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Analysis of transcriptional landscapes and multidrug resistant genes in commensal and diarrheagenic E. Coli strains from stool

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Abstract

Introduction

Antimicrobial drugs are widely used for prophylaxis, metaphylaxis, and treatment. In some areas of the world, they are also utilized to promote growth. A large number of these medications are members of the same classes of antibacterial chemicals that are prescribed to people. According to surveillance studies carried out in several nations, Escherichia coli isolates are becoming more resistant to the main types of antibiotics that are used to treat patients. It has been proposed that the selection pressure that follows antibiotic exposure has a significant role in the formation and spread of resistance. **Aim:** Analysis of transcriptional landscapes and multidrug resistant genes in commensal and diarrheagenic E. Coli strains from stool. **Material and Methods**: A prospective, observational, and cross-sectional study conducted in a hospital setting. This study was conducted in the Department of Microbiology at Index Medical College, Hospital & Research Centre (IMCHRC), located in Indore, Madhya Pradesh over a perid of 3 years, from 2021 to 2023. Submission of a stool sample that reveals an excess of five white blood cells per high-power field (HPF). A total of 180 fecal samples was collected by using disposable container. Each container was inverted and sealed immediately after collection. **Results**: In all three of our research groups, we discovered a significant proportion of distinct DEC co-infection (Table 2). In our investigation, the most prevalent mixed infections found were EAEC, EPEC and ETEC in 54/180 (30%); EAEC and EPEC in 24/180 (13.3%) and EPEC and ETEC in 7/180 (3.9%) isolates. Coexistence of all the four categories of DEC together was present in 3/60 isolates (5%) in Group 1 and 1/60 (1.7%) in Group 2. In healthy controls, the coexistence of EAEC and EPEC occurred in 15/60 isolates (25%). **Conclusion:** DEC was recovered at higher rate from healthy children and children without diarrhea, demonstrating extensive and fast spread of these pathogens in community. Multiplex PCR being time saving can be used for simultaneous detection of pathogenic genes. The occurrence of atypical EPEC in healthy children is of a great concern and cannot be neglected. This study AmpC, ESBL and MBL were the main resistance patterns of the strains evaluated. High prevalence of ESBL and plasmid-mediated AmpC was due to extensive use of third-generation cephalosporins.

Keywords: DEC, Multiplex PCR, EPEC, AmpC, ESBL, MBL, Beta-lactamase, E. coli.

Introduction

Antimicrobial drugs are widely used for prophylaxis, metaphylaxis, and treatment. In some areas of the world, they are also utilized to promote growth. A large number of these medications are members of the same classes of antibacterial chemicals that are prescribed to people. [1] According to surveillance studies carried out in several nations, Escherichia coli isolates are becoming more resistant to the main types of antibiotics that are used to treat patients. [2,3]

Escherichia coli isolates might be a major source of antibiotic resistance factors that propagate human-pathogenic bacteria. Public health is particularly concerned about the reported growth of drug resistance to novel medications, such as fluoroquinolones (FQs) and extended-spectrum β-lactams. There has previously been evidence of the possibility of E. Coli clones spreading from humans to various animal hosts. [4-7] Nevertheless, nothing is now known about the specific pathways of resistance in bacteria grown from hospitalized individuals.

Horizontal gene transfer (HGT) is the collective term for a series of distinct genetic processes that have contributed to the spread of resistance markers. The majority of genes that confer antibiotic resistance are not host-specific. Since resistance determinants often locate inside mobile and mobilizable genetic components including integrons, transposons, and plasmids, HGT has been linked to these structures. Moreover, a lot of the resistance signs continue even when certain antibiotics are no longer used in treatment. [8] These epidemiological statistics may be explained, at least in part, by the physical connections between genes. It has been shown both in vitro and in vivo that bacteria important to patients, such as E. Coli, may transfer resistance plasmids. [9]

The Enterobacteriaceae family includes the facultative anaerobic Gram-negative bacilli that comprise the genus Escherichia, which bears the name of the German physician Theodor Escherich. [11,12]

One major public health concern that greatly increases infant and early childhood morbidity and mortality is diarrhoeal illness. Diarrhoeal illnesses are more common in these nations and may be deadly. Poor living circumstances, including insufficient water supply, unsanitary and unhygienic environments, and inadequate education, are the primary causes of many illnesses. [13]

E. Coli most significant of the several etiological factors that might cause diarrhoea. via the effective persistence of a specific set of traits acquired via horizontal gene transfer, these strains have developed inside their host. [14] The acquired collection of virulence determinants resulted in the creation of specific combinations that identified the pathotypes of E. coli that are now recognised as such; these pathotypes are known as diarrheagenic E. coli (DEC). [15,16]

Material and Methods

This is a prospective, observational, Random and cross-sectional study conducted in the Department of Microbiology at Index Medical College, located in Indore, Madhya Pradesh.

Duration: 3 years, from 2021 to 2023
Data collected from January 2022 to December 2023

Inclusion criteria:

Submission of a stool sample that reveals an excess of five white blood cells per high-power field (HPF).

Exclusion criteria:

1. Patients with known immunological disorders
2. Patients with chronic gastrointestinal disorders.
3. Patients receiving/received probiotic for the last seven days.

Fecal Samples

A total of 180 fecal samples was collected by using disposable container. Each container was inverted and sealed immediately after collection.

We have done Pilot study from March 2021 to May 2021 at Index medical college.

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OBJECTIVE 1

To isolate and characterize E. coli phenotypically from feces of children (OPDand IPD) as well as from normal healthy children existing as commensal.

Phenotypic methods

Laboratory Procedures

Samples All of the children's fresh feces samples were obtained and sent straight to the lab in clean, sterile, labeled, wide-mouthed plastic containers with tight lids (for tiny children, rectal swabs or stools from diapers were collected).

Isolation and identification of E. coli isolates

The protocols for laboratory diagnosis of enteric pathogens were followed in the processing of all the specimens. After being inoculated, specimens were incubated aerobically for 24 hours at 37 °C on MacConkey agar plates. To identify the E. Coli isolates, microscopy, colony morphology, and several sugar fermentation assays were used. On MacConkey agar plates, discrete colonies of lactose fermenters—a typical pink hue associated with

Escherichia coli—were seen. Organisms once identified was stocked in 1ml capacity vials containing stock media (Himedia, Mumbai, India), and stored at room temperature. Every isolate that was first suspected to be E. coli based on its growth characteristics on MacConkey agar was subsequently confirmed phenotypically using standard biochemical tests.

Statistical analysis

The Statistical Package for the Social Sciences (version 29.0) was used to do the statistical analysis. The chi-square test and Fisher's exact test were used to evaluate the statistical significance of the data. It was considered statistically significant when $P < 0.05$.

Results

A total of 180 stool specimens were examined to see whether E. Coli that causes diarrhea was present. Table 1 shows the distribution of DEC in children across different age groups.

Table 1: Age and sex distribution of subjects among three study groups.

Groups	Age group in years	Male/female	EAEC	EPEC	ETEC	EHEC
1	0-1	18/6	15	20	19	10
	1-3	8/15	16	17	15	0
	3-5	8/5	15	10	11	0
(n=60)		34/26	46(76.7)	47(78.3)	45(75)	10(16.7)
2	0-1	16/17	15	21	18	1
	1-3	11/8	9	9	8	0
	3-5	3/5	21	17	13	8
(n=60)		30/30	45(75)	47(78.3)	39(65)	9(15)
3	0-1	19/9	15	16	1	2
	1-3	9/4	4	9	0	0
	3-5	12/7	4	9	0	0
(n=60)		40/20	23(38.3)	34(56.7)	1(1.7)	2(3.3)
(n=180)		104/76	114(63.3)	128(71.1)	85(47.2)	21(11.7)

The most frequent category of DEC detected was EPEC (71.1%) followed by EAEC (63.3%), ETEC (47.2%) and EHEC (11.7%), as shown in Table 1. In ETEC, virulent gene was detected in 40%, 45% and 1.7% whereas elt was observed in 20%, 6.7% and 0% in groups 1, 2 and 3, respectively. In EAEC, eagg (45%, 30% and 33.3%) was more frequently detected than east (10%, 25% and 5%) gene in Groups 1, 2 and 3 respectively. Typical EPEC was detected in 25%, 15% and 1.7% isolates and atypical EPEC was detected in 13.3%, 36.7% and 36.7% isolates in the Groups 1, 2 and 3 respectively. Atypical EPEC was more common in healthy children as compared to diarrheal cases. EHEC was also differentiated into typical (stx + hyla + eae) and atypical (stx with or without eae), however typical EHEC variety was

not detected in any sample as compared to atypical EHEC observed in 1.7% of isolates in children representing Group 2.

Table 2: Distribution of ETEC, EAEC, EPEC and EHEC in three study groups according to the type of virulence genes present.

PATHOGROUPS	GROUP 1 (n=60)	GROUP 2 (n=60)	GROUP 3 (n=60)	TOTAL (n=180)	P value
eagg	27(45)	18(30)	20(33.3)	65(36.1)	0.008*
east	6(10)	15 (25)	3 (5)	24(13.33)	0.01*
Eagg and east	11(18.3)	12(20)	0	23(12.7)	0.003*
****EAEC**	44(73.3)	45(75)	13(38.3)	56(56.7)	0.028*
bfpa	18(30)	6 (10)	11 (18.3)	35(19.4)	0.001*
(bfpa+eaf+eae)	9 (15)	10(16.7)	0 (0)	19(10.5)	0.005
Typical EPEC (eae +bfpa)	15 (25)	9 (15)	1 (1.7)	25(13.9)	0.005*
Atypical EPEC (Eae)	8 (13.3)	22 (36.7)	22(36.7)	42 (23.3)	0.024*
****EPEC**	50(83.3)	41 (68.3)	44(73.3)	135(75)	0.446
elt	12 (20)	4 (6.7)	0 (0)	16 (8.9)	0.002*
est	24 (40)	27 (45)	1 (1.7)	52(28.9)	0.01*
Elt and est	9(15)	6 (10)	0 (0)	15(8.3)	0.070
****ETEC**	45(75)	37 (61.7)	1(1.7)	83(46.1)	0.400
stx	1(1.7)	1(1.7)	0	2(1.1)	0.293
hyla	0	1(1.7)	1(1.7)	2(1.1)	0.728
Stx and hyla	6(10)	1(1.7)	1(1.7)	8(4.4)	0.242
Typical EHEC	0	0	0	0	N.A
Atypical EHEC	0	1(1.7)	0	1(0.56)	1.000
****EHEC**	7(11.7)	4(6.7)	2(3.3)	13(7.2)	1.210

In all three of our research groups, we discovered a significant proportion of distinct DEC co-infection (Table 2). In our investigation, the most prevalent mixed infections found were EAEC, EPEC and ETEC in 54/180 (30%); EAEC and EPEC in 24/180 (13.3%) and EPEC and ETEC in 7/180 (3.9%) isolates. Coexistence of all the four categories of DEC together was present in 3/60 isolates (5%) in Group 1 and 1/60 (1.7%) in Group 2. In healthy controls, the coexistence of EAEC and EPEC occurred in 15/60 isolates (25%).

Table 3: Showing co-infection among diarrheagenic E. coli isolates of three study groups.

Groups	EAEC+ EPEC	EPEC+ ETEC	EAEC+ EHEC	EPEC+ EHEC	ETEC+ EHEC	EAEC+ EPEC+ ETEC+ EHEC	EAEC+ EPEC+ ETEC	EAEC+ EPEC+ EHEC	EAEC+ ETEC+ EHEC	EPEC+ ETEC+ EHEC
1(n=60)	0	3(5)	1(1.7)	0	0	3(5)	30(50)	0	0	0
2(n=60)	9(15)	4(6.7)	1(1.7)	0	1(1.7)	1(1.7	24(40)	1(1.7)	0	0
3(n=60)	15(25)	0	0	0	0	0	0	1(1.7)	0	0
Total (n=180)	24(13.3)	7(3.9)	2(1.1)	0	1(0.56)	4(2.2)	54(30)	2(1.1)	0	0

Table 4: Antimicrobial agent resistance frequency of E. Coli isolates from three research groups

Antibiotics	Group 1 n=60 (%)	Group 2 n=60 (%)	Group 3 n=60 (%)	Total n =180 (%)	P value
Norfloxacin(10µg)	14(23.3)	9(15)	15(25)	38 (21.1)	0.709
Cefotaxime (30µg)	32(53.3)	48(80)	9(15)	89(49.4)	0.00*
Imipenem (10µg)	16(26.7)	9(15)	1(1.7)	26 (14.4)	0.002*
Meropenem (10µg)	3(5)	1(1.7)	0	4(2.2)	0.358
Ceftazidime(30µg)	12(20)	6(10)	0	18(10)	0.011*
Azetronam (30µg)	9(15)	9(15)	0	18(10)	0.044*
Nalidixic acid(30µg)	12(20)	0	0	12(6.7)	0.00*
Amoxicillin(20/10µg)	1(1.7)	3(5)	0	4(2.2)	0.358
Gentamicin (10µg)	22(36.7)	21(35)	3(5)	46(25.6)	0.01*
Ciprofloxacin (5µg)	9(15)	6(10)	1(1.7)	16(8.9)	0.082
Ampicillin(10µg)	27 (45)	15 (25)	6 (10)	48 (26.7)	0.01*

Table 4 shows the antibiotic resistance trend of DEC isolates. Our study's isolates were mostly resistant to many drugs. The majority of DEC isolates shown sensitivity to ceftriaxone, amoxicillin as meropenem, cefotaxime + clavulanic acid, and polymyxin B. High frequency of resistance was seen with cefotaxime (53.3%, 80%, 15%) followed by gentamicin (36.7%, 35% and 5%) and ampicillin (45%, 25%, and 10%) in isolates from three groups respectively.

Discussion

DEC still remains the main cause of childhood diarrhea found most commonly in children <5 years of age. Categories of DEC (a) EAEC: Mechanisms of transmission of enteroaggregative E. coli were not clear. Although, several nosocomial outbreaks have occurred but the food borne transmission was found to be suggestive (Nataro and Kaper, 2014). [16] (b) EPEC: The major mode of enteropathogenic E. coli.

In our study the most frequent category of DEC detected was EPEC (71.1%) followed by EAEC (63.3%), ETEC (47.2%) and EHEC (11.7%), as shown in **Graph** 1. In ETEC, virulent gene was detected in 40%, 45% and 1.7% whereas elt was observed in 20%, 6.7% and 0% in groups 1, 2 and 3, respectively. In EAEC, eagg (45%, 30% and 33.3%) was more frequently detected than east (10%, 25% and 5%) gene in Groups 1, 2 and 3 respectively. Typical EPEC was detected in 25%, 15% and 1.7% isolates and atypical EPEC was detected in 13.3%, 36.7% and 36.7% isolates in the Groups 1, 2 and 3 respectively. Atypical EPEC was more common in healthy children as compared to diarrheal cases. EHEC was also differentiated into typical (stx + hyla + eae) and atypical (stx with or without eae), however typical EHEC variety was not detected in any sample as compared to atypical EHEC observed in 1.7% of isolates in children representing Group 2.

In industrialized countries, although typical EPEC outbreaks in children have disappeared mostly but the diarrheal disease caused by atypical EPEC have emerged drastically (Nguyen, 2016). [17] (c) ETEC: Food and water contaminated by excrement are the main ways in which the disease is spread. (d) EHEC: Consuming infected food, especially undercooked ground beef, drinking contaminated water, coming into touch with farm, and person-to-person transmission in public spaces are the main ways that enterohemorrhagic E. coli is spread (Kassenborg, 2014). [132]

(e) EIEC: A number of outbreaks linked to food have been reported. It may also spread from person to person. (f) DAEC: Before the identification of EAEC, this term was used to describe any HEp-2-adherent E. coli; nevertheless, it is now understood to represent a distinct class of potentially diarrheagenic E. coli strains. The pathogenetic characteristics of diarrhea produced by DAEC are poorly understood (Bilge et al., 2015). [18]

In this study all three of our research groups, we discovered a significant proportion of distinct DEC co-infection (Graph 2). In our investigation, the most prevalent mixed infections found were EAEC, EPEC and ETEC in 54/180 (30%); EAEC and EPEC in 24/180 (13.3%)

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Enterotoxins generated by ETEC include heat-stable ST (STa and STb) and heat-labile LT (LT-1 and LT-2); (Kartsev et al., 2015). [19] EPEC has a pathogenicity island that encodes many proteins responsible for the attaching and effacing lesions on the host cell's intestinal microvilli, as well as the big EPEC adherence factor (EAF) plasmid, which carries the gene cluster for bundle-forming pili (bfp). (Kaper et al., 2004). [20,21]

Conclusion

DEC was recovered at higher rate from healthy children and children without diarrhea, demonstrating extensive and fast spread of these pathogens in community. Multiplex PCR being time saving can be used for simultaneous detection of pathogenic genes. The occurrence of atypical EPEC in healthy children is of a great concern and cannot be neglected.

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