Migration Letters

Volume: 21, No: S6 (2024), pp. 1301-1316

ISSN: 1741-8984 (Print) ISSN: 1741-8992 (Online)

www.migrationletters.com

Molecular Characterization and Antibiogram of Diarrheagenic and Non-Diarrheagenic Escherichia Coli Isolated from Pediatric Patients of Lahore, Pakistan

Ayesha Masood[1], Jawaria Pervaiz[2], Muhammad Qasim Ali[3], Asif Hanif[4], Mohsin Gulzar Barq[5], Syed Zeeshan H. Naqvi[6*](Corresponding Author)

Abstract

Gastrointestinal disturbances, specifically diarrhea, represent a prevalent health concern, with a recognized association with an elevated susceptibility to stunting. This study provides a comprehensive analysis of biological samples collected from various hospitals, highlighting Mayo Hospital, Lahore, as the major contributor. The study encompasses both genders, with 43.9% males and 56.1% females. Age-wise distribution reveals 31.9%, 26.4%, 25%, and 16.7% for individuals below 2, 2-3, 3-4, and 4-5 years, respectively. Subjects were categorized into Group A (Diarrheagenic) and Group B (non-diarrheagenic), each constituting 50% of the total. PCR screenings focused on identifying E. coli pathotypes, emphasizing stx1, stx2, and eae primers for specific gene detection. Three E. coli strains were identified, with three assigned to O121:H19, two to O148:H8, and others denoted as ONT: HNT. DEC strains from diarrhea patients exhibited higher res[1]istance to AMP (69.4%) and AMC (80.6%) compared to those aged 3 years. Detection of EAEC revealed 60% with the aggR gene, and 53.3% characterized as multivirulent isolates. Different E. coli pathotypes (ETEC, EIEC, EPEC, EHEC, and EAEC) exhibited a clonal nature with unique O and O:H serotypes. Virulence factors fell into colonization factors or secreted toxins, emphasizing diverse mechanisms contributing to diarrheal infections. Non-diarrheagenic E. coli constituted 34% of studied strains, while 15.9% were identified as DEC through PCR analysis. Prevalent DEC pathotypes in both groups included AEPEC (6.11%), tEAEC (9.7%), ETEC (8.9%), DAEC (5.3%), and EIEC (5.6%). Clinical management and public health initiatives can benefit from these results, which can help in the creation of more precise plans to avoid and cure diarrhea caused by E. coli. The dynamic nature of E. coli pathotypes and the ways in which they interact with host variables in different population's call for more investigation.

[1]Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, 54000 Pakistan.

[1]Associate professor at The University College of Medicine and Dentistry, The University of Lahore, 54000 Pakistan.

[2]Frontier Medical College, Abbottabad, Pakistan.

[3]Lecturer of Microbiology, Institute of Life Science, Gulab Devi Educational Complex, Ferozpur Road, 54000 Lahore, Pakistan.

[4]Allied Health Sciences (AHS), The University of Lahore, 54000 Pakistan.

[5]Assistant Professor, Department of Microbiology, The University of Central Punjab, Lahore, 54000, Pakistan.

[5]Associate Professor, Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, 54000 Pak istan.

[*6]Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore, 54000 Pakistan.

Keywords: *Escherichia coli, PCR analysis, virulence factors, O serotypes, O:H serotypes, Lahore, Enterotoxigenic E. coli (ETEC), antimicrobial resistance, molecular characteristics*.

Introduction

Gastrointestinal disturbances, specifically diarrhea, represent a prevalent health concern, with a recognized association with an elevated susceptibility to stunting. The consequences of stunting are particularly critical in the context of pediatric mortality, amplifying the vulnerability of children to succumb to additional infectious diseases (Ahmed et al., 2022b). In adults, the manifestation of severe diarrhea is a commonly encountered issue within the purview of family medical practice (Ullah et al., 2023). Viral gastroenteritis stands as the predominant etiological factor, typically presenting as a self-limiting malady. The escalation of global travel, concurrent medical conditions, and instances of foodborne illnesses contribute significantly to the rising incidence of acute bacterial diarrhea (Angulo-Zamudio et al., 2021).

An adept evaluation encompassing a comprehensive scrutiny of the patient's medical history and a meticulous physical examination is imperative (Yusof et al., 2022, Zeshan et al., 2021). This aids in the identification of pertinent risk factors and discerning symptoms indicative of inflammatory diarrhea and/or severe dehydration. The culmination of such an assessment serves as a pivotal guide for the judicious selection of diagnostic testing and subsequent therapeutic interventions (Sohail et al., 2023, Wada et al., 2024).

The prevalence of diarrhea constitutes a significant contributor to morbidity and mortality in the pediatric population, particularly among young children. This predicament assumes heightened significance in low and middle-income nations exemplified by the situation in Mexico, where a paucity of comprehensive studies on diarrheal diseases has been noted over the past two years (Angulo-Zamudio et al., 2021). Conspicuously, a subset of bacteria classified as Diarrheagenic Escherichia coli (DEC) has emerged as a primary causative agent of diarrheal illnesses globally (Ahmed et al., 2019, Mustafai et al., 2023). Notably, common biochemical techniques traditionally employed for the identification of other pathogens responsible for diarrhea, such as Shigella and Salmonella, prove ineffective in diagnosing DEC (Hajihosein-Tabrizi et al., 2018).

Children in underdeveloped countries with inadequate sanitation and hygiene have the added burden of diarrheal infections, which pose a significant threat to public health. A wide variety of pathotypes of the bacterium Escherichia coli (E. coli) are known to cause illness due to their unique infection processes and virulence characteristics (Rabaan et al., 2022b, Rizvi et al., 2022). There are six main pathotypes of diarrheagenic Escherichia coli (DEC): enteropathogenic (EPEC), enterotoxigenic (ETEC), enteroaggregative (EAEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), and broadly adherent (DAEC) (Denamur et al., 2021). Toxins, adhesins, and other virulence factors can be identified using molecular techniques like polymerase chain reaction (PCR), which target specific genes (Liang et al., 2023).

Isolated from diarrhea patients, in addition to DEC, are non-diarrheagenic E. coli (NDEC) strains that can act as commensals or opportunistic infections (Rabaan et al., 2022a, Zeb et al., 2022). Genes that provide antibiotic resistance or enhance survival in the intestinal environment may be carried by NDEC strains, while they lack the traditional virulence genes associated with DEC (Sarowska et al., 2019). Polymerase chain reaction (PCR) was found to be an effective and suitable approach for determining the presence of antibiotic resistance genes, which are present in all isolates. The PCR technique is useful for more than just detecting Diarrheagenic Escherichia coli (DEC) infection; it can also simplify epidemiological surveillance (Yim et al., 2021). On top of that, it works for monitoring water samples for human

consumption and food samples for E. coli contamination. It is crucial to have access to epidemiological data in order to implement preventative measures, such as vaccination programmes and the management of infectious diarrhea in children, because these contaminants can be passed down from adults to them (Zahari et al., 2023, Zahra et al., 2021).

This study aims to conduct a investigation with the following objectives such as to isolate, identify, and profile the antibiotic resistance patterns of Diarrheagenic Escherichia coli (DEC) strains from clinical samples, to determine the phenotypic molecular characteristics and antibiotic resistance profiles of E. coli isolates, to compare the molecular characterization of E. coli strains from pediatric patients, both diarrheagenic and non-diarrheagenic, and to compare the antibiogram profiles of these two types of E. coli strains. The goals of this study are to better understand the genetic variation, phenotypic features, and antibiotic resistance patterns of DEC strains, especially in children so that more effective treatment plans and better patient care can be devised.

Methodology

Study Arrangement

This study was conducted within the Microbiology Laboratory of the Institute of Molecular Biology and Biotechnology (IMBB) at the University of Lahore, Pakistan. The procurement of stool and rectal swab specimens was facilitated through collaboration with the medical and para-medical staff at Mayo Hospital, Lahore.

Sample Assortment & Processing

A total of 684 stool and rectal swab samples were collected from pediatric patients (<5 years old) admitted to tertiary care hospitals, namely Children Hospital Lahore, Indus Hospital Lahore, Saira Memorial Hospital Lahore, Saleem Memorial Hospital and Mayo Hospital Lahore, during the period from March 2018 to December 2019.

Microbiological Analysis and Serotyping of Stool/Rectal Swab Specimens

Stool/rectal swab specimens were subjected to microbiological analysis for Escherichia coli (E. coli) isolation. Inoculation into Lauryl Sulphate Tryptose Broth (LSTB) was followed by overnight incubation. Subsequent sub culturing on MacConkey agar and Eosin Methylene Blue agar, incubated at 37°C for 24-48 hours revealed E. coli colony characteristics. Eosin Methylene Blue agar (EMB) served as selective and differential media indicated lactose and sucrose fermentation with E. coli typically displaying a metallic green sheen (Poirel et al., 2018).

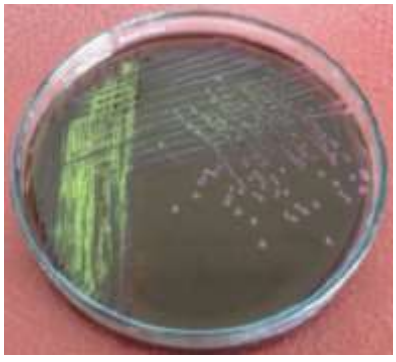
(a)  **(b)** 

Figure 1: (a) Growth of the test culture on MacConkey agar plate, (b) Growth of the test culture on eosin

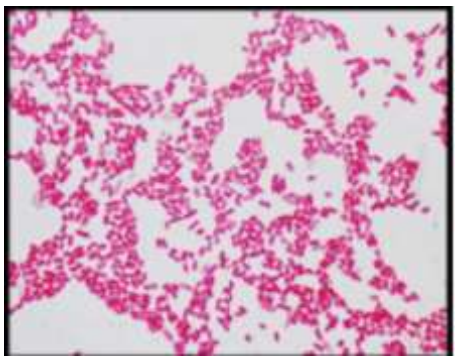


Figure 2: Microscopic examination of the isolates

Biochemical tests, including IMViC, triple sugar iron agar, urease agar, and motility tests were performed on colonies from MacConkey agar. All E. coli isolates exhibited characteristic morphology and biochemical properties, stored at 4°C and -70°C for future use (Zhou et al., 2018). Serotyping involved examining O, H, and K surface antigens based on the modified Kauffman scheme. Serotypes, defined by specific O and H antigen combinations revealed divergent chromosomal markers linked to pathogenic clones (Pang et al., 2018).

Antimicrobial drugs Assay

The susceptibility or resistance to antimicrobial agents was assessed using the Kirby-Bauer disk diffusion method as subsequent in the guidelines established by the Clinical Laboratory Standard Institute (CLSI-M100, 31st Edition) (Humphries et al., 2021). Breakpoints for antibiotics, we followed the recommendations of National Antimicrobial Resistance Monitoring System for E. coli and classified them based on Sensitive (S), Intermediate (I) and Resistance (R) (Ahmed et al., 2020, Ahmed et al., 2022a). The selection of antibiotics was guided by their relevance and availability for treating Gram-negative infections in Pakistan. BD BBL, Sensi-Disc, Becton, Dickinson, USA antimicrobial drugs disk were added in the study (Carvalhaes et al., 2022).

Table 1: Antibiotic Disks and Their Characteristics

Classes	Unit	Antibiotics	Symbols	Disk Content
Aminoglycosides	**µg**	Gentamicin	CN	10
Penicillin		Ampicillin	AMP	10
B-lactamase inhibitor		Amoxicillin-clavulanic acid	AMC	30
Fluoroquinolones		Ciprofloxacin	CIP	5
Folate Pathway Antagonists		Trimethoprim-sulfamethoxazole	SXT	25

***Microgram (µg)**

Molecular characterization & Bioinformatics

Confirmed Escherichia coli (E. coli) samples underwent comprehensive analysis, including DNA extraction using the GeneJET, Thermo Scientific® (Genome DNA purification kit), Gel electrophoresis (Large 50ml), and PCR amplification. Species confirmation was achieved through Sanger sequencing. Phylogenetic trees were generated using online databases such as

NCBI, BLAST, and MEGA 11 bioinformatics tools. The antibiogram analysis of Diarrheagenic E. coli (DEC) and non-DEC involved disc diffusion by the Kirby-Bauer method and antimicrobial susceptibility assays as charted in CLSI-M100, 31st Edition (Humphries et al., 2021). Sequencing of the 16S rRNA gene was conducted, and subsequent analysis involved comparing the obtained sequences with those available in GenBank at NCBI using nucleotide BLAST. This analysis aimed to genetically identify diarrheagenic E. coli strains and detect any sequence variations in the identified strains.

Table 2: Oligonucleotide primer pairs used in the study for detection of diarrheagenic E.coli.

Path-type	Gene	Amplicon size (bp)	Primer sequence	Bibliography
E.coli	uidA(F) uidA(R) AggR(R)	619	GAG TCA TTA AGC AAA AAT AGC GC CCA ACA GGC AAA CAC AGT GCA CAG CAC ATC AGA GAG	(Malberg Tetzschner et al., 2020)
EHEC	Stx 1(R) Stx 1(F) Stx 2(F) Stx 2(R)	614	CTG ACT CCC GTT CGA TCA TG ACA CTG GAT GAT CTC AGT GG	(Tareen et al., 2019)
	HylA(F) HylA(R) PcvD(R) PcvD(F)	778	CCA TGA CGG CAA ACA GCA CTT CAT GTC CTT AAC TCA GCA TG	
		535	GCA TCA AGC TGA CGT GTA TGC AAT TTA GAG AGCTGG CCA AGCT	
		630	CTG AAA GAC GCG TGT ATC AT CAA TGT ATA CGC GAA ATC TGTT	
EAEC	AggR(F)	431	CGC AGA AAG GAT CTA GCC G	(Sanfins, 2019), (Tuo et al., 2020)
EPEC	bfpA(F) bfpA(R) eae(R) eae(F)	450	CGT CAC AGG CGC TAC TGT GA GTT GGC TCA GCA GCT GGA GT	
		228	AAC CTG ATC GTA ACG CAG GC TGA GCA TAA GTC GAA GCT TCC	
ETEC	elt(R) elt(F) estl(R) estl(F)	324	CTA TCT TAC GCA TGT GGA GC TAC CCA CCG TTG TGA CAA T	
		170	TTC TCT TTT TCT CCC ACT CAGTC CAG CAG AGG CAC GAT TAC	

Sample Selection Criteria

Inclusion Criteria: Children (<5 years) admitted for diarrhea.

Exclusion Criteria: Age above 5 years, Non-specific reasons for diarrhea and Systemic disorders.

Data interpretation & Analysis

Statistical analysis was performed using SPSS software. Percentages were compared using either a Pearson chi-square test or Fisher's exact test to assess associations among the DEC pathogroups. A significance level of $p < 0.05$ was considered statistically significant, and odds ratios (OR) along with the 95% confidence interval (95% CI) were calculated.

Results

Study collection

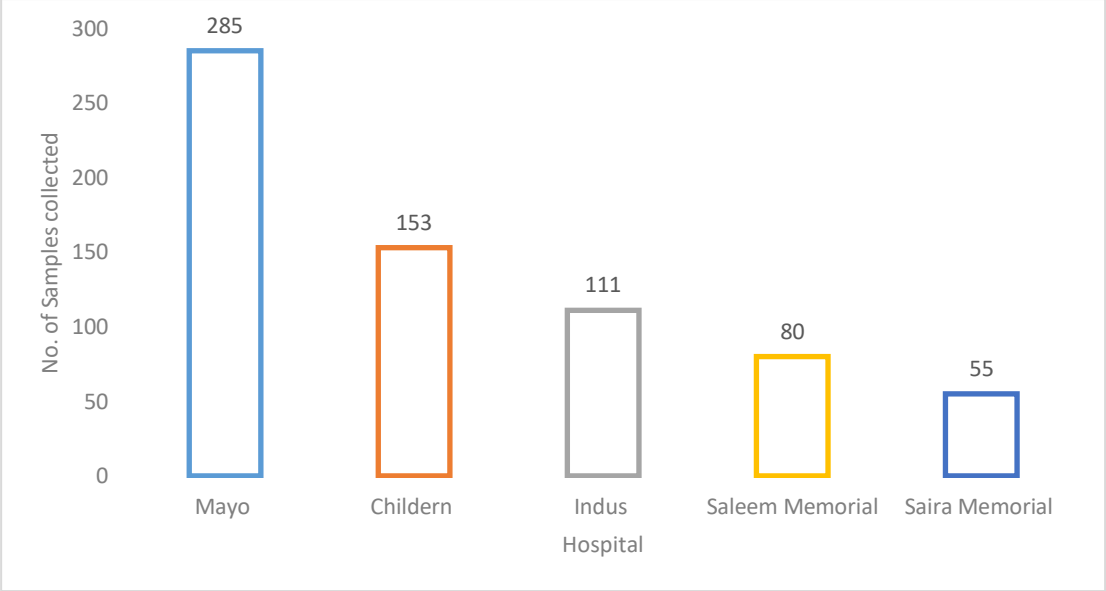


Figure 3: Collection of samples from selected hospitals of Lahore

Demographics

A total of 684 samples were collected, comprising 450 samples identified as diarrheagenic and 234 samples classified as non-diarrheagenic. These samples were obtained from diverse hospitals in Lahore, as detailed in the accompanying table 2.

Table 3: Study Population Demographics

	Variables	Frequency N= 684	Percentage (%)
Gender	Male	300	43.9%
	Female	383	56.1%
Age Group	<1 year	211	30.8%
	1-2 year	146	21.4%
	2-3 year	129	19%
	3-4 year	116	17.7%
	4-5 year	82	12.0%
Groups	A-Diarrheagenic	450	65%
	B-Non-Diarrheagenic	234	34%

Molecular characterization of DEC

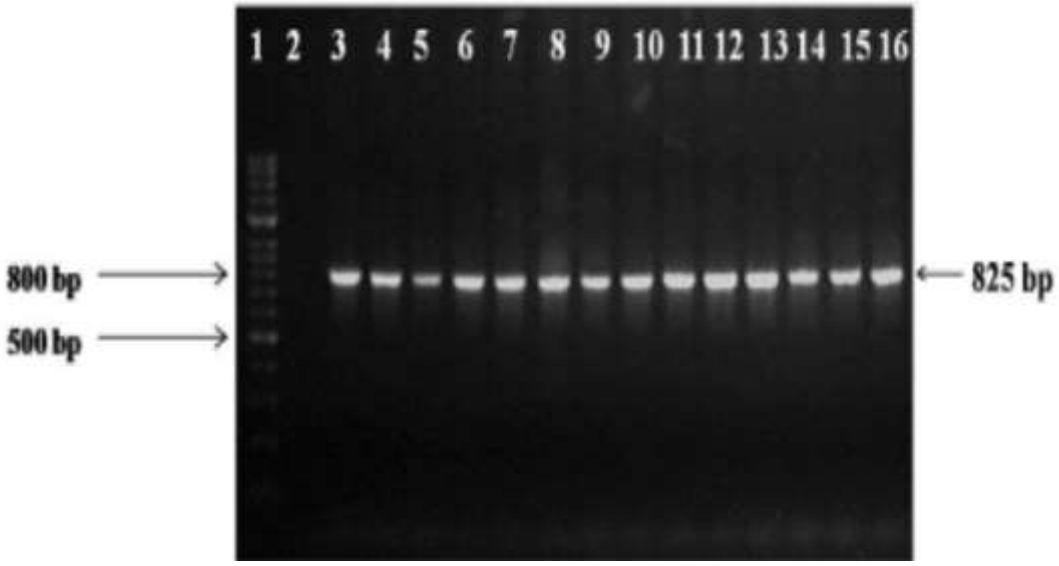


Figure 4: Amplification of mdh Gene by PCR (the PCR products of the mdh gene amplification are presented. Lane 1 displays the molecular weight marker (100bp), while Lane 2 represents the negative control. Lanes 3 to 15 exhibit positive isolates, and Lane 16 serves as the positive control).

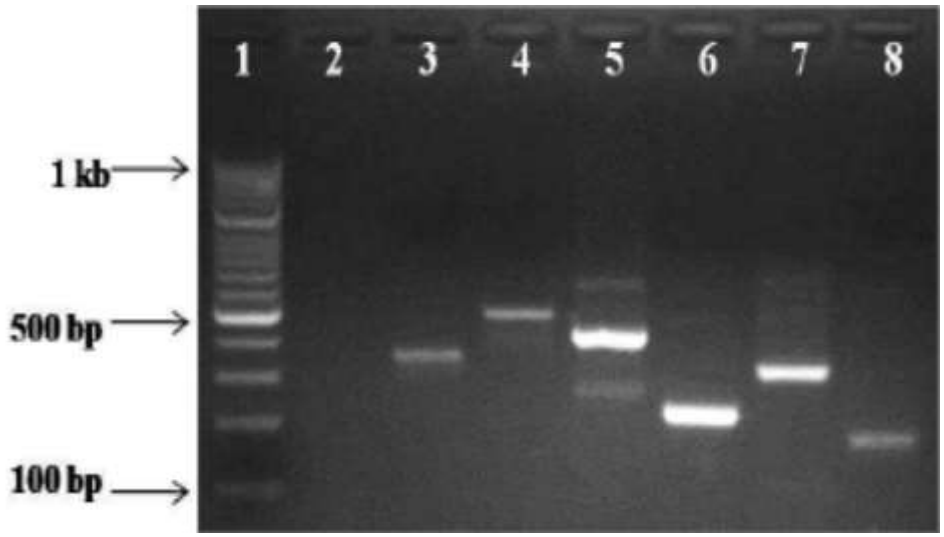


Figure 5: Gel Electrophoresis Analysis of Virulence Genes in E. coli Isolates (a gel electrophoresis image illustrating the virulence gene profile of E. coli isolates. Lane 1 corresponds to the molecular weight marker, while Lane 2 serves as the negative control. Subsequent lanes exhibit specific virulence genes: Lane 3 for stx1 (348 bp), Lane 4 for stx2 (478 bp), Lane 5 for eae A (413 bp), Lane 6 for hlyA (224 bp), and Lane 7 for LT1 (322 bp), and Lane 8 for ST1 (175 bp). This analysis provides insights into the presence and size of virulence-associated DNA fragments in the tested E. coli isolates).

Table 4: Diarrheagenic E. coli Strain Identification

Pathotypes	Serotype	n	Positive genes
ETEC	Ont:Hnt	18	stx 1
EHEC	O121:H19	9	stx 2, pet
	O148:H8	6	astA
EPEC	Ont:Hnt	24	agg3A, aap
	Ont:Hnt	15	ipaH
EIEC	Ont:Hnt	6	aggR, aggA
Nonpathogenic	Ont:Hnt	54	-

Table 5: Distribution of Diarrheagenic Escherichia coli distributed across the different respondents

6666	**No. (%) Positive**								
DEC	**Gender**		**Age (years)**					**Patients with systemic disorders**	**Patients on antimicrobial therapy**
	F (384)	M (300)	<1 (211)	1-2 (146)	2-3 (129)	3-4 (116)	4-5 (82)	(N=100)	(N=80)
EAEC	14 (2.3) †	35 (1.2)	11 (23.1)	45 (14.2)	15 (7.9)	13 (5.6)		34 (3.7)	5 (4.6)
EPEC	35 (4.1)	36 (5.4)	5 (12.3)	25 (2.9)	17 (4.5)	4 (1.6)	0 (0)	16 (34.2)	45 (17.9)
ETEC	30 (2.7)	30 (3.1)	4 (3.9)	20 (4.8)	8 (5.6)	18 (5.9)		50 (51.2)	30 (5.1)
Total DEC	79 (9.1)	101 (9.7)	20 (39.3)	95 (21.9)	40 (18.0)	35 (13.1)		100 (89.1) ‡	80 (27.6)

†Male and female with diarrhea were significantly related to enterohemorrhagic E. coli ($P < 0.02$ OR 6.7). ‡ Patients with systemic disorders had a higher DEC rate than Patients on antimicrobial therapy ($P < 0.05$ OR = 0).

Table 6: Antibiogram Pattern among Subtype of Diarrheagenic Escherichia Coli

Antibiotics	Activity	DEC (n = 180)				
		DEC n = 180	EPEC n = 60	EAEC n = 50	EPEC n =45	ETEC n = 25
AMP	Resistant	125 (69.4)	56 (93.3)	43 (86)	44 (97.8)	21 (84.0)
	Intermediate	21 (11.7)	40 (66.7)	12 (24)	32 (71.1)	22 (88.0)
	Susceptible	24 (13.3)	34 (56.7)	34 (68)	11 (24.4)	24 (96.0)
AMC	Resistant	145 (80.6)	50 (83.3)	45 (90)	12 (26.7)	11 (44.0)
	Intermediate	10 (5.6)	53 (86.3)	32 (64)	34 (75.6)	23 (92.0)
	Susceptible	47 (26.1)	34 (56.7)	32 (64)	23 (51.1)	19 (76.0)
CIP	Resistant	70 (38.9)	34 (56.7)	23 (46)	34 (75.6)	21 (84.0)
	Intermediate	0 (0.0)	58 (96.7)	45 (90)	32 (71.1)	18 (72.0)
	Susceptible	7 (3.9)	23 (38.3)	21 (42)	12 (26.7)	14 (56.0)
SXT	Resistant	7 (3.9)	47 (78.3)	41 (82)	45 (100)	13 (52.0)
	Intermediate	12 (6.7)	34 (56.7)	48 (96)	32(71.1.0)	21 (84.0)
	Susceptible	113 (62.8)	57 (95)	39 (78)	26 (57.8)	17 (68.0)
CN	Resistant	34 (18.9)	32 (53.3)	23 (46)	32 (71.1)	23 (92.0)
	Intermediate	45 (25)	23 (38.3)	12 (24)	41 (91.1)	21 (84.0)
	Susceptible	79 (43.9)	50 (83.3)	35 (70)	38 (84.4)	13 (52.0)

**n (%), Ampicillin (AMP), Amoxicillin-clavulanic acid (AMC), Ciprofloxacin (CIP), Trimethoprim-sulfamethoxazole (SXT), Gentamicin (CN)

Virulence Variables Identified in Diarrheagenic Escherichia coli (DEC)

Table 6 shown the virulence variables identified in Diarrheagenic Escherichia coli (DEC) isolated from individuals who had experienced diarrhea. The aggR gene was found in more than 60% of Enteroaggregative E. coli (EAEC). Among EAEC, 53.3% exhibited multivirulent characteristics, possessing at least three virulence genes. Among the three Enteropathogenic E. coli (EPEC) samples, one exhibited the typical EPEC profile with both eae and bfpA genes. Each Enterotoxigenic E. coli (ETEC) isolate harbored the STp gene, while no LT genes were detected in ETEC. Additionally, three ETEC strains were identified to have colonization factor antigens (CFAs), with 30% displaying CS antigens, including 1 CS6 and 2 CS13

Table 7: Virulence Variables Identified in Diarrheagenic Escherichia coli (DEC) Isolated from Individuals with Diarrhea

Virulence Type & Genes	No. % Positive
EAEC (pCVD) N= 15†	
aggR	9 (60)
aggA	3 (20)
aafA	1 (6.67)
agg3A	4 (26.67)
astA	5 (33.33)
Pet	4 (26.67)
Pic	8 (53.33)
Aap	8 (53.33)
Multivirulent (≥ 3 genes)	8 (53.33)
EPEC (eae) N= 5†	
Typical (eae-positive and bfpA-positive)	3 (60)
Atypical (eae-positive and bfpA-negative)	2 (40)
ETEC (eltB and/or estA) N= 10†	
LT	2 (20)
STh	1 (10)
STp	3 (30)
CS3 (cstA)	0 (0)
CS6 (cssB)	1 (10)
CS13 (cshE)	2 (20)

†Male and female with diarrhea

Molecular Characterization of Escherichia Coli Toxins in Diarrheagenic individuals

Within the cohort of 450 characterized E. coli isolates and Enterotoxigenic E. coli (ETEC) was identified in 125 cases, established 27.8% of the total, as demarcated in Table 5. The majority of ETEC isolates were observed in children below the age of 1 year with a consistent percentage noted across all age categories.

Table 8: Prevalence and Age Distribution of Enterotoxigenic Escherichia coli (ETEC) among individuals

Age (years)	Diarrhea	ETEC positive	p-value
<1 yrs	162 (36.1)	96 (65.0)	< 0.05**
1-2 yrs	111 (24.8)	43 (38.9)	
2-3 yrs	82 (18.2)	21 (22.1)	
3-4 yrs	51 (11.4)	14 (16.3)	
4-5 yrs	42 (9.5)	7 (10.6)	
Total	450	125 (27.8)	

n(%), **Statistical analysis with a significance level of $p < 0.05$ indicated no statistically significant variation in the distribution of ETEC across different age groups, Enterotoxigenic Escherichia coli (ETEC).

Table 9: Distribution of E. coli, Non-Diarrheagenic E. coli, and Diarrheagenic E. coli, Pathotypes in Relation to Clinical Conditions of Patients

E. Coli Types	Total (n = 684)	Diarrhea (n = 450)	Asymptomatic (n = 234)
Non-DEC	180 (50.0)	12 (6.6)	23 (12.8)
DEC	23 (6.9)	34 (18.9)	12 (6.7)
DECs pathotypes	18 (5.0)	32 (17.7)	34 (18.9)
aEAEC	35 (9.7)	14 (7.8)	34 (18.9)
tEPEC	21 (5.8)	24 (13.3)	32 (17.8)
aEPEC	22 (6.11)	8 (4.4)	21 (11.7)
ETEC	32 (8.9)	34 (18.9)	11 (6.1)
DAEC	19 (5.3)	15 (8.3)	7 (3.9)
EIEC	10 (2.7)	7 (3.9)	6 (3.3)

n(%), Non-Diarrheagenic E. coli (non-DEC), and Diarrheagenic E. coli (DEC)

Discussion

Our comprehensive distribution of biological samples collected from various hospitals, with Mayo Hospital, Lahore, contributing the highest number. The study included both male (43.9%) and female (56.1%). Age-wise individuals were categorized into four groups, with 31.9% below 20 years, 26.4% in the 20-30 years range, 25% in the 30-40 years range, and 16.7% above 40 years. The individuals were further divided into two groups: Group A (Diarrheagenic, 50%) and Group B (non-Diarrheagenic, 50%).

In the initial stages of PCR screenings, our focus was on the identification of E. coli pathotypes, with a specific emphasis on utilizing stx1 and stx2, eae primers as discussed by (Hazen et al., 2023). Primers such as ipaH were employed to identify genes linked to plasmid antigen invasion while pCVD432 primers were used to scrutinize specific sequences. Additionally, a PCR test was carried out to target genes associated with various E. coli pathotypes as termed by (Nasir et al., 2020).

Only three distinct E. coli strains were identified. Specifically, three strains were assigned to O121:H19, while the other two were associated with O148:H8. Notably, the available antisera proved inadequate for identifying the remaining strains denoted as ONT:HNT.

We found alignment with (Karambwe et al., 2024) isolated DEC from diarrhea patients exhibited higher resistance patterns to AMP (69.4%) and AMC (80.6%) compared to DEC strains recovered from individuals aged 3 years. However, there were no significant differences in the resistance patterns of DEC isolated from children above the age of 2 and individuals beyond the age of 4 to AMC, GEN, and SXT.

Similarly (Abdulabbas et al., 2023) detected 60% of EAEC exhibited the presence of the aggR gene, 53.3% of EAEC were characterized as multivirulent isolates that influenced three

virulence genes. Among the three EPEC samples, one detected typical EPEC profile while other were eae and bfpA genes. In every ETEC, a gene encoding for STp was observed, while no LT genes were found in ETEC. Furthermore, three ETEC strains were identified to have CS antigens with 30% prevalence, including 1CS6 and 2CS13.

(Lee et al., 2023) also examined different Escherichia coli pathotypes (ETEC, EIEC, EPEC, EHEC, and EAEC) virulence factors in individuals with diarrhea. But our pathotypes demonstrated a clonal nature, belonging to unique O serotypes and O: H serotypes. Virulence determinants for each pathotypes were distinct, falling into categories of either colonization factors (adhesions), facilitating close binding to the intestinal mucosa, or secreted toxins, disrupting host cell physiological processes. This highlights the diverse mechanisms through which E. coli pathotypes contribute to diarrheal infections.

We found concordance with (Agbemavor and Buys, 2023) in term of among our studied strains, of which, 34% (234/684) were categorized as non-diarrheagenic E. coli (non-DEC) due to the absence of DEC-associated virulence factors, while 15.9% (67/450) were identified as DEC strains through PCR analysis. The most prevalent diarrheagenic E. coli pathotypes in both groups were AEPEC (6.11%), tEAEC (9.7%), ETEC (8.9%), DAEC (5.3%), and EIEC (5.6%), followed by ETEC (9.9%), aEAEC (9.7%), and tEPEC (5.8%). Remarkably, aEPEC, the most common pathogen among asymptomatic responders, was the least likely to be identified in individuals with diarrhea. ETEC was exclusively found in patients with diarrhea, distinguishing it from other DEC pathotypes, which were present in both diarrhea cases and asymptomatic individual.

Conclusion

The study contributes valuable insights into the epidemiology and molecular characteristics of E. coli pathotypes in the studied population of Lahore. These findings have implications for both clinical management and public health interventions, aiding in the development of targeted strategies for the prevention and treatment of E. coli-related diarrheal infections. Further research is warranted to explore the evolving landscape of E. coli pathotypes and their interactions with host factors in diverse populations. The identification and categorization of E. coli strains revealed a clonal nature among different pathotypes, each associated with unique O and O:H serotypes. PCR screenings focused on specific virulence factors, showcasing the diversity of mechanisms through which E. coli pathotypes contribute to diarrheal infections. Notably, the prevalence of distinct pathotypes varied between individuals with diarrhea and asymptomatic responders, with Enterotoxigenic E. coli (ETEC) being exclusive to the former group. Resistance patterns of Diarrheagenic E. coli (DEC) strains indicated higher resistance to certain antibiotics in patients with diarrhea, emphasizing the importance of considering age-related variations in resistance profiles. Additionally, the presence of specific virulence genes in EAEC and EPEC further highlighted the complexity of E. coli pathogenicity.

References

ABDULABBAS, H. S., AL-KHAFAJI, N., ABED, S. Y., AL-DAHMOSHI, H. & AL-BAROODY, H. N. 2023. Phylotypes and Pathotypes of Diarrheagenic Escherichia coli of Gastroenteritis.

AGBEMAVOR, W. S. K. & BUYS, E. M. 2023. Dynamic Interactions between Diarrhoeagenic Enteroaggregative Escherichia coli and Presumptive Probiotic Bacteria: Implications for Gastrointestinal Health. Microorganisms, 11**,** 2942.

AHMED, N., ALI, Z., RIAZ, M., ZESHAN, B., WATTOO, J. I. & ASLAM, M. N. 2020. Evaluation of antibiotic resistance and virulence genes among clinical isolates of Pseudomonas aeruginosa from cancer patients. Asian Pacific journal of cancer prevention: APJCP, 21**,** 1333.

AHMED, N., HABIB, S., MUZZAMMIL, M., RABAAN, A. A., TURKISTANI, S. A., GAROUT, M., HALWANI, M. A., ALJELDAH, M., AL SHAMMARI, B. R. & SABOUR, A. A. 2022a. Prevalence of Bacterial Pathogens among Symptomatic–SARS-CoV-2 PCR-Negative Patients. Microorganisms, 10**,** 1978.

AHMED, N., KHAN, M., SALEEM, W., KAROBARI, M. I., MOHAMED, R. N., HEBOYAN, A., RABAAN, A. A., MUTAIR, A. A., ALHUMAID, S. & ALSADIQ, S. A. 2022b. Evaluation of bi-lateral co-infections and antibiotic resistance rates among COVID-19 patients. Antibiotics, 11**,** 276.

AHMED, N., ZESHAN, B., NAVEED, M., AFZAL, M. & MOHAMED, M. 2019. Antibiotic resistance profile in relation to virulence genes fimH, hlyA and usp of uropathogenic E. coli isolates in Lahore, Pakistan. Trop. Biomed, 36**,** 559-568.

ANGULO-ZAMUDIO, U. A., GUTIÉRREZ-JIMÉNEZ, J., MONROY-HIGUERA, L., FLORES-VILLASEÑOR, H., LEON-SICAIROS, N., VELAZQUEZ-ROMAN, J., VIDAL, J. E., TAPIA-PASTRANA, G. & CANIZALEZ-ROMAN, A. 2021. Non-diarrheagenic and diarrheagenic E. coli carrying supplementary virulence genes (SVG) are associated with diarrhea in children from Mexico. Microbial pathogenesis, 157**,** 104994.

CARVALHAES, C. G., KLAUER, A. L., RHOMBERG, P. R., PFALLER, M. A. & CASTANHEIRA, M. 2022. Evaluation of rezafungin provisional CLSI clinical breakpoints and epidemiological cutoff values tested against a worldwide collection of contemporaneous invasive fungal isolates (2019 to 2020). Journal of Clinical Microbiology, 60**,** e02449-21.

DENAMUR, E., CLERMONT, O., BONACORSI, S. & GORDON, D. 2021. The population genetics of pathogenic Escherichia coli. Nature Reviews Microbiology, 19**,** 37-54.

HAJIHOSEIN-TABRIZI, A., HABIBI, M., TABASI, M. & ASADI KARAM, M. R. 2018. Distribution of genes encoding iron uptake systems among the Escherichia coli isolates from diarrheal patients of Iran. Journal of Medical Microbiology and Infectious Diseases, 6**,** 25-30.

HAZEN, T. H., MICHALSKI, J. M., TENNANT, S. M. & RASKO, D. A. 2023. Genomic diversity of non-diarrheagenic fecal Escherichia coli from children in sub-Saharan Africa and south Asia and their relatedness to diarrheagenic E. coli. Nature Communications, 14**,** 1400.

HUMPHRIES, R., BOBENCHIK, A. M., HINDLER, J. A. & SCHUETZ, A. N. 2021. Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. Journal of clinical microbiology, 59**,** 10.1128/jcm. 00213-21.

KARAMBWE, S., TRAORÉ, A. N. & POTGIETER, N. 2024. Epidemiology of Cefotaxime-Hydrolysing β-Lactamase-Producing Escherichia coli in Children with Diarrhoea Reported Globally between 2012 and 2022. Microorganisms, 12**,** 171.

LEE, W., SUNG, S., HA, J., KIM, E., AN, E. S., KIM, S. H., KIM, S. H. & KIM, H.-Y. 2023. Molecular and Genomic Analysis of the Virulence Factors and Potential Transmission of Hybrid Enteropathogenic and Enterotoxigenic Escherichia coli (EPEC/ETEC) Strains Isolated in South Korea. International Journal of Molecular Sciences, 24**,** 12729.

LIANG, C., CUI, H., CHEN, L., ZHANG, H., ZHANG, C. & LIU, J. 2023. Identification, Typing, and Drug Resistance Analysis of Escherichia coli in Two Different Types of Broiler Farms in Hebei Province. Animals, 13**,** 3194.

MALBERG TETZSCHNER, A. M., JOHNSON, J. R., JOHNSTON, B. D., LUND, O. & SCHEUTZ, F. 2020. In silico genotyping of Escherichia coli isolates for extraintestinal virulence genes by use of whole-genome sequencing data. Journal of clinical microbiology, 58**,** 10.1128/jcm. 01269-20.

MUSTAFAI, M. M., HAFEEZ, M., MUNAWAR, S., BASHA, S., RABAAN, A. A., HALWANI, M. A., ALAWFI, A., ALSHENGETI, A., NAJIM, M. A. & ALWARTHAN, S. 2023. Prevalence of carbapenemase and extended-spectrum β-lactamase producing Enterobacteriaceae: a cross-sectional study. Antibiotics, 12**,** 148.

NASIR, N. A., MOHAMMED ALI, S. H., ABU AL-ESS, H. Q. M., HUSSEIN, W. A., WANNAS AL-JANABI, M. K., MOHAMMED, K. I. A. & MOSA, J. M. 2020. The Detection of Escherichiacoli O157: H7 Infections in Children Less than Five Years with Acute Diarrhea. Indian Journal of Public Health Research & Development, 11.

PANG, B., ZHAO, C., LI, L., SONG, X., XU, K., WANG, J., LIU, Y., FU, K., BAO, H. & SONG, D. 2018. Development of a low-cost paper-based ELISA method for rapid Escherichia coli O157: H7 detection. Analytical Biochemistry, 542**,** 58-62.

POIREL, L., MADEC, J.-Y., LUPO, A., SCHINK, A.-K., KIEFFER, N., NORDMANN, P. & SCHWARZ, S. 2018. Antimicrobial resistance in Escherichia coli. Microbiology spectrum, 6**,** 6.4. 14.

RABAAN, A. A., ALHUMAID, S., MUTAIR, A. A., GAROUT, M., ABULHAMAYEL, Y., HALWANI, M. A., ALESTAD, J. H., BSHABSHE, A. A., SULAIMAN, T. & ALFONAISAN, M. K. 2022a. Application of artificial intelligence in combating high antimicrobial resistance rates. Antibiotics, 11**,** 784.

RABAAN, A. A., ELJAALY, K., ALHUMAID, S., ALBAYAT, H., AL-ADSANI, W., SABOUR, A. A., ALSHIEKHEID, M. A., AL-JISHI, J. M., KHAMIS, F. & ALWARTHAN, S. 2022b. An Overview on Phenotypic and Genotypic Characterisation of Carbapenem-Resistant Enterobacterales. Medicina, 58**,** 1675.

RIZVI, A., SAEED, M. U., NADEEM, A., YAQOOB, A., RABAAN, A. A., BAKHREBAH, M. A., AL MUTAIR, A., ALHUMAID, S., ALJELDAH, M. & AL SHAMMARI, B. R. 2022. Evaluation of bi-lateral co-infections and antibiotic resistance rates among COVID-19 patients in Lahore, Pakistan. Medicina, 58**,** 904.

SANFINS, C. D. S. 2019. Escherichia coli potencialmente virulenta em áreas de risco de inundação no município de São Gonçalo, RJ: inundação no município de São Gonçalo, RJ: uma abordagem l aboratorial e espacial.

SAROWSKA, J., FUTOMA-KOLOCH, B., JAMA-KMIECIK, A., FREJ-MADRZAK, M., KSIAZCZYK, M., BUGLA-PLOSKONSKA, G. & CHOROSZY-KROL, I. 2019. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic Escherichia coli isolated from different sources: recent reports. Gut pathogens, 11**,** 1-16.

SOHAIL, M., MUZZAMMIL, M., AHMAD, M., REHMAN, S., GAROUT, M., KHOJAH, T. M., AL-EISA, K. M., BREAGESH, S. A., HAMDAN, R. M. A. & ALIBRAHIM, H. I. 2023. Molecular Characterization of Community-and Hospital-Acquired Methicillin-Resistant Staphylococcus aureus Isolates during COVID-19 Pandemic. Antibiotics, 12**,** 157.

TAREEN, A. M., ULLAH, K., ASMAT, T. M., SAMAD, A., IQBAL, A., MUSTAFA, M. Z., IRSHAD, A. & UR RAHMAN, S. 2019. Incidence of diarrheagenic Escherichia coli pathotypes in children suffering from diarrhea in tertiary care hospitals, Quetta, Pakistan. Pakistan Journal of Zoology, 51**,** 2015.

TUO, X., WANG, S., CUI, D., WANG, F., LIU, Y., WANG, H. & WANG, H. 2020. Antibiotic resistance profiles and virulence markers of Escherichia coli strains isolated from diarrheal lambs in Gansu and Qinghai, China. Pakistan Veterinary Journal, 40**,** 123-126.

ULLAH, M., RASOOL, F., KHAN, N., ALI, S. & SHEIKH, A. A. 2023. Molecular characterization and antimicrobial resistance among diarrheagenic and non-diarrheagenic Escherichia coli from healthy and diseased freshwater farmed fish. Pakistan Journal of Agricultural Sciences, 60.

WADA, Y., AFOLABI, H. A., AL-MHANNA, S. B., BELLO, K. E., IREKEOLA, A. A., WADA, M., AHMED, N., HARUN, A., YEAN, C. Y. & NASIR, N. M. 2024. Global Occurrence of Linezolid-Resistant Enterococcus (LRE): The First Systematic Review and Meta-Analysis. The Microbe**,** 100041.

YIM, J.-H., SEO, K.-H., CHON, J.-W., JEONG, D. & SONG, K.-Y. 2021. Status and prospects of PCR detection methods for diagnosing pathogenic Escherichia coli: A review. Journal of Dairy Science and Biotechnology, 39**,** 51-62.

YUSOF, N. Y., NORAZZMAN, N. I. I., HAKIM, S. N. A. W. A., AZLAN, M. M., ANTHONY, A. A., MUSTAFA, F. H., AHMED, N., RABAAN, A. A., ALMUTHREE, S. A. & ALAWFI, A. 2022. Prevalence of Mutated Colistin-Resistant Klebsiella pneumoniae: A Systematic Review and Meta-Analysis. Tropical Medicine and Infectious Disease, 7**,** 414.

ZAHARI, N. I. N., ENGKU ABD RAHMAN, E. N. S., IREKEOLA, A. A., AHMED, N., RABAAN, A. A., ALOTAIBI, J., ALQAHTANI, S. A., HALAWI, M. Y., ALAMRI, I. A. & ALMOGBEL, M. S. 2023. A Review of the Resistance Mechanisms for β-Lactams, Macrolides and Fluoroquinolones among Streptococcus pneumoniae. Medicina, 59**,** 1927.

ZAHRA, N., ZESHAN, B., QADRI, M. M. A., ISHAQ, M., AFZAL, M. & AHMED, N. 2021. Phenotypic and genotypic evaluation of antibiotic resistance of Acinetobacter baumannii bacteria isolated from surgical intensive care unit patients in Pakistan. Jundishapur Journal of Microbiology, 14.

ZEB, S., MUSHTAQ, M., AHMAD, M., SALEEM, W., RABAAN, A. A., NAQVI, B. S. Z., GAROUT, M., ALJELDAH, M., AL SHAMMARI, B. R. & AL FARAJ, N. J. 2022. Self-medication as an important risk factor for antibiotic resistance: a multi-institutional survey among students. Antibiotics, 11**,** 842.

ZESHAN, B., KAROBARI, M. I., AFZAL, N., SIDDIQ, A., BASHA, S., BASHEER, S. N., PEERAN, S. W., MUSTAFA, M., DAUD, N. H. A. & AHMED, N. 2021. The usage of antibiotics by COVID-19 patients with comorbidities: the risk of increased antimicrobial resistance. Antibiotics, 11**,** 35.

ZHOU, Y., ZHU, X., HOU, H., LU, Y., YU, J., MAO, L., MAO, L. & SUN, Z. 2018. Characteristics of diarrheagenic Escherichia coli among children under 5 years of age with acute diarrhea: a hospital based study. BMC infectious diseases, 18**,** 1-10.