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Molecular epidemiology of multidrug-resistant *Escherichia coli* in paediatric patients: Focus on extended spectrum beta lactamases, quinolones, tetracyclines and sulfonamides resistance genes

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Abstract: In this study, the molecular determinants of antimicrobial resistance (AMR) among extended spectrum beta lactamase (ESBLs) producing *Escherichia coli* isolated from children were reported. The samples (n=183) were collected from Children Hospital, Faisalabad-Pakistan and subjected to molecular identification followed by antibiotic susceptibility testing against routine antibiotics. Different ESBLs and antimicrobial resistance genes (ARGs) were detected for quinolones, tetracyclines and sulfonamides. The study showed significant resistance to amoxicillin-clavulanate, ceftriaxone, ampicillin, trimethoprim-sulfamethoxazole, levofloxacin and ciprofloxacin. Molecular analysis showed higher prevalence of β-lactamases i.e. bla_{SHV} (38.9%), bla_{TEM} (100%) and bla_{CTX-M} (100%); quinolones resistance genes as i.e. *qnr*A (19.4%), *qnr*B (66.7%), *gyr*A (47.2%) and *par*C (36.1%), tetracyclines resistance gene *tet*B (19.4%) and sulfonamides resistance genes *Sul*-1 and *Sul*-2 (100%). The age-group-specific pattern indicated the presence of bla_{TEM}, bla_{CTX-M}, *Sul*1 and *Sul*2 in children <4 years. *gyr*A was more frequently identified in children >4 years. A significant negative correlation was found between ciprofloxacin MIC and *qnr*B (-0.434, p= 0.010) indicating higher ciprofloxacin resistance. However, a strong negative association was observed between *qnr*A and *qnr*B *(-0.789, p < 0.001)*. This study highlighted increasing resistant *E. coli* infections in children particularly >4 years, emphasizing the strengthening of national antimicrobial stewardship and interventions.

Keywords: Antimicrobial resistance; ESBLs; *Escherichia coli;* Paediatric patients

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INTRODUCTION

Escherichia coli, one of the diverse bacteria, is a natural inhabitant of the gastrointestinal tract (GIT) of humans having a significant contribution to healthy life; however pathogenic strains cause severe infections particularly among children (Lai *et al*., 2021). In Pakistan, *E. coli* is reported as second most common causative agent for gastrointestinal and urinary tract infections and resulted in sepsis among the paediatric population. There is a marked increase in morbidity among young children (<5 years), where the immature immune system increases the susceptibility towards *E. coli* infections (Snehaa *et al*., 2021). The World Health Organization (WHO) focused on improving therapeutic approaches to control the increasing infections associated with resistant *E. coli* (Adamus-Białek *et al*., 2018).

The significantly increased antimicrobial resistance (AMR) pattern of *E. coli* among children is largely attributed to high prevalence of multidrug-resistant (MDR) strains isolated from paediatric patients. A recent study reported that approximately 80% of *E. coli* isolates *originated from* paediatric patients having UTIs were resistant to at least one antibiotic with commonly prescribed drugs like amoxicillin and ciprofloxacin to levels of 65% and 60%, respectively, Bangladesh (Ahsan and Islam, 2019). This trend is alarming that complicates the treatment options and increases the risks of treatment failure leading to prolonged hospitalization and increased health care costs (Sukumaran and Hatha, 2021). Similarly, it has been reported that the overuse/ misuse of antibiotics in paediatric settings has significantly contributed in spread of AMR in Pakistan and there is a need to strictly monitor public health (Razzaq *et al*., 2024).

Besides MDR, extensive or pan-drug resistance (XDR and PDR) *E. coli* strains are considered as significant concern as XDR strain are resistant to all classes of antibiotics except one or two, while PDR strains are resistant to all classes of the antibiotics (Iqbal *et al*., 2021). XDR strains have been identified in clinical settings with an increased morbidity and mortality as these limit the treatment options creating complicated management strategies, as reported in community-acquired infections which necessitate close observation and successful empirical treatment approaches (Gundeslioglu *et al*., 2024). These antibiotic-resistant strains, particularly in children, pose a significant concern by reducing treatment options and increasing chances of developing severe infections (Bumbangi *et al*., 2022). According to another study conducted in Karachi- Pakistan, the increased prevalence of UTIs among children was observed caused by XDR *E. coli strains* (Dela *et al*., 2022).

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Another crucial aspect is the distribution of antimicrobial resistance genes (ARGs) among the *E. coli* strains in children. A study conducted in Taiwan has been able to establish that genes encoding for extended spectrum beta lactamases (ESBLs) including bla_{TEM} and $bla_{CTX\text{-}M}$ are widely prevalent among the *E. coli* isolates originated from pediatric patients (Chen *et al*., 2020). These resistance genes suggest that antibiotic utilization not only applies selective pressure but also allows for horizontal transfer of various ARGs within bacterial populations and further complicating the concerns of AMR spread. The presence of MDR *E. coli* strains among apparently healthy children identifies the need for continued surveillance and monitoring of resistance trends to inform treatment recommendations and public health efforts including national action plan on antimicrobial resistance (Afridi *et al*., 2021). In Pakistan, several of the previous studies reported prevalence of AMR *E. coli* infections among pediatric patients, however, sufficient information is not available regarding the genetic determinants of AMR along with demographic distribution in different regions including Faisalabad. Hence, the current study fills the gap by identifying different resistance genes along with phenotypic patterns of resistance among children of different ages.

Despite the increasing rate of global or national MDR *E. coli* occurrence among pediatric patients, there have been few reports describing distribution of antibiotic resistance determinants at the genetic level particularly among children. The scarcity of age-specific information regarding the co-occurrence of resistance genes and the contribution to antibiotic susceptibility makes it difficult to design treatment strategies targeting specific needs. This research fills the gap by examining the prevalence and distribution of AMR and ARGs in *E. coli* isolated from children in Faisalabad. Finally, the current study aims to determine age- and gender- based variations and correlations among phenotypic and genotypic characteristics emphasizing the urgent need for enhanced antimicrobial stewardship and precision medicine to combat pediatric AMR spread and minimizing the unnecessary use of antibiotics.

MATERIALS AND METHODS

Sample collection and transportation

A total of 183 urine samples were collected from suspected paediatric patients aged up to 12 years admitted/ attending Children Hospital, Faisalabad, from January 2023 to December 2024. The children suspected of urinary tract infections including lower UTIs and febrile upper UTIs were included in the current study. Patients with other aggravating conditions or antibiotic use within past 2 weeks were excluded. The urine samples were categorized into 3 groups: Group 1= <4 years, Group 2= 4-8 years and Group 3= >8 years. Urine samples were obtained in sterile containers using the standard midstream clean catch approach to avoid cross-contamination (Shafquat *et al*., 2025). All samples were transported immediately to the Postgraduate Laboratory, Institute of Microbiology (IOM-GCUF) under cold storage for further processing.

Processing and initial identification

Samples were initially inoculated on cysteine lactose-deficient agar (CLED, BD, Germany) and followed by an incubation at 37°C for 24 hours. Samples were also streaked onto MacConkey agar (Oxoid-UK). Eosin methylene blue agar (EMB) (Oxoid-UK) was also used to subculture the lactose fermenter isolates (Campos *et al*., 2018). To further confirm the presence of *E. coli*, biochemical profiles including the catalase test, oxidase test, indole production, Voges-Proskauer test and methyl red were performed followed by API 20E (BioMérieux, France) as described (AL-Joda and Jasim, 2021).

Molecular identification of isolates

To identify *E. coli* isolates at the molecular level, DNA was isolated using commercial DNA extraction kit (Thermo Scientific™ GeneJET Genomic) according to manufacturer's described protocol. DNA purity was further determined using NanoDrop™ by measuring absorbance at 260 and 280 nm (Thermo Fisher Scientific, Crawley, UK). *E. coli*-specific amplification of *uidA* gene was targeted using specific primers. The amplicons were subjected to agarose gel electrophoresis and visualized using a UV transilluminator.

Antimicrobial susceptibility profile

The Kirby-Bauer disc diffusion method was used to perform antimicrobial susceptibility testing following the CLSI 2023 guidelines, as described recently (Madni *et al*., 2025). A panel of antibiotic discs (Oxoid, UK) was tested including imipenem (10 µg), gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), nitrofurantoin (300 µg), amoxicillin-clavulanate (20/10 µg), ceftriaxone (30 µg), ampicillin (10 µg), trimethoprim-sulfamethoxazole (1.25/23.75 µg), fosfomycin (200 µg), doxycycline (30 µg) and levofloxacin (5 µg). Results were then interpreted according to CLSI-2023 and categorized as susceptible (S), intermediate (I), or resistant (R). The minimum inhibitory concentrations (MICs) were performed for ampicillin, ceftriaxone, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole and ciprofloxacin. For the disc diffusion method, the bacterial suspensions were adjusted to a 0.5 McFarland standard and inoculated onto Mueller-Hinton agar plates (Oxoid, UK). Antibiotic discs were applied on the inoculated plates with the help of a disc dispenser and zones of inhibition were recorded. (Masar *et al*., 2023).

Minimum inhibitory concentration

Additionally, MICs of a panel of antimicrobial agents comprising amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, ceftriaxone, ampicillin and ciprofloxacin were determined using the broth

microdilution method (MIC of tetracyclines was not determined, due to least clinical importance among paediatric patients). In a 96-well microtiter plate, successive two-fold dilutions of each antimicrobial drug were made in cation-adjusted Mueller-Hinton broth. After standardizing the bacterial suspension to a final concentration of approximately 5×10^5 CFU/mL, 100 µL was applied to each well. The plates were incubated for 16-20 hours at 37°C. Growth was evaluated visually and optical density was measured with a spectrophotometer by using a wavelength of 600 nm. Visible bacterial growth was taken as the cutoff for determining the MIC as the lowest concentration of the antibiotic inhibiting growth. Results were interpreted according to CLSI-2023 guidelines (Madni *et al*., 2025).

Detection of antimicrobial resistance genes in E. coli

The specific resistance genes among the *E. coli* isolates were determined through polymerase chain reaction in conjunction with phenotypic testing. The genes targeted for the PCR assays were those encoding resistance, including ESBLs (*bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$), quinolone resistance genes (*qnr*A, *qnr*B, *qnr*S, *gyr*A, *par*C), *sul*fonamide resistance genes (*sul*1, *sul*2) and tetracycline resistance genes (*tet*A and *tet*B) as shown in table 1. The following conditions were used for PCR: an initial denaturation at 95°C for 5 minutes, denaturation at 94°C for 1 minute, annealing for 30 seconds and extension at 72°C for 1 minute, followed by a final extension at 72°C for 5 minutes. PCR was performed for ARGs along with validated ARG-positive strains as positive control and ARG-negative strains as negative control. All controls were processed in each batch to verify accuracy and specificity. The amplification of *uidA* and ARGs of *E. coli* isolates was analyzed with the PCR products using agarose gel electrophoresis. Estimation of sizes of PCR products was based on size comparison with a 100 bp DNA ladder. Positive amplification of the genes was confirmed by the presence of bands corresponding to expected sizes for the target genes.

Statistical analysis

A Microsoft Excel 365 spreadsheet was used by inserting the collected data and initial statistical analyses were performed including frequencies and percentages. Fisher's exact tests were used to examine the prevalence of AMR and ARGs among different groups using SPSS Statistics version 27.0. *P*-values < 0.05 were regarded as statistically significant. Spearman's correlation was used to determine the association between particular resistance genes and MIC patterns.

RESULTS

Number of E. coli isolates

The samples were collected from 183 individuals, with the majority (70.5%) being under 4 years old. 17.6% and 11.7% of samples were collected from children aged 4-8 years old and above 8 years old, respectively. According to gender distribution, 44.1% were male and 55.8% were female.

Antibiotic resistance pattern

The antibiotic resistance patterns that were seen in this study showed important heterogeneity in the susceptibility of *E. coli* isolates from children to different antibiotics. Fig. 1 showed differing sensitivity and resistance between the different antibiotics. Fosfomycin was reported to be sensitive (91.7%), while amoxicillin clavulanate, ceftriaxone, ampicillin and trimethoprim/ sulfamethoxazole had a reported a 100% resistance rate. However, other notable antibiotics with their high resistance included Ciprofloxacin 86.1%, Levofloxacin 88.9% and Doxycycline 66.7%. On the other hand, chloramphenicol 83.3%, imipenem 77.8% and amikacin 69.4% were significantly sensitive.

The antibiotic resistance profiles among various age groups showed considerable differences, with children <4 years being more resistant to some antibiotics. In the analysis, significant resistance was observed for levofloxacin, with a *p*-value of 0.027 (Fisher's Exact Test) for the <4 years group, indicating a notable age-related variation in resistance. Ciprofloxacin also showed a high resistance rate in the <4 years group, but the *p*-value (0.616) indicated no significant association as shown in table 2. For amikacin**,** a significant difference was found (*p*-values of 0.049), with the highest resistance seen in the <4 years group. On the other hand, fosfomycin and nitrofurantoin showed no significant variation across age groups (*p*-values > 0.7). Most other antibiotics, including doxycycline and ciprofloxacin, did not exhibit significant age-related differences in resistance. The analysis revealed varying resistance patterns, with some antibiotics showing significant age-related trends, while others displayed stable resistance across all groups.

Comparative analysis of MIC values among the different age groups

MIC values obtained with regard to comparison among the age groups in this study were analyzed comparatively. This data illustrated that resistance levels of younger children to MIC values of specific antibiotics were higher. The presence of multiple resistances suggested an impending threat in a trend of multidrug-resistant *E. coli* strains within pediatric patients. The spread of resistance genes was also distributed in an age-dependent manner (Fig. 2).

Frequency of antibiotic resistance genes

The results of the analysis indicated varying distributions of resistance genes among the *E. coli* isolates. In the category of beta-lactam resistance, the prevalence of *bla*$_{SHV}$ was 38.9%, whereas the distribution of *bla*$_{TEM}$ and *bla*$_{CTX-M}$ was 100% in all samples as presented in fig. 3. In

the class of quinolone resistance genes, *qnr*A had an occurrence rate of 19.4% among the isolates, whereas the figures for *qnr*B distribution were at 66.7%. *qnr*S was absent in all samples (0%). *gyr*A and *par*C were present in 47.2% and 36.1% of the isolates, respectively. For sulfonamide resistance genes, *Sul*-1 and *Sul*-2 are ubiquitously found in all isolates (100%). Tetracycline resistance genes were reported with the presence of *tet*A in 0% of samples and that of *tet*B in 19.4% of the isolates (Fig. 3).

This indicated that in *E. coli*, there is a highly prevalent infection with the simultaneous presence of multiple resistance genes, further outlining that resistance is multifactorial.

All age groups harboured resistance genes for quinolones, which included *qnr*A, *qnr*B, *qnr*S, *par*C and *gyr*A. None of these showed statistical linkage to age at either a chi-square or Fisher's exact test, where the p-values ranged from 0.540 to 1.000. tet A and tet B, the genes for tetracycline resistance, had no resistance in children under 4 years of age (Table 3), but increased with age in the older children, especially the grou*p between* 9 and 12 years. Tet B had no significant variation with age (p-value = 1.000). *Sul*fonamide resistance genes, *sul* 1 and *sul* 2, presented with age-related trends where the older age groups, especially the >8 years group, showed a higher frequency of positive cases, though p-values did not indicate any statistical significance. Beta-lactam resistance genes, such as $bla_{\text{CTX-M}}$, $bla_{\text{TEM and}}$ bla_{SHV}, had higher frequencies with increasing age, especially of $bla_{\text{CTX-M}}$ and bla_{TEM}, but bla_{SHV} showed no significant age-related change.

Spearman's correlation analysis of E. coli

Logistic regression was avoided as small and unbalanced subgroups would yield unstable estimates with wide confidence intervals. Spearman's rank correlation was used instead, a non-parametric test more appropriate for our dataset (Gong *et al*., 2022). Spearman's rho analysis of *E. coli* revealed a highly significant negative correlation (-0.434, p= 0.010) between ciprofloxacin MIC and *qnr*B genes, indicating higher levels of resistance in ciprofloxacin MIC to be found in the presence of *qnr*B; however, no correlation was shown to be between ciprofloxacin MIC with *qnr*A (p= 0.736) as well as the *qnr*S gene (Table 4). Besides, the relationship between the *qnr*A and *qnr*B genes was strongly and significantly negative (-0.789, p< 0.001). Overall, these genetic associations describe specific relationships that affect ciprofloxacin resistance in *E. coli.*

Spearman's rho analysis did not find a significant correlation between CRO (Ceftriaxone resistance) and tested genes for resistance. There was an anti-correlation in a weak negative value of -0.087 between CRO and bla_{SHV}, though it was statistically not relevant (p= 0.623). The correlation values of CRO with bla_{TEM} and $bla_{\text{CTX-M}}$ were not disclosed and cannot be analyzed further. As such, the re*sul*ts altogether did not suggest a meaningful association of CRO resistance with the genes that were tested.

Co-occurrence of multiple resistance genes in E. coli isolates

There was a difference in the pattern of co-occurrence of ARGs across the different age groups. The most common combination of resistance genes observed in children less than 4 years of age was bla_{TEM}, $bla_{\text{CTX-M}}$, *Sul*1 and *Sul*2, in 24 cases. In the 4-8 years age group, co-occurrence of *qnr*B, bla_{TEM}, $bla_{\text{CTX-M}}$, *Sul*1 and *Sul*2 was observed in 6 cases as shown in (Fig. 4). This shows the presence of quinolone resistance along with beta-lactam and *sul*fonamide resistance genes. In the >8 years age group, the most common co-occurrence was of *gyr*A, bla_{TEM}, $bla_{\text{CTX-M}}$, *Sul*1 and *Sul*2, found in 4 cases and the quinolone resistance gene *gyr*A was added to the combination. This result indicates that there is an age-related increase in the diversity of co-occurring ARGs, especially in older children who have quinolone resistance genes.

DISCUSSION

This research emphasizes the prevalence of multidrug-resistant *E. coli* isolates, including ESBLs producing *E. coli* among paediatric patients in Faisalabad, Pakistan. The incidence of *E. coli* infections in children is a major public health concern worldwide with varying rates being reported in different regions. Studies in Pakistan have revealed extremely high levels of antibiotic resistance among *E. coli* isolates from children, especially in urban settings where healthcare and sanitation may be suboptimal (Shaikh *et al*., 2016). MASOOD *et al*., 2022 reported a greater prevalence of 54.5% in children younger than five years old in Lahore, with enteroaggregative *E. coli* being the most common strain at 36%. The increased spread is critical in low- & middle-income countries having increased infection and malnutrition index creating a cycle that threatens child development.

In the current study, amoxicillin-clavulanate, ceftriaxone, ampicillin and trimethoprim/sulfamethoxazole showed complete resistance, concerning the effectiveness of some of the common antibiotics used to treat infections in targeted population. Other key resistances were also observed with ciprofloxacin at 86.1%, levofloxacin at 88.9% and doxycycline at 66.7%. This confirms to current research, in which resistance to beta-lactams was between 70–85% and fluoroquinolone resistance between 60-80% in *E. coli* isolates among pediatric and adult patients from Pakistan and other regions (Nji *et al*., 2021). A significant resistance rate was found in children <4 years of age with p= 0.027. There is an alarming age-related variation in resistance.

Table 1: Details of primers for amplification of genome

Genes	Primers	Sequence (5' to 3')	Aimed product (bp)	Annealing temperature (^{0}C)	References
uidA	*uidA*-F	CAACGAACTGAACTGGCAGA	162	50	(Bej *et al*., 1991)
	uidA-R	CATTACGCTGCGATGGAT			
bla_{TEM}	TEM-F	TCAACATTTCCGTGTCG	860	46	(Cullik *et al*., 2010)
	TEM-R	CTGACAGTTACCAATGCTTA			
bla_{SHV}	SHV-F	ATGCGTTATATTCGCCTGTG	896	49	(Paterson *et al*., 2003)
	SHV-R	AGATAAATCACCACAATGCGC			
bla_{CTX-M}	CTXMU-F	ATGTGCAGYACCAGTAARGT	593	53	(Tofteland *et al*., 2007)
	CTXMU-R	TGGGTRAARTARGTSACCAGA			
*tet*A	tetA-F	GTGAAACCCAACATACCCC	888	50	(Ng *et al*., 2001)
	tetA-R	GAAGGCAAGCAGGATGTAG			
*tet*B	tetB-F	CCTTATCATGCCAGTCTTGC	774	50	(Ng *et al*., 2001)
	tetB-R	ACTGCCGTTTTTTCGCC			
*sul*1	*sul*1-F	CGGCGTGGGCTACCTGAACG	433	58	(Kozak *et al*., 2009)
	*sul*1-R	GCCGATCGCGTGAAGTTCCG			
*sul*2	*sul*2-F	GCGCTCAAGGCAGATGGCATT	293	58	(Kozak *et al*., 2009)
	*sul*1-R	GCGTTTGATACCGGCACCCGT			
qnrA	*qnrA*-F	GGGTATGGATATTATTGATAAAG	492	50	(Öktem *et al*., 2008)
	qnrA-R	CTAATCCGGCAGCACTATTTA			
qnrB	*qnrB*- F	GATCGTGAAAGCCAGAAAGG	264	46	(Öktem *et al*., 2008)
*sul*2	*sul*2-F	GCGCTCAAGGCAGATGGCATT	293	58	(Kozak *et al*., 2009)
qnrS	*qnrS*-F	ACGACATTCGTCAACTGCAA	428	50	(Öktem *et al*., 2008)
	qnrS-R	TAAATTGGCACCCTGTAGGC			
*gyr*A	*gyr*A-F	GACCTTGCGAGAGAAATTACAC	647	49	(Qiang *et al*., 2002)
	*gyr*A-R	GATGTTGGTTGCCATACCTACG			
*par*C	parC-F	CGGAAAACGCCTACTTAAACTA	395	49	(Qiang *et al*., 2002)
	parC-R	GTGCCGTTAAGCAAAATGT			

uidA = β-glucuronidase gene (*E. coli* identification marker); bla_{TEM} = β-lactamases TEM-type; bla_{SHV} = β-lactamases SHV-type; bla_{CTX-M} = cefotaximase; *tet*A = tetracycline resistance gene A; *tet*B = tetracycline resistance gene B; *sul*1 = sulfonamide resistance gene 1; *sul*2 = sulfonamide resistance gene 2; *qnrA* = quinolone resistance gene A; *qnrB* = quinolone resistance gene B; *qnrS* = quinolone resistance gene S; *gyr*A = DNA *gyr*ase subunit A; *par*C = DNA topoisomerase IV subunit C.

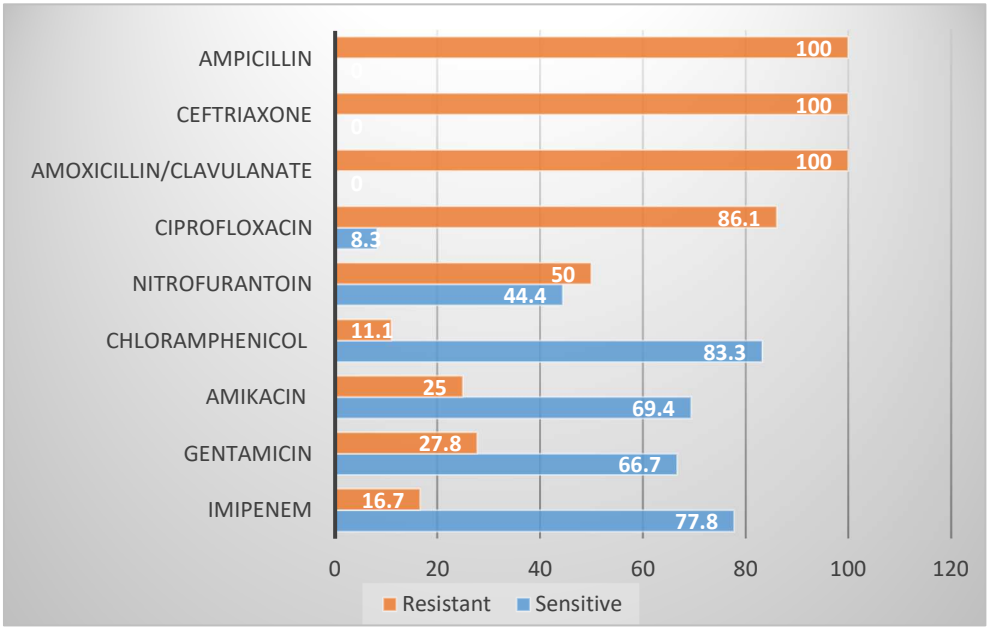

Fig. 1: Antimicrobial resistance pattern in *E. coli* isolates

Table 2: Age related variation in antibiotic resistance patterns

Antibiotic	Age group	Sensitive (n)	Resistant (n)	Total (n)	*p*- value (Fisher's exact test)
LEV 5	<4 years	0	25	25	0.027
	4-8 years	1	6	7	
	>8 years	1	1	2	
DOX 30	<4 years	6	19	25	0.147
	4-8 years	2	5	7	
	>8 years	2	0	2	
FOS 50	<4 years	24	1	25	1.000
	4-8 years	7	0	7	
	>8 years	2	0	2	
STX 25	<4 years	0	11	11	
	4-8 years	0	7	7	
	>8 years	0	16	16	
AMP 10	<4 years	0	15	15	
	4-8 years	0	8	8	
	>8 years	0	11	11	
CRO 30	<4 years	0	3	3	
	4-8 years	0	6	6	
	>8 years	0	25	25	
AMC 30	<4 years	0	18	18	
	4-8 years	0	10	10	
	>8 years	0	6	6	
CIP 5	<4 years	2	23	25	0.616
	4-8 years	1	6	7	
	>8 years	0	2	2	
F 300	<4 years	12	13	25	1.000
	4-8 years	3	4	7	
	>8 years	1	1	2	
AK 30	<4 years	19	6	25	0.049
	4-8 years	6	1	7	
	>8 years	0	2	2	
CN 30	<4 years	17	8	25	1.00
	4-8 years	5	2	7	
	>8 years	2	0	2	
IMP 10	<4 years	20	5		1.000
	4-8 years	6	1		
	>8 years	2	0		

$P < 0.005$ = significant

LEV 5 = Levofloxacin; DOX 30 = Doxycycline; FOS 50 = Fosfomycin; STX 25 = Trimethoprim–Sulfamethoxazole; AMP 10 = Ampicillin; CRO 30 = Ceftriaxone; CIP 5 = Ciprofloxacin; F 300 = Nitrofurantoin; AK 30 = Amikacin; C 30 = Chloramphenicol; CN 30 = Gentamicin; IMP 10 = Imipenem.

This result concurs with the recent findings indicating that there has been a growing resistance of fluoroquinolones in pediatric populations, suggesting that early age exposure to antibiotics might be responsible for the development of resistance (Zhang *et al*., 2022). Conversely, though ciprofloxacin also had similarly high resistance rates in the same age group not having significant association ($p = 0.736$ and $p = 0.616$ for genetic and phenotypic resistance respectively). These values are in line with (Tuem *et al*., 2018) which described variable resistance patterns in children in Ethiopia, suggesting the need for further surveillance to update empirical treatment decisions. The substantial resistance noted for Amikacin (AK 30) in the <4 years group, at $p = 0.046$ and $p = 0.050$, highlights that younger children are more susceptible to multidrug-resistant infections. This is consistent with the previous findings in Nigeria (Galadima *et al*., 2022), that there was a similar trend of resistance pattern in pediatric patients and indicated that age-specific factors could be influencing the susceptibility to specific antibiotics.

The presence of resistance genes showed multiple determinants of resistance in the *E. coli* isolates. The presence of that bla_{TEM} and $bla_{CTX\text{-}M}$ genes and sulfonamide resistance genes, *sul*1 and *sul*2 in all the isolates poses a serious threat. These genes are known to be involved in extended spectrum beta lactamase making treatment difficult. This prevalence is significantly higher than found in human clinical studies in Pakistan, where $bla_{CTX\text{-}M}$ was detected among 86.2% of isolates (Abdullah *et al*., 2023) and sulfonamide resistance genes (*sul*1, *sul*2)

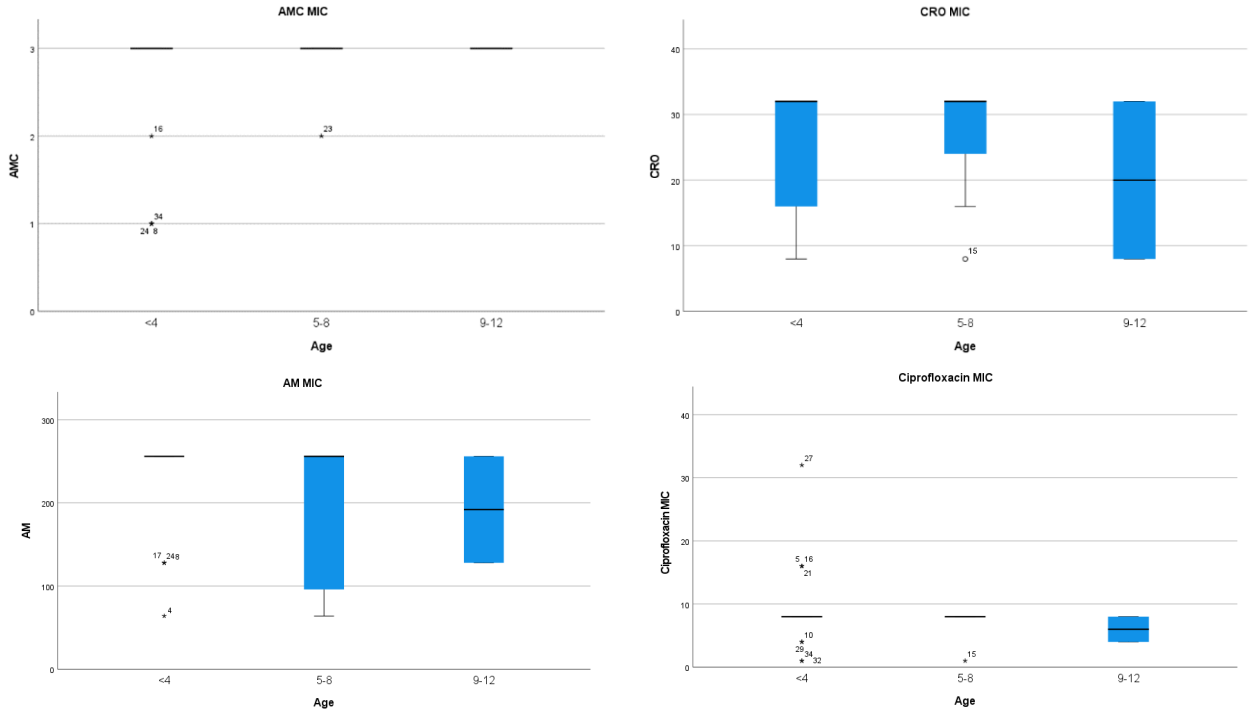

Fig. 2: Box plots of comparative analysis of MIC values among the different age groups

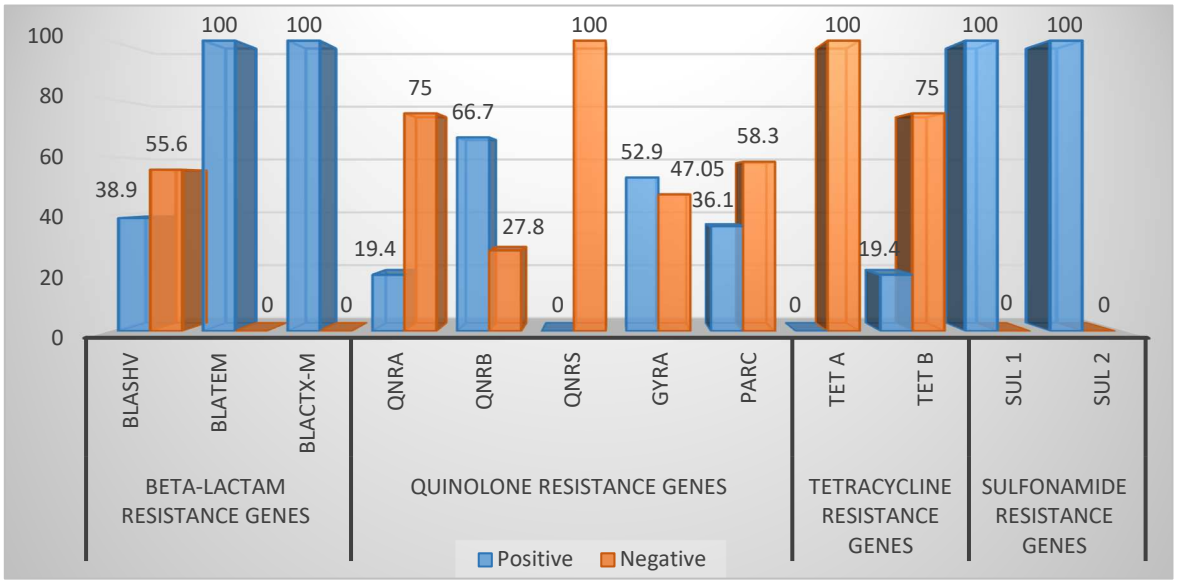

Fig. 3: Distribution of different classes of antibiotic resistance genes among children

varied from 21% to 60.5% (Shafquat *et al*., 2025). This high prevalence could be indicative of age-related antibiotic use, local prescribing preferences and regional epidemiological factors.

Quinolone resistance genes were distributed: *qnr*B was detected in 66.7% of the isolates, *qnr*A was in 19.4% and *qnr*S was not detected. Conversely, in a study from Iran, the following rates of prevalence among *E. coli* isolates in a tertiary referral hospital were reported: *qnr*A in 18%, *qnr*B in 30.9% and *qnr*S in 25.4%. (Badamchi *et al*., 2019). This finding is consistent with previous studies in which *gyr*A mutations occurred in 40-50% of isolates and *par*C in 30-35% (Adekunle *et al*., 2020).

Results of Spearman's rho analysis in *E. coli* strains provided critical genetic associations that may direct the occurrence of ciprofloxacin resistance. An important finding is that MIC ciprofloxacin was negatively associated with the presence of the *qnr*B gene (-0.434, p= 0.010). This means that higher ciprofloxacin resistance levels are associated with the presence of the *qnr*B gene, known to pose low-level resistance to fluoroquinolone. This is consistent with the studies by

Table 3: Age dependent variations in resistance genes

Quinolone resistance genes	Age group	Positive (n)	Negative (n)	Total (n)	p-value (Chi- square test)	p- value (Fisher's exact test)
*qnr*A	<4 years	5	2	25	0.540	0.562
	4-8 years	1	6	7		
	>8 years	1	1	2		
*qnr*B	<4 years	18	7	25	0.819	0.827
	4-8 years	5	2	7		
	>8 years	1	1	2		
*qnr*S	<4 years	0	4	25		
	4-8 years	0	12	7		
	>8 years	0	18	2		
parC	<4 years	10	15	25	0.805	0.846
	4-8 years	2	5	7		
	>8 years	1	1	2		
*gyr*A	<4 years	13	12	25	0.913	1.000
	4-8 years	3	4	7		
	>8 years	1	1	2		
Tetracycline resistance genes						
tetA	<4 years	0	5	5		
	4-8 years	0	8	8		
	>8 years	0	21	21		
tetB	<4 years	6	19	25	0.528	1.000
	4-8 years	1	6	7		
	>8 years	0	2	2		
Sulfonamide resistance genes						
*Sul*1	<4 years	7	0	7		
	4-8 years	9	0	9		
	>8 years	18	0	18		
*Sul*2	<4 years	5	0	5		
	4-8 years	11	0	11		
	>8 years	18	0	18		
Beta- lactam resistance genes						
$bla_{\text{CTX-M}}$	<4 years	6	0	6		
	4-8 years	10	0	10		
	>8 years	18	0	18		
bla_{TEM}	<4 years	11	0	11		
	4-8 years	8	0	8		
	>8 years	15	0	15		
bla_{SHV}	<4 years	11	14	25		
	4-8 years	2	5	7		
	>8 years	1	1	2		

P<0.005=significant

This table summarize the antimicrobial resistance genes found in *E. coli*, categorized by antibiotic class. Quinolone resistance genes (*qnr*A, *qnr*B, *qnr*S, *par*C, *gyr*A) are chromosomally or plasmid-mediated determinants; tetracycline resistance genes (*tet*A, *tet*B) translate to efflux pumps; sulfonamide resistance genes (*sul*1, *sul*2) translate to alternative dihydropteroate synthases; and β-lactam resistance genes ($bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}) translate to extended-spectrum β-lactamases.

(Kariuki *et al*., 2023) which established that there was the presence of multiple PMQR genes, including *qnr*B, among the ciprofloxacin non-susceptible *E. coli* and Klebsiella spp. recovered from children (Caracciolo *et al*., 2011, Bryce *et al*., 2016). The existence of *qnr*B may promote the development of high-level resistance due to mutations in genes encoding *gyr*A and topoisomerase enzymes, which are important targets for fluoroquinolones

On the other hand, the examination did not find a significant association between ciprofloxacin MIC and the *qnr*A gene (p= 0.736) or the *qnr*S gene (not shown). This could mean that *qnr*A and *qnr*S do not significantly contribute to the resistance process of ciprofloxacin in the population under study, similar to most studies that show the prevalence and expression of these genes are variable across different geographical locations as described by (Forster *et al*., 2017, Owumi *et al*., 2014).

Table 4: Spearman's rank correlation between MIC values and resistance genes in *E. coli*

Spearman's rho		Ciprofloxacin MIC	*qnr*A Ciprofloxacin Gene	*qnr*S Ciprofloxacin Gene	*qnr*B Ciprofloxacin Gene
Ciprofloxacin MIC	Correlation coefficient	1.000	.060	.	-0.434*
	Sig. (2-tailed)	.	.736	.	0.010
	N	34	34	34	34
*qnr*A Ciprofloxacin Gene	Correlation coefficient	.060	1.000	.	-0.789**
	Sig. (2-tailed)	.736	.	.	0.000
	N	34	34	34	34
*qnr*S Ciprofloxacin Gene	Correlation coefficient	.	.	.	.
	Sig. (2-tailed)	.	.	.	.
	N	34	34	34	34
*qnr*B Ciprofloxacin Gene	Correlation coefficient	-.434*	-.789**	.	1.000
	Sig. (2-tailed)	.010	.000	.	.
	N	34	34	34	34
Spearman's rho		CRO MIC	bla_{SHV} of CRO	bla_{TEM} of CRO	bla_{CTX-M} of CRO
CRO MIC	Correlation coefficient	1.000	-.087	.	.
	Sig. (2-tailed)	.	.623	.	.
	N	34	34	34	34
bla_{SHV} of CRO	Correlation coefficient	-.087	1.000	.	.
	Sig. (2-tailed)	.623	.	.	.
	N	34	34	34	34
bla_{TEM} of CRO	Correlation coefficient	.	.	.	.
	Sig. (2-tailed)	.	.	.	.
	N	34	34	34	34
bla_{CTX-M} of CRO	Correlation coefficient	.	.	.	.
	Sig. (2-tailed)	.	.	.	.
	N	34	34	34	34

Spearman's rho (ρ) describes the direction and strength of the monotonic relationship between two variables. A positive value indicates a direct relationship, while a negative value indicates an inverse relationship. $p < 0.05$ is statistically significant (*), and $p < 0.01$ is highly significant (**).

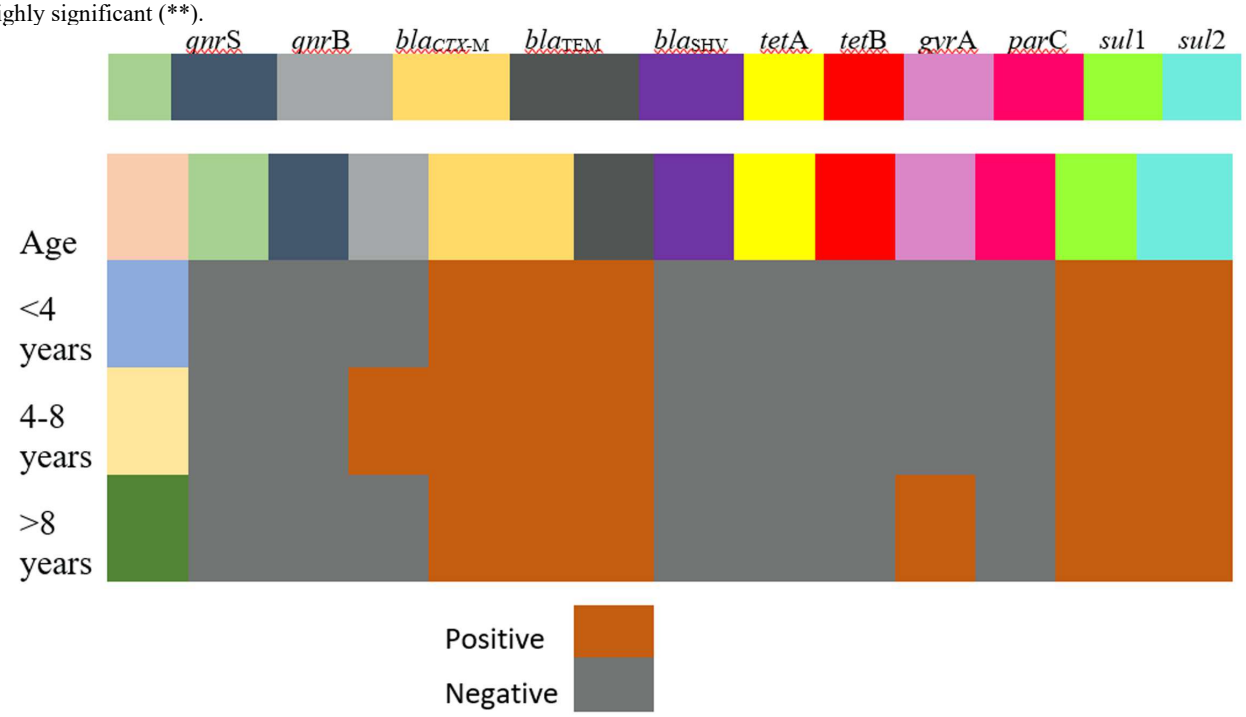

Fig. 4: Heat map showing the co-existence of multiple genes in different age groups

The lack of substantial data relative to the association between *qnr*A and *qnr*S with resistance to ciprofloxacin highlights that more studies are needed to better describe the roles these genes play specifically in varying scenarios.

We also found an extremely strong negative correlation (-0.789, $p< 0.001$) between *qnr*A and *qnr*B genes. These results indicate some type of competitive relationship that may affect prevalence in clinical isolates. Some similar reports were shown by previous studies that indicated a low frequency of *qnr*A co-occurring with *qnr*B among different populations and populations could be possibly affected due to selective pressure of particular environmental factors as shown in study conducted by (Gong *et al*., 2022, Principi and Esposito, 2016).

The analysis also considered the relationship between resistance to ceftriaxone (CRO) and the tested genes of resistance. The results do not show an association between the CRO studied and the assayed resistance genes. A nonsignificant poor negative correlation of -0.087 was found with bla_{SHV} and CRO, where $p= 0.623$. This nonsignificant association indicates that the tested resistance genes might not play an important role in ceftriaxone resistance in the *E. coli* isolates investigated. (Schrier *et al*., 2018) has shown different effects of β-lactamase genes on resistance to ceftriaxone. It may suggest that the local epidemiological factor is an important discriminating factor.

The differences seen in antimicrobial resistance patterns and prevalence of genes among different age groups can be explained by differences in exposure to antibiotics, maturity of the immune system and gut microbiota composition among children of different ages. Young children (<4 years) receive antibiotics more frequently for typical infections and this may favor selection of MDR *E. coli* Secondly, local prescribing habits and environmental conditions in Faisalabad, including sanitation and access to healthcare, may have affected the distribution of resistance determinants.

The widespread resistance determined in this study indicate that conventionally used first-line drugs might fail to have the desired effect in specific subgroups, especially children younger than 4 years. The inclusion of local resistance data into treatment guidelines can maximize empirical therapy, minimize treatment failure and restrict selection pressure for multidrug-resistant strains. These results indicate that national guidelines might fail to reflect the local resistance pattern, highlighting a call for regional surveillance and alternative antibiotic use in empirical therapy.

Limitations and recommendations

This single-center analysis from Faisalabad was limited due to the absence of molecular typing and clinical outcome information, limiting understanding of strain relatedness and the clinical significance of resistance. Future multicenter studies combining molecular characterization and patient outcomes are needed. The age- and gender-specific patterns of resistance found here should guide focused antimicrobial stewardship initiatives.

CONCLUSION

This study reports disturbingly increasing incidents of multidrug-resistant *E. coli* infections in paediatric patients; younger children face the highest threats. The resistance genes strongly associated with antibiotic susceptibility patterns call for strengthening antimicrobial stewardship and targeted interventions. Urgent public health efforts are needed immediately to curb spread of resistance so that effective therapies are not further compromised for common infections in childhood.

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Authors' contributions

HJ: Methods, statistical analysis and initial draft writing, MAZ: Concept, study design, supervision and critical review of the manuscript, ZN: statistical analysis, graphs and tables, SM: methods and initial draft.

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Data availability statement

The complete data has been included in this manuscript.

Ethical approval

The current study was approved by the Ethics Review Committee (Ref No. GCUF/ERC/640), Government College University Faisalabad. The samples were collected by the hospital staff according to the routine sampling guidelines of the hospital along with written consent of parents/ guardians and their identification was not disclosed.

Conflict of interest

The authors have no conflict of interest relevant to this study.

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