Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.3416

DETECTION AND MOLECULAR CHARACTERIZATION OF DIARRHEAGENIC ESCHERICHIA COLI ISOLATED FROM CHILDREN WITH DIARRHEA IN TERTIARY CARE HOSPITAL OF LAHORE, PAKISTAN

Ayesha Masood[1], Dr Bushra Adeel[2], Sehr Syed[3], Muhammad Qasim Ali[4], Asif Hanif[5], Dr. Syed Zeeshan Haider Naqvi[6]*, Hafiza Ayesha Andleeb[7], Dr Amina Mahmood[8], Dr. Fatima Masood[9], Dr. Izah Tahir[10]

[1]Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore -Pakistan.
Email: ayesha.masood@ucm.uol.edu.pk
[2]Rashid Latif Medical College, Lahore - Pakistan
[3]University Teaching Hospital, The University of Lahore - Pakistan
[4]Lecturer of Microbiology, Institute of Life Science, Gulab Devi Educational Complex, Ferozpur Road, Lahore - Pakistan
[5]University College of Medicine and Dentistry, The University of Lahore - Pakistan
[6*]Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore - Pakistan.
[7]M. Phil Microbiology, The University of Lahore, Pakistan
[8]University College of Medicine and Dentistry, The University of Lahore - Pakistan
[9]University College of Medicine and Dentistry, The University of Lahore - Pakistan
[10]University College of Medicine and Dentistry, The University of Lahore - Pakistan

***Corresponding Author:** Dr. Syed Zeeshan H. Naqvi
*Associate Professor, Research Supervisor & Principle Investigator Institute of Molecular Biology & Biotechnology (IMBB), The University of Lahore, Pakistan.
Email: zeeshan.haider@imbb.uol.edu.pk

Abstract

Background: Diarrhea is a ruling general health issue in developing countries. The prevailing rate of diarrhea in individuals below the age of 5 years is increasing every year. The current study aims to find out the prevalence of *Escherichia coli* strains causing diarrhoea in off-springs and children of 5 years or below in the region of Lahore, Pakistan. The purpose of this study is to reveal the underline cause of diarrhoea in children and to monitor the anti-microbial treatment of the children.

Material and Methods: Over the course of 9 months, samples were obtained from the Medical Center for Children and Sir Ganga Ram Hospital in Lahore. Participating children were less than 5 years old and were having diarrhea. For determining the prevalence of Diarrheagenic *Escherichia coli* (DEC) among children aged five and under, a total of 268 rectal swab specimens were collected from kids who were ill with diarrhoea. All specimens were received in sterile containers and cultured. Standard biochemical assays were used for identifying *E. coli*. Confirmed *E. coli* samples were further studied using multiplex PCR for identification of its particular genes by amplification of toxic genes. Pearson correlation analysis was done for the present study.

Results: 146 out of 268 samples were derived as positive for *E. coli.* 36 % isolates had the entero-aggressive (EAEC) pathogenic gene. EAEC revealed 10 % co-infection with entero-hemorrhagic E. coli (EHEC). Enteropathogenic (EPEC) virulent gene was found in 19% of total samples that were observed positive for *E. coli*. Older *Escherichia coli* (DEC) strains had a stronger negative correlation with age groups, suggesting that lower-aged infants may have more chances of getting the strains.
Conclusion: The current study found that EAEC is significantly linked to diarrheal disease in children under the age of 5 years. The present study reveals strain-specific analysis of diarrheagenic *E. coli* at a molecular level by multiplex PCR plays a pivotal role in finding out the prevalence of *E. coli* strains at a regional level. Moreover, the current study will also play a positive role in the anti-microbial monitoring of diarrheal patients, with a focus on the increased prevailing ratio of the disease. To the best of our knowledge, this is a foremost report from Punjab Pakistan that enlists isolated EAEC, EHEC and EPEC genes in the region.

Keywords: Diarrhea, Diarrheagenic *Escherichia coli* (DEC), Multiplex PCR, Infection.

BACKGROUND

Diarrhea is a dominant general illness of developing countries. While in Pakistan, issues of this disease are higher and only particular investigations are made. *Escherichia coli* is dependent and basically lives in human intestine after birth hours. The frequency of this problem in children under 5 years of age is increasing every year. 1600 children died in the year 2012 due to diarrheal infection that expanded day after day (1).

In children, diarrhea is known as subordinate major reason of death globally. Whereas in Ethiopia, the number of newborns less than age of five years, dying due to this health problem is about half-million. There are many reasons for this including foul environment, contaminated water and poor personal hygiene which cause almost 90% of disease spread (2).

It causes 5,25,000 deaths of children below five years of age and cases of 1.7 billion throughout the globe and is a crucial health problem of people of under-developed countries (3). The average age ratio of mortality among children due to diarrheal disease is 72% under 2 years of age. Most of the times, children that live in downtown areas of the cities are more vulnerable to develop diarrhea (4). More distance from health and hygiene facilities also plays a major role to raise illnesses from diarrhea in children in many areas of Pakistan such as Matiari (5). There are many other factors too that take part in extended ratio of diarrhea. Changes in seasons and monthly variations occur in frequency of growth of disease growth but in general, diarrhea has comparatively the highest frequency (59.1%) for most of the months in Pakistan (6).

According to the proceeding of World Health Organization (WHO), Pakistan is ranking 23 today out of 194 countries that were monitored for mortality of lives of children due to this trouble between 0 to 4 years of age which means that diarrhea is the reason for six deaths in every thousand live births of children in Pakistan (7). E. coli pathogenic types living in the gut of human body like entero-pathogenic (EPEC) and entero-toxigenic E. coli (ETEC) are mostly infectious in developing nations, whilst strains of ETEC are crucial reason for developing diarrheal disease in travelers of these under developed nations. They are responsible for diarrhea and also for other critical clinical issues such as hemolytic uremia (HUS) or hemorrhagic colitis (8). Unusual EPEC and entero-aggregative E. coli (EAEC) are the chief pathogenic agents that cause diarrhea in children among diarrheagenic E. coli pathotypes (9). EPEC is the prominent sources of water consistency diarrhea in children (10). Different kinds of E. coli give rise to acute level of diarrheal illness in human and create massive load of this sickness worldwide. Detection techniques and categorizations of DEC pathotypes are beneficial but not perfect. The application of methods at molecular stage, based on

order processes and fine-formed research studies in epidemiology will proceed to many progressive comprehensions of these pathotypes (11). DEC, still is a crucial germ that cause diarrhea and leads to admission in the hospital and then death particularly in children (12) and many research studies mention it as a main cause of diarrheal problem. According to many studies, EPEC and EAEC affect children below period of 5 years. Resistance to multi-drugs amid DEC pathotypes has capability for becoming a great reason to worry (13). They are also a root of gastroenteritis in children but, still minimum information is accessible for phylogenetics, serotyping, epidemiology, also antibiotic drug sensitivity of DEC in offspring living in the United States (US). Methods based on DNA to detect these subclasses require more investigations to guide in differentiations between colonizing and pathogenic strains (14).

The diversification of DEC is accountable to develop distinct kinds of diarrhoea in children chiefly in emergent nations. The occurrence of DEC pathotypes in our state causing diarrhea especially in children is distressing. Many studies highlight the use of PCR test for the best differentiation (15). *ETEC* is a very typical reason for bacterial attack that can lead at stage of severe watery consistency diarrhea in children and youngsters and also inside travelers of *ETEC* endemic areas of states. Ciprofloxacin, is a far-ranging agent of antimicrobe these days that is used for the cure of diarrhea (16). According to an estimation, almost 79,420 cases of *ETEC* occur in the United States each year (17).

Regardless of irregular E. coli record restored from diarrheal sufferers in the country Pakistan, the patho-typing along with molecular characterization of analytical isolates are insufficient. Survey like this is critical to realize the pathogenicity of E. coli insulates and can aid the legislators in advance for discovering the efficacious approach to control it. The current research work is directed to find out the approximate pervasiveness of five key types of *DEC* in children with age range less than 5 years.

RESULTS

A total of 268 rectal swab samples were collected from children with diarrheal sickness to find out the DEC prevalence within children aged five years or below.

For documenting the current rate of DEC in patients, samples were examined using traditional and new diagnostic procedures. By using a selective EMB agar medium, 146 samples out of 268 were found positive for *E. coli*. Positive samples included common colonies with a blue-black core and a greenish metallic sheen under mirrored light. A specific PCR assay was used to establish biochemically verified and purified *E. coli* isolates.

Table 1: Primer sequences of virulent genes of the *E. coli*

Patho-type	Gene	Amplicon size (bp)	Primer sequence
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
EHEC	Stx 1(R) Stx 1(F) Stx 2(F) Stx 2(R) HylA(F) HylA(R) PcvD(R) PcvD(F)	614 778 535 630	CTG ACT CCC GTT CGA TCA TG ACA CTG GAT GAT CTC AGT GG CCA TGA CGG CAA ACA GCA CTT CAT GTC CTT AAC TCA GCA TG GCA TCA AGC TGA CGT GTA TGC AAT TTA GAG AGCTGG CCA AGCT 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

EPEC	bfpA(F) bfpA(R) eae(R) eae(F)	450 228	CGT CAC AGG CGC TAC TGT GA GTT GGC TCA GCA GCT GGA GT AAC CTG ATC GTA ACG CAG GC TGA GCA TAA GTC GAA GCT TCC
ETEC	elt(R) elt(F) estl(R) estl(F)	324 170	CTA TCT TAC GCA TGT GGA GC TAC CCA CCG TTG TGA CAA T TTC TCT TTT TCT CCC ACT CAGTC CAG CAG AGG CAC GAT TAC

Multiplex PCR was used to confirm the samples *of E. coli* by running gene-specific PCR reactions to identify *E. coli* strains with their extremely pathogenic genes. The following table revealed the percentage prevalence of *E.coli* strains in diarrheagenic patients of 5 years of age or below.

Table 2: **Frequency of Diarrheagenic *Escherichia coli* (DEC) strains among individuals suffering from diarrhea.**

***Escherichia coli* (DEC) strains**	**Total Samples**	**Positive Samples (n)**	**Percentage**	**P Value**
EAEC	146	52	36%	0.003
EPEC	146	28	19%	0.001
EHEC	146	14	9%	0.001
EAEC + EHEC	146	15	10%	0.002
EPEC + EHEC + EAEC	146	20	14%	0.001
EAEC + ETEC + EHEC	146	17	12%	0.003

According to the findings of the multiplex PCR, 36 percent of isolates had the EAEC pathogenic gene, as indicated in Table 2. In particular, EAEC revealed 10 % co-infection with entero-hemorrhagic *E. coli* (EHEC). EPEC virulent gene was found in 19% of total samples that were observed positive for *E. coli*. In 9% of all positive E. coli cases, the EHEC pathotype indicating Shiga-like toxin 2 (Stx2) genes were amplified. EHEC's Stx1 was found only in samples that got infected with EAEC. In the same way, the pathotype of the ETEC exhibiting gene was exclusively discovered in EAEC and EHEC-infected samples. Combined infections, were found in about 12 % of the *E. coli* confirmed cases that tested positive. Furthermore, EHEC and EAEC stx2 were detected combined in 10% of the samples with positive results. Co-infection of EPEC with EAEC and EHEC was discovered in 14% of the cases.

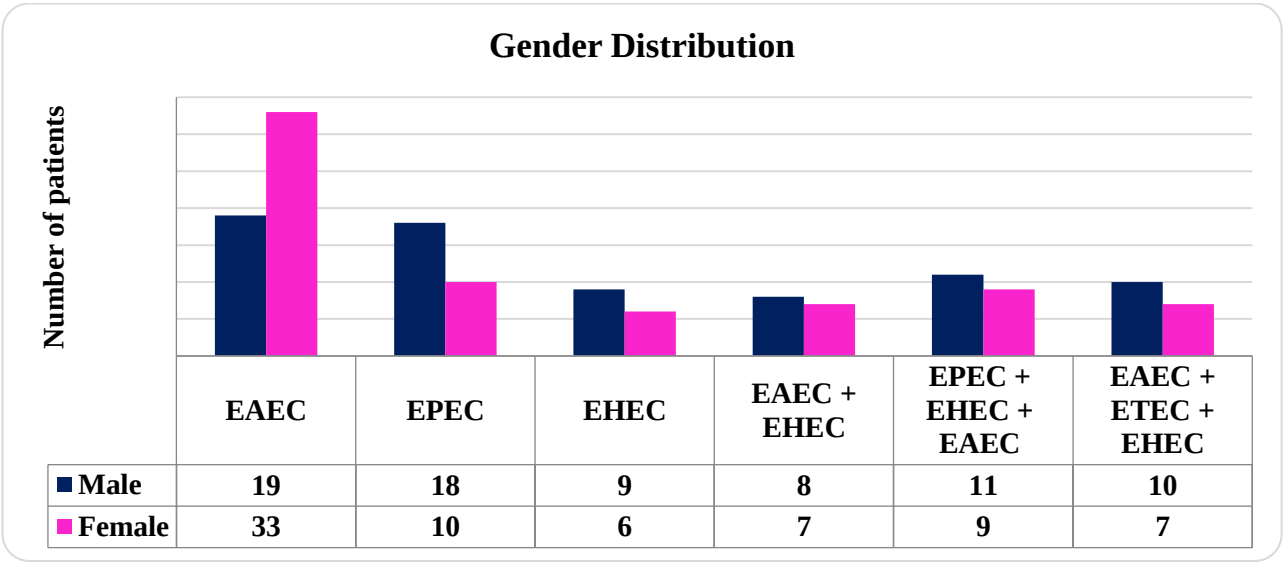

Figure 1: Diarrheal *Escherichia coli* prevalence (DEC) strains sub-categorized in accordance with gender of the children having diarrhea.

From one hundred and forty-six positive DEC samples, 75 (51%) of them were males whereas, 71 (49%) were females. The most prevalent among children was the virulent gene of EAEC. The females were more affected by this as compared to other strains where a trend of males is more prevalent.

Table 3: Prevalence of Diarrheagenic *E. coli* (DEC) strains detected by multiplex PCR, sub-categorized in accord with age of suffering infant from diarrhea.

***Escherichia coli* (DEC) strains**	**Age of Children**					**P Value**
	<1 year	**1-2 years**	**2-3 years**	**3-4 years**	**4-5 years**	
EAEC	18	21	11	2	0	0.002
EPEC	14	7	5	0	2	0.001
EHEC	6	7	1	0	0	0.002
EAEC + EHEC	5	4	4	2	0	0.002
EPEC + EHEC + EAEC	8	7	3	0	2	0.001
EAEC + ETEC + EHEC	5	6	3	2	1	0.003
Total	56	52	27	6	5	0.002

According to the findings, the patients' age groups were under the age of 12 months (38%), 1 - 2 years of age (35%), 2-3 years of age (18%), between 3 to 4 years of age (4%) and 4-5 years of age (3%). This has been tabulated in Table 3. These findings suggest that infants under the age of 5 years suffering from diarrhea have a significant DEC burden. Importantly, the EAEC strain was shown to be the most common in the current study.

Table 4: Pearson's correlation matrix of DEC strains and age in years.

		***Escherichia coli* (DEC) strains**	**Age (Number of Years)**
Escherichia coli (DEC) strains	Pearson Correlation	1	-0.792**
	Sig. (2-tailed)		.000
	N	146	146
(Age) Number of Years	Pearson Correlation	-0.792**	1
	Sig. (2-tailed)	.000	
	N	146	
**. Correlation is significant at the 0.01 level (2-tailed).			

The correlation analyses of the present results are shown in Table 4.4. In this study, we found that older *Escherichia coli* (DEC) strains had a stronger negative correlation with age groups, suggesting that lower-aged infants may have more chances of getting the strains.

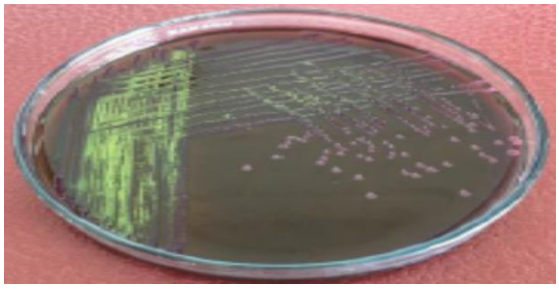

Figure 2: Test culture on EMB

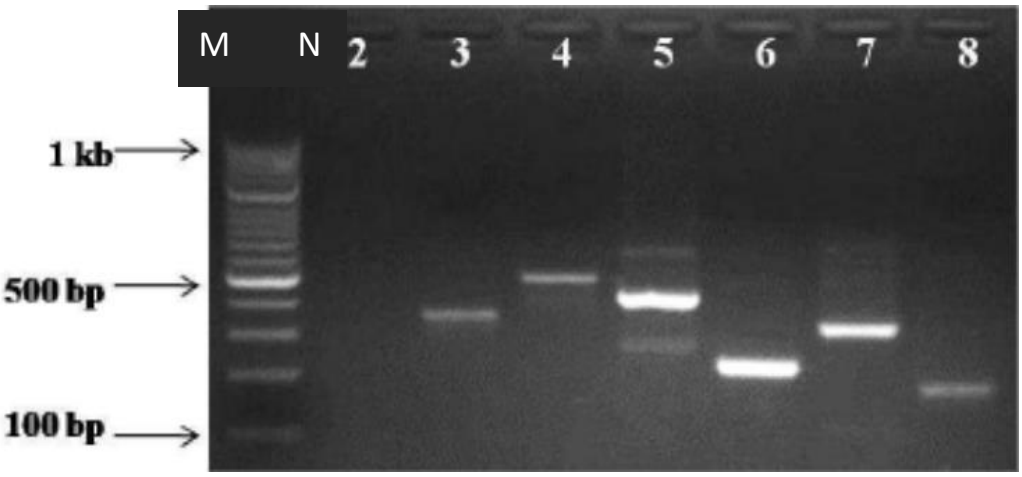

Figure 3: A representative gel electrophoresis image showing virulence genes profile of E. coli isolates. Lane 1: molecular weight marker (100bp), Lane 2: negative control, Lane 3: stx1 (348 bp), Lane 4: stx2 (478 bp), Lane 5: eae A (413bp), Lane 6: hlyA (224 bp), Lane 7; LT1 (322 bp), Lane 8: ST1 (175 bp).

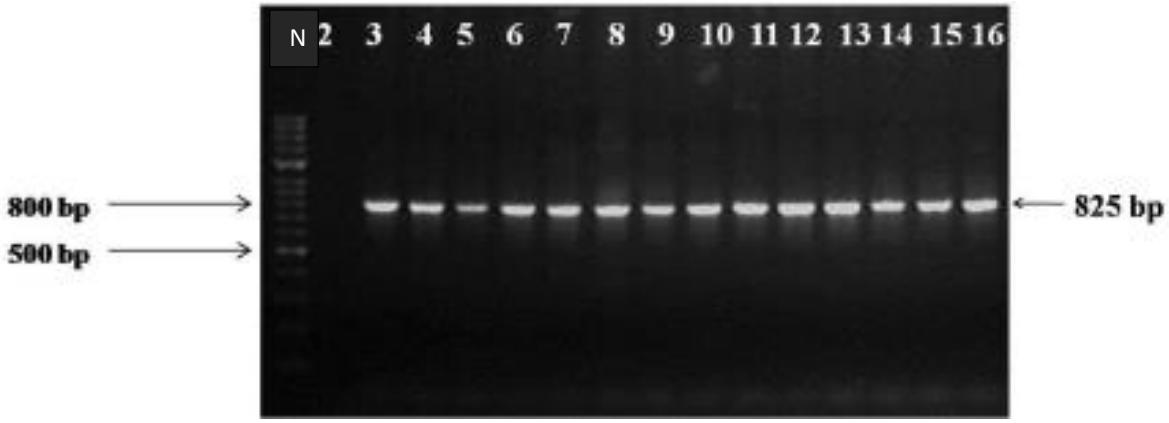

Figure 4: PCR products of the amplification of mdh gene. Lane1: Molecular weight marker (500bp); Lane 2: negative control; Lanes 3-15: positive isolates; Lane 16: positive control.

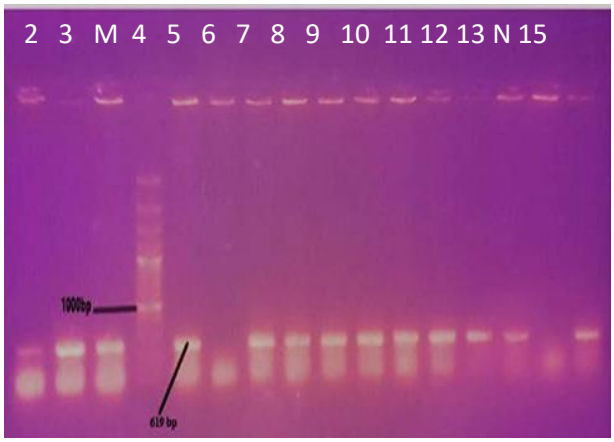

Figure 5: Polymerase chain reaction analysis for detection of the virulence gene profile of *E.coli* Isolates. Lanes 1-3: positive Isolates (619bp); Lane 4: molecular weight marker (1000bp); Lanes 4-13: positive Isolates (619bp); Lane 14: negative control; Lane 15: (619bp).

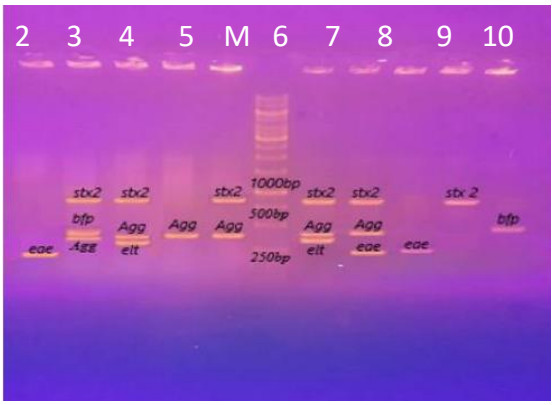

Figure 6: Ethidium bromide-strained agarose gel Electrophoresis of PCR-amplified product from extracted diarrheagenic *E. coli* DNA amplified with primers, DNA molecular size marker (250bp).

DISCUSSION

Strains of DEC are not routinely pursued as stool parasites in developing countries, but they are instead treated based on symptoms. DEC infections are serious, especially in newborns, therefore appropriate tests should be carried out before any type of medicine is prescribed. In this research, the frequency of *E. coli* is 54% among all the diarrhea-genic cases obtained. This may rely on many conditions. It may be due to the fact that anti-microbial medication might be prescribed prior to the sample collection. Medications can cause E. coli pathogens to reduce significantly. Our findings are consistent with prior research and reveal significant DEC detected in specimens taken from children tolerating diarrhea (21). Similar findings were also observed in research carried out by Indian researchers, where only 25.6% of results pointed to a proportionate causation for DEC (22). Contradictory results were found in Hasan *et al.*, study where the frequency of incidence of DEC was almost 87 percent (15).

EAEC is one of the most common emerging viruses linked to diarrhea and is considered to cause chronic diarrheal problem in children living in poor countries. *EAEC* is also found in individuals contaminated with the immunodeficiency virus in human, particularly in children and tourers in underdeveloped countries. *EAEC* was most prevalent among all the DEC strains. Imdad *et al.*, saw similar outcomes in their research as well (14). Zhou *et al.*, study revealed contradicting results, with the EPEC strain having the highest prevalence (23). In another study, the most prevalent among the under developed countries was EIEC (21).

EAEC class is a diverse bacterial collection with a diverse set of virulence factors. The diarrhea started by it, is hydrous with no blood, assisted by irregular stomach pain with no fever, as mentioned before. It has been observed internationally, and the findings of this study are consistent with it. Resisting ability against anti-microbial agents was also much noticeable in EAEC class. Related end-results were also noticed by Lima *et al.* The study indicated of similar results along with resistance to drugs as well (24). A huge number was resistant to one antibiotic at the minimum, as reported by Imdad *et al*., in a study (14). Strains of EHEC are linked with illnesses caused by food, affected most of the times by consuming uncooked grated meat and meat substituents, dairy products that are not cooked properly, or impure foods contaminated with feces. The most frequent DEC in diarrheal isolates in the study carried out by Mabika *et al.*, were ETEC and EHEC, which opposes out research as well (25).

EPEC-caused diarrhea is generally watery and may contain mucous, although it does not always involve blood. Fever, vomiting, and dehydration are some of the symptoms. Typical EPEC is a prevalent cause of gastroenteritis in newborns. ETEC exposure is a frequent source for tourist's diarrhoea in certain nations. Majority of the diarrheal children produced non-bloody, lucid thick liquid stool without pus. In our study, the prevalence of ETEC (12 percent in the diarrheal class as mixed infection) was lessened than other nations' studies (26).

Zhou *et al.,* mentioned in his study about age that DEC likely to affect young individuals. Similar results were also noticed in this study (23). There is the majority of the children were under the age of 12 months (38%).

It's critical to correctly diagnose DEC so that the disease's spectrum, infection source, and transmission routes may all be understood. The practitioner will be able to correctly monitor and treat the condition by recognizing this path. DEC strains are frequently resistant to common antibiotics. As a result, it's critical to identify the pathogen quickly and complete the experiment in under 24 rush hours. If multiple PCR assays are implemented in peculiar laboratories, they must undoubtedly give quick and / or easy way for detecting DEC.

DEC strains are not routinely sought as stool pathogens but are instead treated symptomatically in poor countries. Since DEC-related infections and consequences are particularly dangerous for newborns, thorough research should precede medication prescribing. One possible explanation for the relatively low rate of E. coli isolation (50%) in this study is that some of the children with diarrhoea may have received antimicrobial therapy before sample collection; it is well-established that such treatment reduces the rate of isolation of bacterial enteropathogens (27). Our findings are consistent with those of other research indicating significant DEC in samples taken from children with diarrhoea. This research established four distinct types of DEC. EAEC is one of the most important new viruses linked to diarrhoea, and it is the leading cause of severe, long-lasting diarrhoea in children in low-income countries. In addition, EAEC has been detected in HIV-positive patients, particularly young patients and international visitors to underdeveloped countries. The EAEC bacteria are diverse and exhibit several virulence characteristics. Consistent with our results, previous worldwide documentation describes EAEC-caused diarrhoea as a watery diarrhoea without blood accompanied by intermittent stomach pains but no temperature (28). We observed that among DECs, EPEC was the second most common, occurring in 15% of cases. EPEC-related diarrhoea is often watery and may include mucous but does not typically include blood. Flu-like symptoms, including high body temperature, nausea, and vomiting, often accompany dehydration. Typical EPEC causes diarrhoea in people and animals and is a frequent cause of gastroenteritis in newborns because ETEC generate heatlabile (LT) and/or heat-stable (STa and STb) toxins. Exposure to ETEC is a major contributor to traveler's diarrhoea in certain regions. In comparison to research from other countries, found a decreased incidence of ETEC (11% in the diarrhoea group as a mix infection) (28).

Materials and Methods

Collection of Sample and its Microbiology

All the cases were gathered from the medical center for Children and Sir Ganga Ram Hospital, Lahore in period of nine months. Kids < 5 years of age experiencing diarrhea were part of this study. Participants which were detected with some other kinds of infectious diseases besides diarrhea were excluded from study. All specimens were carried in sterile canisters from children's rectums having diarrhea with the support of sterilized cotton wipes and then were arranged in Cary Blair Transport instrument, then moved to lab to culture as well as isolate E. coli in two hours. Selected cases were infused, then banded on Eosin Methylene Blue (EMB), then MacConkey agars to create the territory. Colonies were examined for characteristics of E. coli after the nightlong incubation at 37^{o} Celsius of temperature. The expected E. coli colonies were striped later over the fresh antiseptic nutrient agar for performing regular biochemical experiments to identify E. coli.

E. coli was documented by tests that include Positive Methyl Red, Indole, Citrate, Urease and Negative Voges Proskauer. All isolates from every rectal swab showing biochemical features and common properties of morphology of colony were sustained in lab in a nutrient stock at the temperature of 4^{o}C and were stored at -70^{o}C till further usage.

DNA genomic extractions and differentiation

The taking out of genetic code process (DNA) occurred using cetyletrimethyle ammonium bromide (CTAB) technique (18). The derived DNA was then retained at – 70^{o} C till usage like polymerase

chain reaction (PCR) pattern. PCR test was performed for the confirmation of E. coli with the help of common E. coli primer (uidA). The overall environment for polymerase chain reaction (PCR) was as: 94^{o} C for five minutes to denature the starting template, 35 cycles at heat of 98^{O}C just for at least 10 seconds for last denaturation, annealing of primer at the temperature of 68^{o}C for almost thirty-five seconds, primer expansion at 72^{o}C for 45 seconds and then at last, it was performed at 72^{o} Celsius for approximately 7 minutes for concluding extension (19). To inspect amplicons on electrophoresis, Agarose gel (2 % w/v) was smeared. It was then complemented with assistance of streaking by the use of Ethidium Bromide (20)

Phenotypic Characteristics (inclusion and exclusion area)

Inclusion Criteria

- Patients included in this study were children of 5 years or below.
- Patients included in this study were diarrheagenic.

Exclusion Criteria

Patients above 5 years of age were not included in this study.

ABBREVIATIONS

WHO: World Health Organization
EPEC: Entero-pathogenic *E. coli*;
ETEC: entero-toxigenic E. coli;
HUS: Hemolytic Uremia;
EAEC: entero-aggregative E. coli;
DEC: diarrheagenic E. coli;
EMB: Eosin Methylene Blue;
CTAB: Cetyletrimethyle Ammonium Bromide;
PCR: Polymerase Chain Reaction;
EHEC: entero-hemorrhagic E. coli;
Stx2: Shiga-like toxin 2

DECLARATION

Ethical approval and consent for participation

This research investigation was accepted by the Ethical Committee of 'University of Lahore' and all the processes mentioned were carried out as stated by the regional institutional ethical instructions. Written consent was taken from legalized guardians and parents of the participants prior to inclusion in study.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

Authors do not have any competing interests.

Funding

This study has not received any funding for this study.

Authors' contributions

AM and AH designed the work; HRH and FL acquired data; **MM and SS** interpreted the data; **AM, SS and SZH** drafted the manuscript**; SZH** revised final manuscript.

Acknowledgements
Authors acknowledge the clinical staff and experimental laboratory staff of Sir Ganga Ram hospital, Lahore in facilitating the collection of stool samples.

Reference

1. Baloch DGMJP-EJoM, Sciences L. An Overview of Diarrheagenic Escherichia coli Among Infants in Pakistan. 2021;4(Special Is):S11-S24.
2. Melese B, Paulos W, Astawesegn FH, Gelgelu TBJBPH. Prevalence of diarrheal diseases and associated factors among under-five children in Dale District, Sidama zone, Southern Ethiopia: a cross-sectional study. 2019;19(1):1-10.
3. Shine S, Muhamud S, Adanew S, Demelash A, Abate MJBid. Prevalence and associated factors of diarrhea among under-five children in Debre Berhan town, Ethiopia 2018: a cross sectional study. 2020;20(1):1-6.
4. Khaliq A, Jameel N, Krauth SJJM, journal ch. Knowledge and Practices on the Prevention and Management of Diarrhea in Children Under-2 Years Among Women Dwelling in Urban Slums of Karachi, Pakistan. 2022;26(7):1442-52.
5. Corden E, Siddiqui SH, Sharma Y, Raghib MF, Adorno III W, Zulqarnain F, et al. Distance from healthcare facilities is associated with increased morbidity of acute infection in pediatric patients in Matiari, Pakistan. 2021;18(21):11691.
6. Khan MA. Prevalence of several infectious diseases in the District Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan. 2022.
7. Organization WH. Global antimicrobial resistance and use surveillance system (GLASS) report: 2021. 2021.
8. Rojas-Lopez M, Monterio R, Pizza M, Desvaux M, Rosini RJFim. Intestinal pathogenic Escherichia coli: insights for vaccine development. 2018;9:440.
9. Eltai NO, Al Thani AA, Al Hadidi SH, Al Ansari K, Yassine HMJBm. Antibiotic resistance and virulence patterns of pathogenic Escherichia coli strains associated with acute gastroenteritis among children in Qatar. 2020;20(1):1-12.
10. Younas M, Siddiqui F, Noreen Z, Bokhari SS, Gomez-Duarte OG, Wren BW, et al. Characterization of enteropathogenic Escherichia coli of clinical origin from the pediatric population in Pakistan. 2016;110(7):414-20.
11. Jesser KJ, Levy KJCoiid. Updates on defining and detecting diarrheagenic Escherichia coli pathotypes. 2020;33(5):372.
12. Abdul-hussein ZK, Raheema RH, Inssaf AIJJoP, Microbiology A. Molecular diagnosis of diarrheagenic E. coli infections among the pediatric patients in Wasit Province, Iraq. 2018;12(4):2229-41.
13. Khairy RM, Fathy ZA, Mahrous DM, Mohamed ES, Abdelrahim SSJBID. Prevalence, phylogeny, and antimicrobial resistance of Escherichia coli pathotypes isolated from children less than 5 years old with community acquired-diarrhea in Upper Egypt. 2020;20(1):1-9.
14. Imdad A, Foster MA, Iqbal J, Fonnesbeck C, Payne DC, Zhang C, et al. Diarrheagenic Escherichia coli and acute gastroenteritis in children in Davidson County, Tennessee, United States: a case-control study. 2018;37(6):543.
15. Hasan HK, Yassin NA, Eassa SHJSJoUoZ. Bacteriological and Molecular Characterization of Diarrheagenic Escherichia Coli Pathotypes From Children in Duhok City, Iraq. 2020;8(2):52-7.
16. Dewanggana MN, Evangeline C, Ketty MD, Waturangi DE, Magdalena SJSr. Isolation, characterization, molecular analysis and application of bacteriophage DW-EC to control Enterotoxigenic Escherichia coli on various foods. 2022;12(1):1-10.
17. Kipkirui E, Koech M, Ombogo A, Kirera R, Ndonye J, Kipkemoi N, et al. Molecular characterization of enterotoxigenic Escherichia coli toxins and colonization factors in children under five years with acute diarrhea attending Kisii Teaching and Referral Hospital, Kenya. 2021;7(1):1-7.

18. Abduraimova A, Molkenova A, Duisembekova A, Mulikova T, Kanayeva D, Atabaev TSJN. Cetyltrimethylammonium bromide (CTAB)-loaded SiO2–Ag mesoporous nanocomposite as an efficient antibacterial agent. 2021;11(2):477.
19. Zhang J, Xu Y, Ling X, Zhou Y, Lin Z, Huang Z, et al. Identification of diarrheagenic Escherichia coli by a new multiplex PCR assay and capillary electrophoresis. 2020;49:101477.
20. Tareen AM, Ullah K, Asmat TM, Samad A, Iqbal A, Mustafa MZ, et al. Incidence of diarrheagenic Escherichia coli pathotypes in children suffering from diarrhea in tertiary care hospitals, Quetta, Pakistan. 2019;51(6):2015.
21. David EE, Yameen MA, Igwenyi IO, Okafor AC, Obeten UN, Obasi DO, et al. The frequency of virulent genes and antimicrobial resistance patterns of diarrheagenic Escherichia coli isolated from stools of children presenting with diarrhea in a tertiary hospital in Abakaliki, Nigeria. 2020;6:147-52.
22. Moharana SS, Panda RK, Dash M, Chayani N, Bokade P, Pati S, et al. Etiology of childhood diarrhoea among under five children and molecular analysis of antibiotic resistance in isolated enteric bacterial pathogens from a tertiary care hospital, Eastern Odisha, India. 2019;19(1):1-9.
23. Zhou Y, Zhu X, Hou H, Lu Y, Yu J, Mao L, et al. Characteristics of diarrheagenic Escherichia coli among children under 5 years of age with acute diarrhea: a hospital based study. 2018;18(1):1-10.
24. Lima FM, de Paulo Daurelio F, Mucci ER, Ahagon CM, dos Santos Carmo AM, Eterovic A, et al. Epidemiology and genetic screening of diarrheagenic Escherichia coli among symptomatic and asymptomatic children. 2019;68(7):1033-41.
25. Mabika RM, Liabagui SLO, Moundounga HK, Mounioko F, Souza A, Yala JFJOJoMM. Molecular Prevalence and Epidemiological Characteristics of Diarrheagenic E. coli in Children under 5 Years Old in the City of Koula-Moutou, East-Central Gabon. 2021;11(3):157-75.
26. Rahman MM, Ahmed P, Kar A, Sakib N, Shibly AZ, Zohora FT, et al. Prevalence, antimicrobial resistance, and pathogenic potential of enterotoxigenic and enteropathogenic Escherichia coli associated with acute diarrheal patients in Tangail, Bangladesh. 2020;17(7):434-9.
27. de Castro AFP, Betancor A, Mercado EC, Cataldi A, Parma AEJPEciLABS, Bolingbrook. Escherichia coli animal reservoirs, transmission route and animal disease. 2010:223-48.
28. Shrivastava AK, Kumar S, Mohakud NK, Suar M, Sahu PSJGp. Multiple etiologies of infectious diarrhea and concurrent infections in a pediatric outpatient-based screening study in Odisha, India. 2017;9(1):1-12.