* among Children in a Tertiary Care Centre, South India

Santhosh J. Thattil[1,2], Thekkuttuparambil A. Ajith[3] and Suresh Dhanaraj[1,*]

[1]*Department of Microbiology, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, 600117, Tamil Nadu, India;* [2]*Department of Microbiology, Nyle Hospital, Kaiparambu, Thrissur, 680546, Kerala, India;* [3]*Department of Biochemistry and Amala Integrated Medical Research, Amala Institute of Medical Sciences, Amala Nagar, Thrissur, 680555, Kerala, India*

ARTICLE HISTORY

Received: November 08, 2024
Revised: January 16, 2025
Accepted: January 21, 2025

DOI:
10.2174/0122113525363630250203053527

Abstract: ***Introduction*:** The incidence of Extended-spectrum Beta-lactamase (ESBL)- and carbapenemase-producing enterobacteriaceae in enterobacterales is increasing globally, posing a threat to health. Schoolchildren under adolescence show a gradual increase in immunity, and respiratory infections like pneumonia and others are more prevalent in children under five-years of age. However, gastrointestinal disorders primarily affect children under one-year of age.

***Aims*:** The study aimed to analyze the ESBL and carbapenemase-producing cephalosporin-resistant strain of *Escherichia coli* and *Klebsiella pneumoniae*, isolated from various specimens of hospitalized children below ten years of age.

***Methods*:** A cross-sectional study on laboratory reports of positive culture specimens from children below ten years of age was conducted to analyze the cephalosporin-resistant strains of *E. coli* and *K. pneumoniae* between July, 2023 and July, 2024 in the Microbiology Department of a tertiary care hospital central Kerala, South India. The cephalosporin-resistant strain was analyzed for carbapenemase or ESBL presence using a double disc synergy test. The data were subjected to statistical analysis.

***Results and Discussion*:** A total of 1636 culture reports were analyzed, and growth was found in 672 (41%) specimens. Among the cephalosporin-resistant 187 strains (66%), *E. coli* and *K. pneumoniae* were 106 (57%) and 81 (43%), respectively. A significant difference (p <0.05) was noted between cephalosporin-resistant and sensitive *E. coli* among the various age groups. The difference between cephalosporin-resistant and susceptible *K. pneumoniae* across different age groups, however, was negligible (p>0.05). Carbapenemase and ESBL-producing *E. coli* and *K. pneumoniae* were 39%, 29%, and 48%, respectively. The carbapenemase and ESBL-producing strains were more in children below one year. An insignificant difference (p>0.05) between ESBL (+) and ESBL (-) *E. coli* or *K. pneumoniae* was found among various age groups. A similar observation was found between carbapenemase [+] and carbapenemase (-) *E. coli* or *K. pneumoniae*. According to the effect value (Cramer's V) determined for each comparison, the association was either very weak or negligible.

***Conclusion*:** The cephalosporin-resistant strains (66%) of *E. coli* and *K. pneumoniae* were 57% and 43%, respectively. Specimens of children below one year of age showed more incidence of ESBL-producing *K. pneumonia* (67%) and carbapenemase-producing *E. coli* (68%).

Keywords: Carbapenemase, enterobacteriaceae, extended-spectrum beta-lactamase, *Escherichia coli*, *Klebsiella pneumoniae*, statistical analysis.

1. INTRODUCTION

The advent of multidrug-resistant bacterial infections has been demonstrated in recent research, indicating the need for cautious antibiotic administration and laboratory-based patient care [1]. It has become more commonplace globally, posing a threat to public health. According to World Health Organization estimates, by 2050, antibiotic resistance-related deaths will account for 10 million annual deaths worldwide if appropriate control and preventive measures are not implemented. Gram-negative Enterobacteriaceae in enterobacterales, such as *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*), are commonly treated with β-lactam antibiotics. Carbapenems are the first-choice medication when treating *K. pneumoniae*-related infections associated

*Address correspondence to this author at the Department of Microbiology, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, 600117, Tamil Nadu, India;
E-mail: sureshdhanaraj.sls@velsuniv.ac.in

2211-3533/25 $65.00+.00

with healthcare settings. The frequent use of broad-spectrum antibiotics and carbapenems in hospitals facilitates the development of these resistant strains. Numerous mechanisms are known to contribute to resistance, including the formation of metallo-β-lactamases (MBLs) and extended-spectrum β-lactamases (ESBLs). Depending on the study population, carbapenemase-producing Enterobacteriaceae in enterobacterales can cause significant infections in immunocompromised and disabled individuals. Longer hospital stays and increased mortality rates—which can approach 70%—are associated with these illnesses [2]. Carbapenemase genes have been acquired by *K. pneumoniae* and *E. coli* to demonstrate drug resistance [3]. Despite efforts to manage infections and steward drugs, the prevalence of ESBL and carbapenemase-producing bacteria is rising, and incurable infections are developing [4, 5]. Previous studies have shown that individuals who undergo empirical treatment only for UTIs develop more drug resistance, making it more challenging to treat bacterial UTIs that are acquired in hospitals and community [1, 6].

Reducing the empirical drug use can be achieved by comprehending the growth of ESBL and carbapenemase-resistant bacteria. In research, children also showed serious treatment complications from ESBL-producing *E. coli* and *K. pneumoniae* [7]. The ESBL-producing strains showed resistance to third-generation cephalosporins, such as ceftriaxone (100%), cefotaxime (100%), and ceftazidime (70.6%) [8]. Devi *et al*. demonstrated the presence of Gram-negative bacilli that produce carbapenemase and ESBL among neonates in a tertiary care hospital in Dibrugarh, Assam, India [9]. Enterobacteriaceae in enterobacterales are the common producers of MBL and ESBL [10]. As a steady rise in antibiotic-resistant cases is observed worldwide, which is associated with geographical variations, clinicians face a dilemma in using first-line antibiotics while treating newborn sepsis or meningitis in children. The majority of studies on bacteria that produce carbapenemase and ESBL were conducted on adult subjects. There are limited studies available in this area to determine the prevalence of *E. coli* and *K. pneumoniae* that produce carbapenemase and ESBL in children of various ages. We had previously shown that 42% of the *E. coli* and *K. pneumoniae* isolates in children under ten years of age were ESBL producers, and 52% of the isolates were cephalosporin-resistant [11]. The study did not, however, take into account carbapenemase or ESBL producers in various age groups under the age of ten or adolescence. Thus, this study aimed to analyze the ESBL and carbapenemase-producing cephalosporin-resistant strain of *K. pneumoniae* and *E. coli* isolated from various specimens of hospitalized children at a tertiary care hospital in Central Kerala.

2. MATERIALS AND METHODS

2.1. Study Design

A cross-sectional study was designed using the laboratory data of hospitalized children in a tertiary care centre in central Kerala between July 2023 and July 2024. The study included children aged between one day to ten years with positive cultures of *E. coli* or *K. pneumoniae* resistant to cephalosporin isolated from specimens, such as urine, blood, throat swabs, cerebrospinal fluid, and respiratory aspirate. Positive cultures other than *E. coli* and *K. pneumoniae* and children above ten years of age were excluded. The research was carried out in compliance with the 1975 Helsinki Declaration, which underwent revisions in 2013. As it is a retrospective study on laboratory reports, consent was waived, and the study was approved by the institutional ethics committee (VISTAS-SPS/IE/1/2022/07).

2.2. Sample Size Calculation

The sample size was calculated based on the previous study by Thattle *et al*. [11]. The prevalence (p) of ESBL positive cephalosporin-resistant *E. coli* and *K. pneumoniae* strains was 42%, with relative precision (d) 20% of p and Zα-1 of 1.96, and the minimum sample size was calculated as 138 using the formula $N= Z\alpha\text{-}1^{2} pq/d^{2}$.

2.3. Study Procedure

Data were collected from the laboratory register of the Microbiology department. The standard operating procedures were followed for the collection, processing, culture, identification of organisms, and testing for antibiotic sensitivity.

The approach of Forbes *et al*. was used to identify the bacteria [12]. The guidelines of the Clinical and Laboratory Standards Institute and the technique of Bauer *et al*. were followed for testing antibiotic sensitivity [13, 14]. In the Kirby-Bauer disc diffusion method used in testing the antibiotic sensitivity, a zone diameter of <23 mm against the third-generation cephalosporin, cefotaxime was defined as resistant to cephalosporin. The resistant strains were further tested for ESBL and carbapenemase production using a double disc synergy test.

ESBL presence was tested using Amoxicillin/clavulanic acid and cefotaxime disc, which were placed 20 mm apart on lawn cultures (made by spreading 0.5 Mac Farland bacterial inoculum) prepared on Muller-Hinton agar plates. After incubating the plates at 37°C overnight, an enhancement in the zone of inhibition between amoxicillin/clavulanic acid and cephalosporins antibiotics disc was interpreted as ESBL-positive [15, 16]. For carbapenemase testing, an imipenem disc at the center and a disc carrying ten-microlitre (750 μg) ethylenediaminetetraacetic acid were placed 20 mm apart. After incubation overnight at 37°C, the presence of an inhibitory zone was interpreted as carbapenemase-positive [17]. The standard tester stains of *K. pneumonia* (ATCC 700603) and *E. coli* (ATCC 25922) were used to check the quality assurance. Children under the age of ten were divided into three age groups: under one year, one to five years, and five to ten years.

2.4. Statistical Analysis

Data were expressed in frequency and percent. A statistical package for social sciences (v16, IBM, Illinois, US) was used for the analysis. To examine the significant difference between the positive culture specimen's age and gender, the chi-square (X^2) test of independence was used. $p<0.05$ was considered significant. The Creamer's V effect size (ES) value was used for the chi-square test of independence to determine the strength of association between the variables. In a 2x2 con-

tingency table, an ES value around 0 denotes little to no correlation, and a value near 1 denotes higher associations between two category variables. For a 3x2 contingency table, a value of 0.1 to 0.2 suggests a weak association, while 0.2 to 0.4 indicates a moderate association, and values greater than 0.4 signify a strong association.

3. RESULTS

A total of 1636 specimen culture reports were analyzed during the study period and found growth in 672 (41%) samples. STROBE flow chart of sample collection is depicted in Fig. (**1**). Among the total specimens that showed growth, 144 (21%) and 141 (20%) samples were *E. coli* and *K. pneumoniae,* respectively (Fig. **2**). The specimens from males and females in the *E. coli* positive culture (144) were 82 (67%) and 62 (43%), respectively. Out of 141 *K. pneumoniae*-positive specimens, 86 (61%) were from males and 55 (39%) were from females. No significant difference was found between gender and culture positivity (X^2 =0.4825, p = .487285). Among the cephalosporin-resistant 187 strains (66%), *E. coli* and *K. pneumoniae* were 106 (57%) and 81 (43%), respectively. The remaining 98 (34%) strains (*E. coli*, 39% and *K. pneumoniae*, 61%) were sensitive to cephalosporin (Fig. **3**). A significant difference was found between resistant strains of *E. coli* and *K. pneumoniae* (X^2 =8.2504, p = .004074). However, the Cramer's effect size value (0.1701) revealed a relatively weak strength of association between the variables.

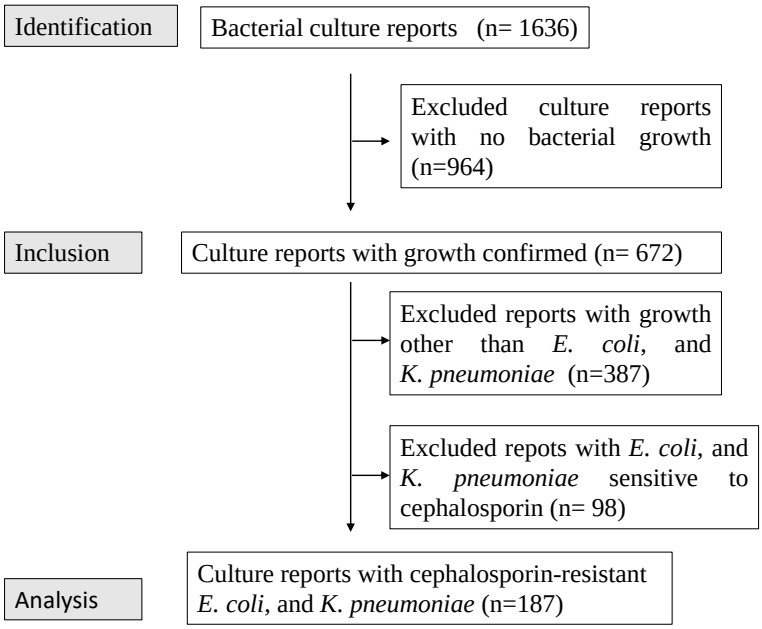

Fig. (1). STROBE flow chart of sample collection and analysis. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

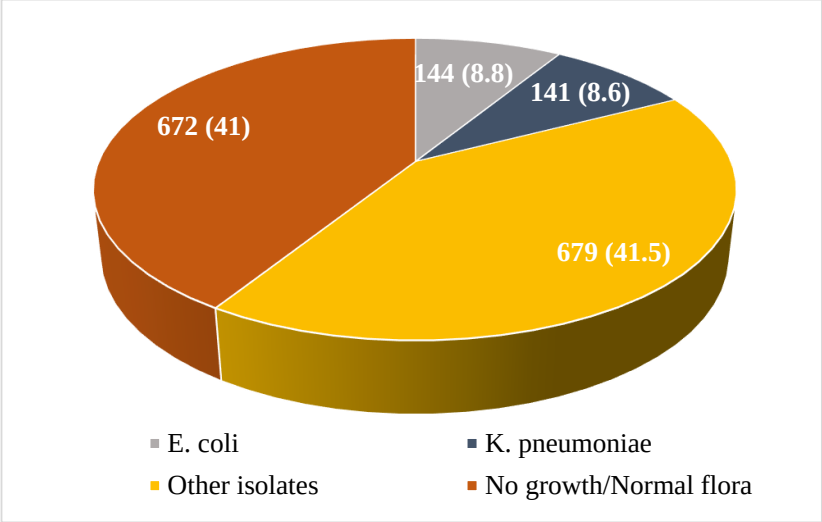

Fig. (2). Distribution frequency of bacterial strains in samples collected from children below ten years of age. The percentage is given in parentheses. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

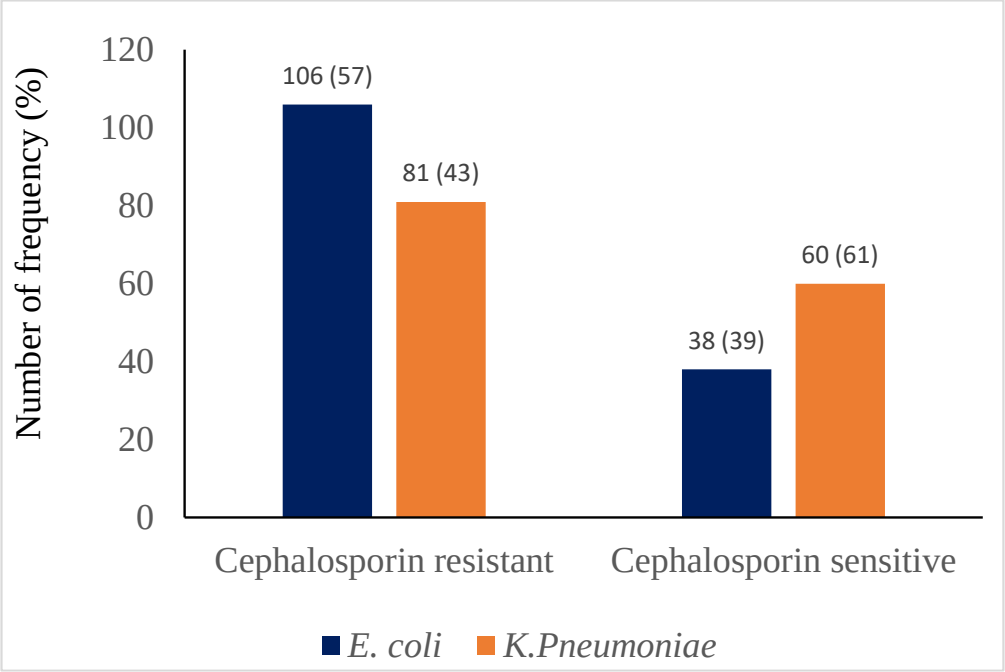

Fig. (3). Distribution of cephalosporin-resistant and sensitive *E. coli* and *K. pneumoniae.* X^2 (1, N=285) = 8.2504, p = .004074; a significant difference was found between resistant strains of *E. coli* and *K. pneumoniae.* Cramer's effect size = 0.1701 (95% CI, 0.1502-0.1898) indicated a weak association between variables. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

Table 1. Distribution of cephalosporin-resistant and sensitive *E. coli* and *K. pneumoniae* among children in various age groups.

Age (Years)	Cephalosporin Resistant *E. coli* (%)	Cephalosporin Sensitive *E. coli* (%)	Cephalosporin-resistant *K. pneumoniae* (%)	Cephalosporin-sensitive *K. pneumoniae* (%)
Below 1	65 (62)	15 (38)	53 (65)	30 (51)
1-5	26 (25)	15 (38)	20 (24)	25 (42)
5-10	14 (13)	9 (23)	9 (11)	4 (7)
Total	105 (73)	39 (27)	82 (58)	59 (42)

Note: X^2 (2, N=144) = 6.3779, p=0.04121 significant difference between cephalosporin-resistant and sensitive *E. coli* among various age groups; effect size 0.2106 (95% CL, 0.1761, 0.2451) indicated weak association between variables. X^2 (2, N=141) =5.2397, p=0.07281 insignificant difference between cephalosporin-resistant and sensitive *K. pneumoniae* among various age groups. Cramer's V effect 0.1928, (95% CL, 0.1609 to 0.2243) indicates a small to moderate association between variables.

The distribution of cephalosporin-resistant and sensitive *E. coli* and *K. pneumoniae* among various age groups is depicted in Table **1**. Cephalosporin-resistant *E coli* was found to be 62% in children below one year of age, whereas cephalosporin-resistant *K. pneumoniae* was 65%, which is more prevalent. A significant difference (p <0.05) was noted between cephalosporin-resistant and sensitive *E. coli* among various age groups. While the difference between *K. pneumoniae*-producing cephalosporin-resistant and sensitive strains was statistically insignificant (p>0.05). Similarly, no statistically significant difference was found between the cephalosporin-resistant *E. coli* and *K. pneumoniae* in the age groups studied (Chi-square = 0.265, p= .875887). Among the 105-cephalosporin resistant *E. coli,* ESBL producing strain (64%) was more in children below one- year of age (Table **2**). In the same age group, among the 82 isolates of cephalosporin-resistant *K. pneumoniae,* the ESBL-producing strain was 67%. No statistically significant (p>0.05) difference was found among the ESBL-producing strains in the age groups. Furthermore, the difference between ESBL [+] *E. coli* and *K. pneumoniae* in the age groups was statistically insignificant (Chi-square= 0.0911, p = .955471). Carbapenemase-producing strains of *E. coli* and *K. pneumonia* are depicted in Table **3**. A greater number of carbapenemase-producing strains of *E. coli* were found in children below one year of age. No statistically significant difference was found between the strains in any age group (p>0.05). Furthermore, the difference between carbapenemase-producing strains of *E. coli* and *K. pneumonia* among various age groups was insignificant (Chi-square = 0.165, p = .92079). According to the effect value (Cramer's V) determined for each comparison, the association was either very weak or negligible.

Table 2. Distribution of ESBL producing strains of *E. coli* and *K. pneumoniae* among children in various age groups.

Age (Years)	ESBL [+] *E. coli* (%)	ESBL [-] *E. coli* (%)	ESBL [+] *K. pneumoniae* (%)	ESBL [-] *K. pneumoniae* (%)
Below 1	32 (64)	33 (66)	26 (67)	27 (63)
1-5 y	12 (24)	14 (25)	9 (23)	11 (26)
5-10 y	6 (12)	8 (15)	4 (10)	5 (12)
Total	50 (48)	55 (52)	39 (48)	43 (52)

Note: X^2 (2, N=105) =0.2173, p=0.89702) insignificant difference between ESBL (+) and ESBL (-) *E. coli* among various age groups. Cramer's V effect 0.0455, (95% CL, 0, 0.131) indicates a very weak association between variables. X^2 (2, N=82) =0.13517, p=0.93464) insignificant difference between ESBL (+) and ESBL (-) *K. pneumoniae* among various age groups. Cramer's V effect 0.0455, (95% CL, 0.0318 to 0.0494) indicates a very weak association between variables.

Table 3. Distribution of carbapenemase-producing *E. coli* and *K. pneumoniae* among various age groups.

Age (Years)	Carbapenemase [+] *E. coli* (%)	Carbapenemase [-] *E. coli* (%)	Carbapenemase [+] *K. pneumoniae* (%)	Carbapenemase [-] *K. pneumoniae* (%)
Below 1	28 (68)	37 (58)	12 (63)	41 (65)
1-5	9 (22)	17 (27)	5 (26)	15 (24)
5-10	4 (10)	10 (16)	2 (11)	7 (11)
Total	41 (39)	64 (61)	19 (23)	63 (77)

Note: X^2 (2, N=105) =1.30357, p= 0.52111) insignificant difference between carbapenemase [+] and carbapenemase (-) *E. coli* among various age groups. Cramer's V effect 0.1114, [%95CL, 0.0900 to 0.1328] indicates a very weak association between variables. X^2 (2, N=82) =0.05048, p=0.97507) insignificant difference between carbapenemase [+] and carbapenemase (-) *K. pneumoniae* among various age groups. Cramer's V effect 0.0248, [%95CL, 0.0193 to 0.0303] indicates a very weak association between variables.

4. DISCUSSION

The findings of the study detailed the prevalence of *K. pneumonia* and *E. coli* in different age groups of children under ten. Overall, the incidence of cephalosporin-resistant *E. coli* (57%) was higher than that of *K. pneumonia* (43%). Cephalosporin-resistant *K. pneumoniae* (65%) and *E. coli* (62%) were found more in children with the age group below one year. However, no significant difference was observed between carbapenemase or ESBL-producing strains of *E. coli* and *K. pneumonia* in any of the age groups studied. Cephalosporin-resistant *E. coli* strains were shown to be 69% prevalent in newborns in a previous investigation [18], which is found consistent with the result of this study (62%). Similarly, a prior study in children under five years of age revealed that 31% of the identified *E. coli* strains were resistant to imipenem [18], while in the present study, it becomes 35% (37/105), and this indicates a progressive increase in carbapenem resistance.

The subjects were categorized as infants (below one year of age), toddlers and preschoolers (1 to 5 years of age), and school children below adolescent (below 5 to 10 years of age) [19]. This classification was chosen in order to identify three child phases where a higher likelihood of infection was suspected. A previous study reported that the prevalence of pneumonia was most common among children aged < 10 years. In comparison, respiratory infection was more common among children below five- years of age. Gastrointestinal and bloodborne infections mostly affect children aged < 1 year and 1–3 years, respectively [20].

A retrospective study conducted among children with UTI in southern China demonstrated that both *K. pneumoniae* and *E. coli* that produce ESBLs have resistance rates (greater than 98%) to cephalosporin drugs, such as cefpodoxime, cefaclor, cefazolin, and ceftriaxone, with a higher prevalence for *E. coli* [21]. The intestinal-dwelling bacteria, *Klebsiella* spp., have been found to carry multidrug resistance plasmids and have high carriage rates on patient hands and nasopharynges [22]. Carbapenemase production was detected in isolates of *Klebsiella* (66.7%) and the lone isolate of *E. coli.* The development of resistance to carbapenem can also be due to the production of extended-spectrum cephalosporin-like Amp C [9].

In this investigation, *E. coli* and *K. pneumoniae* that generate carbapenemase were detected in 39% and 29% of children under the age of ten, respectively, while ESBL producers were found in 48%. Among the ESBL producers, *K. pneumonia* (67%) and among the carbapenemase producers, *E. coli* (68%) were observed more in age groups below one year. According to our earlier research, 42% of children under the age of ten were ESBL producers of *K. pneumoniae* and *E. coli* [11]. Approximately 60% of the *Klebsiella* isolates and 75% of the *E. coli* isolates developed ESBLs, according to a study conducted in India on neonates with septicemia [23]. A study of neonatal sepsis in West India showed that 95% and 92% of the bacteria were ESBL-producing, respectively, *K. pneumoniae* and *E. coli* [24]. According to Vijayakanthi *et al*., the most common ESBL producers in neonates were *Klebsiella* (60%) and E. coli (30%) [25]. We found ESBL producer *Klebsiella* (67%) followed by *E. coli* (64%) in neonates.

Antibiotic use is directly linked to the occurrence of ESBLs and carbapenemases in bacteria, especially when antibiotics, such as cephalosporins, carbapenems, and fluoroquinolones

are misused or overused. The overuse and abuse of these antibiotics put bacterial populations under selective pressure, which helps resistant types of bacteria survive and proliferate [26]. A prior study found that a statistically and clinically significant increase in the incidence of ESBL-positive *K. pneumoniae* strains was linked to increased use of third-generation cephalosporins [27]. Resistance was caused by the rapid accumulation of chromosomal mutations along with the insertion of genetic elements encoding resistance. The mobility of genetic elements carrying carbapenemase genes contributes to their increased dissemination. Carbapenem resistance is rising as a result of the effective growth of Enterobacteriaceae clonal groupings and the frequent horizontal gene transfer of plasmids encoding carbapenemase [28]. It was demonstrated that the bla-oxacillin hydrolyzing enzymes-48 gene is connected to an effective and widespread plasmid that conjugates at high rates within the enterobacteriaceae family [29]. A recent study demonstrated that the prevalence of carbapenemase genes was higher in the age group of 0-21 years (45.8%) followed by 21-40 years (27%). This indicated their prevalence among youngsters. The common carbapenemase-producing gene was New Delhi metallo-β-lactamase in *K. pneumoniae* (58.2%) and *E. coli* (22.4%) [30]. Genes like CTX-M-15 and CTX-M-1 were previously found to be prevalent in ESBL-positive *E. coli*, whereas CTX-M-U and CTX-M-15 were found to be prevalent in *K. pneumoniae* in specimens taken from children under the age of ten [11]. *K. pneumoniae* evolved into the indicator species for plasmid-encoding genes imparting resistance to medicines other than β-lactams, as well as ESBLs, primarily TEMs and SHVs, active against more recent cephalosporins [22].

The rise of β-lactamases producers due to the use of β-lactam antibiotics is a major issue for physicians as these bacteria are resistant to several drugs, which can lead to therapeutic failure. For such ESBL-producing organisms and life-threatening infections, carbapenems are the preferred medication. This has most likely caused the organisms to become resistant to carbapenem, posing yet another significant therapeutic issue. The current investigation also concluded that the isolates that were Gram-negative were resistant to carbapenem. Carbapenemase was confirmed in 66.7% of the *Klebsiella* isolates in neonates [9]. In this study, we found that 63% of *Klebsiella* isolates from infants were carbapenemase producers. Enterobacteriaceae in enterobacterales that produce carbapenemase primarily target hospitalized patients and frequent visitors, but they are also increasingly seen in long-term care institutions [31]. These bacteria are frequently treated with tigecycline and colistin. The novel β-lactamase inhibitor, when combined with either ceftazidime or aztreonam, is a promising treatment for carbapenem-resistant Enterobacteriaceae, in contrast to the traditional regimens of colistin or tigecycline and avibactam [24]. The prevalence of ESBL production of *K. pneumoniae* and *E. coli* was successfully reduced by substituting piperacillin/tazobactam for extended-spectrum cephalosporins [32].

A diversified strategy is needed to defeat antimicrobial resistance (AMR). This entails avoiding the unnecessary prescription of antibiotics, using the right dosage, duration, and administration method in accordance with clinical guidelines, using the narrowest spectrum of antibiotics that are effective against the identified pathogen, de-escalating or stopping therapy when necessary, and educating patients about the importance of completing the prescribed antibiotic course and non-antibiotic alternatives in cases of mild infection. It will be helpful to create and execute an antibiotic stewardship program in a clinical environment, monitor local trends of resistance, and adjust prescribing practices accordingly. Additional measures include following infection control procedures like isolating infected patients, sterilizing procedures, and practicing good hand hygiene; taking part in local, national, and international programs to monitor AMR trends; staying up to date on AMR trends through frequent review of guidelines; and supporting public health campaigns to increase awareness of AMR. To direct treatment and stop the spread of resistant diseases, a mix of molecular analysis, microbiological testing, and suitable clinical management techniques is essential. Hence, working together with infection control experts, pharmacists, and microbiologists can provide comprehensive treatment.

5. LIMITATIONS OF THE STUDY

The present investigation was constrained by its small sample size and monocentric and retrospective study design. Even though it could demonstrate the rise of ESBL and carbapenemase among frequent bacterial isolates in children, no molecular characterization was done for the presence of carbapenemase-encoding genes. Hence, future research directions, such as multicentric studies, longitudinal analyses to track resistance trends, or incorporating next-generation sequencing to uncover the genetic landscape of resistance, are warranted.

CONCLUSION

The cephalosporin-resistant strains (66%) of *E. coli* and *K. pneumoniae* were 57% and 43%, respectively. The resistant *K. pneumoniae* and *E. coli* were more in children of age below one year, with a frequency of ESBL-producing *K. pneumonia* (67%) and carbapenemase-producing *E. coli* (68%). Therefore, physicians should consider the rise of carbapenemase-producing *E. coli* and ESBL-positive *K. pneumoniae* when selecting antibiotics as an empirical treatment for children mainly below one year of age. This emphasizes the necessity of careful antibiotic stewardship, efficient infection control procedures, and routine surveillance.

AUTHORS' CONTRIBUTIONS

The study conception and design, along with the analysis and interpretation of results and manuscript draft, were contributed by SJT, TAA, and SD. Data collection was performed by SJT. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

AMR	=	Antimicrobial Resistance
CLSI	=	Clinical and Laboratory Standards Institute
CTX-M	=	Cifotaxime-munich
ESBL	=	Extended-spectrum Beta-lactamase

MBL	=	Metallo-β-lactamases
PER	=	Pseudomonas Extended Resistance
SHV	=	Sulphydral Variable
TEM	=	Temoniera
UTI	=	Urinary Tract Infection

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the Institutional ethics committee of Vels Institute of Science, Technology and Advanced Studies, India (VISTAS-SPS/IES/I/2022/07).

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Informed consent was the waived by the institutional ethics committee, due to retro scopic nature of the study.

STANDARDS OF REPORTING

STROBE guidelines were followed.

AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this study are available within the article.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Shafiq, M.; Zeng, M.; Permana, B.; Bilal, H.; Huang, J.; Yao, F.; Algammal, A.M.; Li, X.; Yuan, Y.; Jiao, X. Coexistence of blaNDM–5 and tet(X4) in international high-risk *Escherichia coli* clone ST648 of human origin in China. *Front. Microbiol.,* **2022**, *13*, 1031688.
http://dx.doi.org/10.3389/fmicb.2022.1031688

[2] Zarkotou, O.; Pournaras, S.; Tselioti, P.; Dragoumanos, V.; Pitiriga, V.; Ranellou, K.; Prekates, A.; Themeli-Digalaki, K.; Tsakris, A. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clin. Microbiol. Infect.,* **2011**, *17*(12), 1798-1803.
http://dx.doi.org/10.1111/j.1469-0691.2011.03514.x
PMID: 21595793

[3] Tzouvelekis, L.S.; Markogiannakis, A.; Psichogiou, M.; Tassios, P.T.; Daikos, G.L. Carbapenemases in *Klebsiella pneumoniae* and other Enterobacteriaceae: An evolving crisis of global dimensions. *Clin. Microbiol. Rev.,* **2012**, *25*(4), 682-707.
http://dx.doi.org/10.1128/CMR.05035-11 PMID: 23034326

[4] Bonomo, R.A.; Burd, E.M.; Conly, J.; Limbago, B.M.; Poirel, L.; Segre, J.A.; Westblade, L.F. Carbapenemase-producing organisms: A global scourge. *Clin. Infect. Dis.,* **2018**, *66*(8), 1290-1297.
http://dx.doi.org/10.1093/cid/cix893 PMID: 29165604

[5] Li, C.; Claeys, K.C.; Justo, J.A. No Crystal Ball? Using risk factors and scoring systems to predict extended-spectrum beta-lactamase producing Enterobacterales (ESBL-E) and Carbapenem-Resistant Enterobacterales (CRE) infections. *Entropy (Basel),* **2022**, *24*, 147-158.

[6] Patel, G.; Bonomo, R.A. Status report on carbapenemases: Challenges and prospects. *Expert Rev. Anti Infect. Ther.,* **2011**, *9*(5), 555-570.
http://dx.doi.org/10.1586/eri.11.28 PMID: 21609267

[7] Castanheira, M.; Mills, J.C.; Costello, S.E.; Jones, R.N.; Sader, H.S. Ceftazidime-avibactam activity tested against *Enterobacteriaceae* isolates from U.S. hospitals (2011 to 2013) and characterization of β-lactamase-producing strains. *Antimicrob. Agents Chemother.,* **2015**, *59*(6), 3509-3517.
http://dx.doi.org/10.1128/AAC.00163-15 PMID: 25845862

[8] Abayneh, M; Tesfaw, G; Abdissa, A. Isolation of extended-spectrum β-lactamase-(ESBL-) producing *Escherichia coli* and *Klebsiella pneumoniae* from patients with community-onset urinary tract infections in Jimma University Specialized Hospital, Southwest Ethiopia. *Canadian J. Infect. Dis. amp., 2018***2018**, , 4846159.
http://dx.doi.org/10.1155/2018/4846159

[9] Devi, U.; Bora, R.; Das, J.K.; Mahanta, J. Extended-spectrum β-lactamase & carbapenemase-producing Gram-negative bacilli in neonates from a tertiary care centre in Dibrugarh, Assam, India. *Indian J. Med. Res.,* **2018**, *147*(1), 110-114.
http://dx.doi.org/10.4103/ijmr.IJMR_1288_16 PMID: 29749369

[10] Mathur, P.; Kapil, A.; Das, B.; Dhawan, B. The prevalence of ESBL producing gram negative bacteria in a tertiary care hospital. *Indian J. Med. Res.,* **2002**, *115*, 153-157.
PMID: 12239838

[11] Thattil, S.J.; Dhanaraj, S.; Ajith, T.A. Molecular characteristics of Cephalosporin resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from children below ten years of age. *Antiinfect. Agents,* **2024**, *22*, 77-83.
http://dx.doi.org/10.2174/0122113525296665240304071400

[12] Forbes, B.A.; Sahm, D.F.; Weissfeld, A.S. Bacteriology. In: *Bailey and Scott's diagnostic Microbiology,* 12th ed; Mosby, Elsevier: St. Louis, Missouri, **2007**, 216-533.

[13] Bauer, A.W.; Kirby, W.M.M.; Sherris, J.C.; Turck, M. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.,* **1966**, *45*(4_ts), 493-496.
http://dx.doi.org/10.1093/ajcp/45.4_ts.493 PMID: 5325707

[14] *Peter, S. Performance standards for antimicrobial susceptibility testing,* 29th Ed; Clinical and laboratory standards Institute, **2019**.

[15] Duttaroy, B.; Mehta, S. Extended spectrum b lactamases (ESBL) in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli*. *Indian J. Pathol. Microbiol.,* **2005**, *48*(1), 45-48.
PMID: 16758790

[16] Datta, P.; Thakur, A.; Mishra, B.; Gupta, V. Prevalence of clinical strains resistant to various beta-lactams in a tertiary care hospital in India. *Jpn. J. Infect. Dis.,* **2004**, *57*(4), 146-149.
PMID: 15329445

[17] Suryavanshi, K.T.; Lahiri, K.K. Assessment of Rapidec Carba NP test, as screening test to detect carbapenemase, specially metallo-β-lactamase producing Gram negative bacteria at tertiary care centre. *Int. J. Health Sci. Res.,* **2018**, *8*, 56-61.

[18] Thattil, S.J.; Ajith, T.A. Emergence of third generation cephalosporin resistant *Escherichia coli* in Infants: Retrospective study from a tertiary care hospital in South India. *Antiinfect. Agents,* **2021**, *19*(2), 215-219.
http://dx.doi.org/10.2174/2211352518999200925153722

[19] Jaworska, N.; MacQueen, G. Adolescence as a unique developmental period. *J. Psychiatry. Neurosci.,* **2015**, *40*(5), 291-293.
http://dx.doi.org/10.1503/jpn.150268 PMID: 26290063

[20] Wang, W.; Wang, H.; Song, K.; Wang, B.; Xue, F.; Zhao, L.; Cao, W. Epidemiological features of infectious diseases in children and

adolescents: A population-based observational study in shandong province, China, 2013–2017. *Children ,* **2024**, *11*(3), 309. http://dx.doi.org/10.3390/children11030309 PMID: 38539344

[21] Keshi, L.; Weiwei, X.; Shoulin, L.; Xiaodong, L.; Hao, W.; Junhai, J.; Xiangwei, W.; Rui, W.; Pei, Z. Analysis of drug resistance of extended-spectrum beta-lactamases-producing *Escherichia coli* and *Klebsiella pneumoniae* in children with urinary tract infection. *Saudi Med. J.,* **2019**, *40*(11), 1111-1115. http://dx.doi.org/10.15537/smj.2019.11.24547 PMID: 31707407

[22] Podschun, R.; Ullmann, U. Klebsiella spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin. Microbiol. Rev.,* **1998**, *11*(4), 589-603. http://dx.doi.org/10.1128/CMR.11.4.589 PMID: 9767057

[23] Roy, S.; Gaind, R.; Chellani, H.; Mohanty, S.; Datta, S.; Singh, A.K.; Basu, S. Neonatal septicaemia caused by diverse clones of *Klebsiella pneumoniae* & *Escherichia coli* harbouring blaCTX-M-15. *Indian J. Med. Res.,* **2013**, *137*(4), 791-799. PMID: 23703349

[24] Patel, D.; Nimbalkar, A.; Sethi, A.; Kungwani, A.; Nimbalkar, S. Blood culture isolates in neonatal sepsis and their sensitivity in Anand District of India. *Indian J. Pediatr.,* **2014**, *81*(8), 785-790. http://dx.doi.org/10.1007/s12098-013-1314-2 PMID: 24408399

[25] Vijayakanthi, N.; Bahl, D.; Kaur, N.; Maria, A.; Dubey, N.K. Frequency and characteristics of infections caused by extended-spectrum beta-lactamase-producing organisms in neonates: A prospective cohort study. *BioMed Res. Int.,* **2013**, *2013*, 1-8. http://dx.doi.org/10.1155/2013/756209 PMID: 24175299

[26] Mancuso, G.; Midiri, A.; Gerace, E.; Biondo, C. Bacterial antibiotic resistance: The most critical pathogens. *Pathogens,* **2021**, *10*(10), 1310. http://dx.doi.org/10.3390/pathogens10101310 PMID: 34684258

[27] Urbánek, K.; Kolář, M.; Lovečková, Y.; Strojil, J.; Šantavá, L. Influence of third-generation cephalosporin utilization on the occurrence of ESBL-positive *Klebsiella pneumoniae* strains. *J. Clin. Pharm. Ther.,* **2007**, *32*(4), 403-408. http://dx.doi.org/10.1111/j.1365-2710.2007.00836.x PMID: 17635342

[28] Potter, R.F.; D'Souza, A.W.; Dantas, G. The rapid spread of carbapenem-resistant Enterobacteriaceae. *Drug Resist. Updat.,* **2016**, *29*, 30-46. http://dx.doi.org/10.1016/j.drup.2016.09.002 PMID: 27912842

[29] Potron, A.; Poirel, L.; Nordmann, P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* : Mechanisms and epidemiology. *Int. J. Antimicrob. Agents,* **2015**, *45*(6), 568-585. http://dx.doi.org/10.1016/j.ijantimicag.2015.03.001 PMID: 25857949

[30] Vamsi, S.K.; Moorthy, R.S.; Hemiliamma, M.N.; Chandra Reddy, R.B.; chanderakant, D.J.; Sirikonda, S. Phenotypic and genotypic detection of carbapenemase production among gram negative bacteria isolated from hospital acquired infections. *Saudi Med. J.,* **2022**, *43*(3), 236-243. http://dx.doi.org/10.15537/smj.2022.43.3.20210809 PMID: 35256490

[31] Haque, M.; Sartelli, M.; McKimm, J.; Abu Bakar, M.B. Health care-associated infections: An overview. *Infect. Drug Resist.,* **2018**, *11*, 2321-2333. http://dx.doi.org/10.2147/IDR.S177247 PMID: 30532565

[32] Lee, J.; Pai, H.; Kim, Y.K.; Kim, N.H.; Eun, B.W.; Kang, H.J.; Park, K.H.; Choi, E.H.; Shin, H.Y.; Kim, E.C.; Lee, H.J.; Ahn, H.S. Control of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a children's hospital by changing antimicrobial agent usage policy. *J. Antimicrob. Chemother.,* **2007**, *60*(3), 629-637. http://dx.doi.org/10.1093/jac/dkm225 PMID: 17599919