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ANTIMICROBIAL RESISTANCE PATTERN OF ENTEROAGGREGATIVE *ESCHERICHIA COLI* ISOLATED FROM ACUTE DIARRHEA OF PEDIATRIC PATIENT UNDER FIVE IN KANPUR REGION

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ABSTRACT: Enteroaggregative *Escherichia coli* (EAEC) strains are causal agents of persistent diarrhea in developing countries. Enteroaggregative *Escherichia coli* strains (EAEC) are an emerging type of diarrheagenic *E. coli*. We aimed to isolate, identify and characterize the *E. coli* pathotypes and study their antibiotic resistance in children under five with diarrhea in Kanpur region, India. Children under 5 years suffering from acute diarrhea and attending an outpatient clinic or admitted in Rama Hospital, Kanpur were enrolled for the study. Severe diarrhea is a public health problem and is responsible for morbidity and mortality, especially in developing countries. DEC was detected by multiplex PCR. Among 38 *E. coli* isolates, 12 samples contain Cvd432 gene of EAEC diarrheagenic *E. coli*. Most of the strains are resistant to ampicillin, Co-trimoxazole, gentamicin, azithromycin, imipenem. Ciprofloxacin, Norflex and chloramphenicol are relatively more sensitive. The high antimicrobial resistance observed in our study raises a broad discussion on the indiscriminate or improper use of antimicrobials, besides the risks of self-medication.

INTRODUCTION: Diarrhea is one of the leading causes of child death in both developing and developed countries, causing an estimated 1.87 million deaths of children aged under 5 years globally each year ¹. Reason for mortality and morbidity in developing countries have been increased by *E. coli* infection in Diarrhea ^{2, 3} diarrheagenic *Escherichia coli* being the most common cause of acute diarrhea in children ⁴.

The study was to isolate, identify and characterize the *E. coli* pathotypes and look for their antibiotic resistance in young children under five with diarrhea in Kanpur, India. Examination of stool samples from 104 children less than 5 years of age indicated that 38 (36.5%) children harbored one *E. coli* pathotype.

Among 38 *E. coli*, Enteroaggregative *E. coli* (EAEC) accounted for 31.57%, Antibiotic sensitivity assay in a subsample indicated that more than 50% of the *E. coli* isolates were resistant to Ampicillin, Gentamicin, Co-Trimoxazole, Azithromycin, Imipenem. Emerging bacterial pathogen like *E. coli* O157: H7 was isolated from diarrheal patients during the study through the conventional method.

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In general *E. coli*, pathotypes were resistant to Ampicillin, Gentamicin, Co-Trimoxazole, Azithromycin, Imipenem. The progressive increase in antibiotic resistance among enteric pathogens in developing countries is of great concern. Overuse and misuse of antibiotics in the treatment of diarrhea could lead to a rise in antibiotic resistance. The most common cause of acute diarrhea in children is *E. coli*. Around the world resistance of antibiotics is very common in bacterial isolates⁵. *Escherichia coli* are found as normal flora of the human intestine^{5, 6}. Across the world, *E. coli* has shown increasing antimicrobial resistance to most antibiotics, but susceptibility pattern has varied from region to region⁶⁻⁸. This investigation aimed to determine the susceptibility pattern of DEC to different conventional and commonly available antibiotics.

MATERIALS AND METHODS: Stool samples from pediatric patient less than five years were collected from January 2017 to May 2018 from the outdoor and indoor patient department of Rama Medical College and Hospital, Kanpur. Inclusion criteria were to include Stool samples from patients who had not received antibiotic treatment prior at the time of collection of samples in sterile containers and taken to the Department of Microbiology, Rama Medical College for bacterial analyses. Identification of *E. coli* cultures was made by morphology, cultural characteristics, biochemical reactions and Molecular characterization of DEC were done by Multiplex PCR.

All the stool samples were cultured into MacConkey agar, Eosin Methylene agar and Sorbitol MacConkey agar for primary isolation of common intestinal pathogens and incubated at 37°C for 24 h. The isolates were subjected to Gram's - staining, motility, urease production, oxidase, mannitol, and TSI, indole and citrate utilization tests. All *E. coli* isolates were gram-negative, rod-shaped, motile, and indole-positive, mannitol- positive urease-negative strains that produced acid on Triple Sugar Iron agar slants⁹.

Categorization of *Escherichia coli* was done by multiplex PCR using primers for detection of *sltA* or *elt* gene for ETEC, *bfpA* or *eaeA* gene for EPEC and *cvd432* gene for EAEC. The PCR was carried

out in a various number of genes combinations. PCR assays were performed in a DNA thermal cycler. Each PCR was carried out comprised of preheating at 95 °C for 15 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 90 sec, elongation at 72 °C for 1-2 min and a final extension at 60 °C for 90 sec. Amplified products were run on to horizontal gel electrophoresis on 1.5% (w/v) agarose gel at 100v (50 mV) for 30 to 35 min and visualized with a Gel doc after ethidium bromide staining.

The sample was considered harmful for DEC when DNA template result was found to be negative by gel electrophoresis. Three thousand bp DNA size standards were used as a marker to measure the molecular size of the amplified products. Only the presence of the amplified product with the correct size was interpreted as a test positive. All identified DEC isolates were tested for susceptibility testing which was done by Kirby-Bauer modified disk diffusion technique¹⁰.

The antimicrobial susceptibility assay was performed on Mueller-Hinton agar (Oxoid) by the disc diffusion method, and growth inhibition zones were interpreted according to the Clinical Laboratory Standards Institute¹¹. The identified DEC were tested against ampicillin (10 µg), nalidixic acid (30 µg), Gentamicin (10 µg), imipenem (10 µg), cotrimoxazole (25 µg), chloramphenicol (30 µg), azithromycin (15 µg), ciprofloxacin (5 µg), cefotaxime (10 µg), Norfloxacin (10 µg). *Escherichia coli* ATCC 25922 was tested along with the isolates for quality control purpose. The result was documented according to the performance range of inhibition zone of control strain.

RESULT: In this cross-sectional study, during one year, stool samples were collected from 104 patients aged 0 to 5 years, and among them 51 (49%) were males, and 53 (51%) were females. Among 104 stool samples, *E. coli* was isolated in 38 (36.5%). Among those *E. coli* isolates, 12(11.5%) were identified as EAEC DEC by Multiplex PCR. The result for antimicrobial susceptibility testing of the different groups of DEC strains is shown in **Table 1**. The trend of resistance was different among Diarrheagenic *E. coli* types.

EAEC strains were highly resistant to ampicillin, cotrimoxazole and gentamicin. Moderately resistance was detected towards azithromycin, imipenem,

chloramphenicol and nalidixic acid. The Diarrheagenic *E. coli* isolates were least resistant to cefotaxime, ciprofloxacin and norfloxacin.

TABLE 1: ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF EAEC STRAIN OF DIARRHEAGENIC *E. COLI* ISOLATES

Antibiotics	Resistant		Intermediate Sensitive		Sensitive	
	Number	Percentage	Number	Percentage	Number	Percentage
Ampicillin AMP (1)	12	100%	-	-	-	-
Imipenem IPM (2)	6	50%	-	-	6	50%
Cotrimoxazole SXT (3)	9	75%	-	-	3	25%
Nalidixic acid NA (4)	5	41.7%	1	8.3%	6	50%
Norflox NOR (5)	2	16.7%	2	16.7%	8	66.6%
Cefotaxime CTX (6)	3	25%	-	-	9	75%
Ciprofloxacin CIP (7)	1	8.3%	1	8.3%	10	83.4%
Chloramphenicol C (8)	5	41.7%	-	-	7	58.4%
Azithromycin AZM (9)	7	58.4%	-	-	5	41.7%
GentamycinGM (10)	9	75.0%	-	-	3	25.0%

In EAEC strain of DEC *E. coli* Ampicillin 12(100%), Co-trimoxazole 9 (75%), Gentamicin 9 (75%) was found to be highly resistant

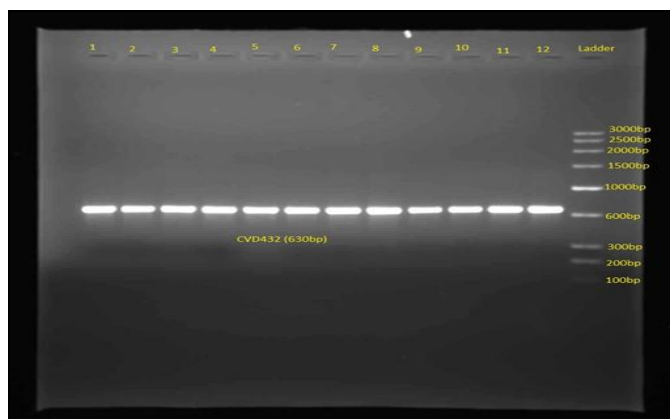


FIG. 1: IMAGE OF MULTIPLEX PCR OF PEDIATRIC PATIENT. Lane 1: 12 EAEC (CVD432 amplicon size 630bp, lane 13 is Ladder 100bp to 3000bp

DISCUSSION: Diarrheagenic *E. coli* strains have been recognized as important pathogens worldwide¹². The misuse of antibiotics has been the cause of bacterial resistance to antimicrobial drugs worldwide¹³. In the present study, resistance to ampicillin, cotrimoxazole, and gentamicin was shown by 100% and 75% in EAEC strains. In the present study, 11.5% EAEC were resistant to cotrimoxazole. The levels of resistance observed for trimethoprim-sulfamethoxazole could be due to widespread use in the treatment of diseases associated with Gram-negative bacteria, especially in children under two years of age with acute infectious diarrhea¹⁴. Resistance to ciprofloxacin 8% in EAEC strains. Varying rates of resistance against ciprofloxacin could be due to the high prescription of this drug in some countries as a treatment for enteric infections caused by gram-negative bacteria^{15, 16}.

In the present study, the sample contained one pathogenic strain of DEC, are resistant to more than one antimicrobial drug.

The high antimicrobial resistance observed in our study could be due to indiscriminate or improper use of antimicrobials which is becoming an alarming situation in drug resistance. Clinicians can be helped by timely monitoring of drug resistance patterns of *E. coli* which will help them regarding therapeutic regimens to be adopted against individual cases. As the solution to the problem of resistance among diarrheagenic bacteria especially *E. coli* but more effective measures, such as health education and further research on the prevention of infections through quality sanitation.

CONCLUSION: This study has established that diarrhea is higher among younger children than adults and also monitors drug resistance patterns of *E. coli* which will give vital clues to clinicians regarding therapeutic regimens to be adopted against individual cases and will be an essential tool to devise comprehensive chemoprophylaxis. Newer antibiotics development might offer a short term solution to the problem of resistance among diarrheagenic bacteria especially *E. coli* but more effective measures, such as health education and further research on the prevention of infections through quality sanitation

Ethical Approval: Study is ethically approved by Rama University, Kanpur U.P with reference no 90 Res/Reg/RUK/2017.

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CONFLICT OF INTEREST: Nil

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