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IDENTIFICATION OF ENTEROPATHOGENIC E.COLI (EPEC)IN PATIENTS OF ACUTE DIARRHEA IN A TERTIARY CARE HOSPITAL FROM THE FOOTHILLS OF HIMALAYAS

Microbiology

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ABSTRACT

Diarrheagenic *Escherichia coli* (DEC) is an important cause of diarrhea in children, adolescents and adults. Studies from all over the world have documented the high prevalence and varying pattern of antimicrobial resistance in DEC. There is very inadequate information regarding prevalence and antibiotic resistance patterns about Enteropathogenic *E.coli* (EPEC) from our region. **Materials and Methods:** The EPEC was diagnosed By conventional polymerase chain reaction (PCR)assays using the target genes *bfpA(charactisticfeature of tEPEC)* . Antibiotic resistance was analyzed by Kirby Bauer method (disk diffusion) of antibiotic susceptibility testing .**Results:** Out of total 100 patients of acute diarrhea presented in OPD and IPD of tertiary care hospital EPEC was found in 12% of the analyzed samples. Significant number of isolates of EPEC was resistant to one or more drugs. **Conclusion:** DEC are important cause of acute diarrhea. The PCR assays can be used to identify EAEC. The current study highlights the necessity for continuous monitoring of antibiotic resistance in EAEC and other bacterial pathogens.

KEYWORDS

Enteropathogenic E.coli (EPEC), molecular analysis, PCR, Antibiotic resistance

INTRODUCTION

Infectious diarrhea is caused by various etiological agents like bacteria, parasites, viruses, and fungi is the leading cause of morbidity and mortality worldwide[1-3] Among these pathogens, *Escherichia coli (E. coli)* is one of the most common causes of bacterial diarrhea in developing countries.[3,4] Enteropathogenic *Escherichia coli* (EPEC), one of the diarrheagenic *E. coli* pathotypes, are among the most important pathogens infecting children worldwide because of their high prevalence in both the community and hospital setting [2], and because they are one of the main causes of persistent diarrhea [3]

Though number of methods have been devised for the identification of disease causing *E.coli* but only PCR helps to differentiate between commensal *E.coli* and diarrheagenic *E.coli*[5,6]

Due to prompt use of antimicrobials to treat acute diarrhea especially in young children, this rampant use of antimicrobials has led to problem of the antimicrobial resistance worldwide. The need of hour is to monitor the susceptibility pattern of common bacterial isolates for drugs used in diarrheal disease to formulate guidelines for the empirical treatment.[7]

The aim of this study is to identify the prevalence of Enteropathogenic E.coli (EPEC) and to compare the antibiotic susceptibility pattern among the same in the foothills of Himalayas.

MATERIALS AND METHODS

Study design

This study was conducted for a period of 1 year in patients of acute diarrhea presenting in Pediatric and Gen Medicine OPD and IPD of AIIMS, Rishikesh. The study was approved by Institutional Ethics committee.

Sample collection and identification of pathogens

5–10 ml of freshly passed single stool sample were collected in a clean, dry, and leak- proof wide mouth plastic container and transported to the laboratory within 2 hours. Specimen from hospital pan and rectal swabs were not collected. The stool sample was first observed for gross appearance and subjected to microscopy to screen for leukocytes, red blood cells, ova , cysts or any segments of parasites.

Stool samples were inoculated on MacConkey Agar , Xylose lysine deoxycholate (XLD) agar and Thiosulfate bile salt agar (Hi Media ,Mumbai).Lactose fermenting colonies were then subjected to battery of biochemical tests as per standard protocol.[(8,9,10)] Bacterial isolates obtained from pure culture were stocked in two ampoules and stored for future testing.

Antimicrobial sensitivity testing

Escherichia coli isolates were submitted for susceptibility testing toward sixteen antimicrobials by disk diffusion method.[(11)] *Escherichia coli* ATCC 25922 was taken as the control.0.5McFarland suspension was prepared and then inoculated on Mueller – Hinton agar (Hi Media, Mumbai) using sterile swab.

Following antibiotics were used – levofloxacin , ciprofloxacin, cefoxitin, amikacin, ampicillin, aztreonam, ceftriaxone, ceftazidime, cefotaxime, piperacillin-tazobactam ,ampicillin - sulbactam, gentamicin, cefepime,imipenem, meropenem and amoxyclavulanic acid. Antibiotic susceptibility was classified as sensitive, intermediate or resistant on the basis of the CLSI Zone diameters.**(Figure1)**

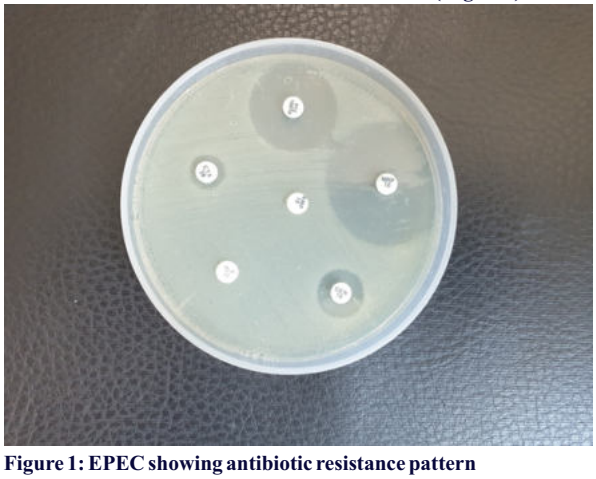

Figure 1: EPEC showing antibiotic resistance pattern

Molecular Diagnostic Methods for Enteroaggregative *Escherichia coli*

A loopful of pure bacterial growth (confirmed *E.coli*) was suspended in 1X TE buffer and then boiled for 10-15 minutes in water bath. After

boiling, the broth was immediately put in -20 degree Celsius for 5 minutes(snap chilling), followed by centrifugation at 14000rpm for 5minutes to pellet down the cell debris .The supernatant was separated and used as DNA template for PCR assays.[12] The DNA templates were subjected to conventional PCR with specific primers for the detection of virulence marker genes such as *bfpA* . DH5 alpha *E.coli* was used as negative control.

Reaction was performed under standardized cycling conditions in GeneAmp 96 well veriti PCR system (AB Applied Biosystem).The amplified product was separated by electrophoresis on a 2% agarose gel (Lonza, Rockland, USA) along with a molecular mass marker (100 bp DNA ladder; Solis Biodyne,India) in TAE buffer [100 mM Tris/HCl (pH 8.3), 10 mM EDTA], stained with 0.5 mg ethidium bromide visualized using a gel documentation system [13](Syngene G box)

Statistical analysis:
Data were collected, tabulated and analysed using Microsoft excel and SPSS version 20. The qualitative data was represented as frequency and percentages and the association between two quantitative data was done using Chi-square test .

RESULTS

A.Identification of *E.coli* isolates

Identification of *E. coli* pathotypes was performed on the basis of conventional biochemical methods, and confirmed by appearance of mauve colored colonies on chromogenic agar (Biomeriux CPSE media)(Figure 2).

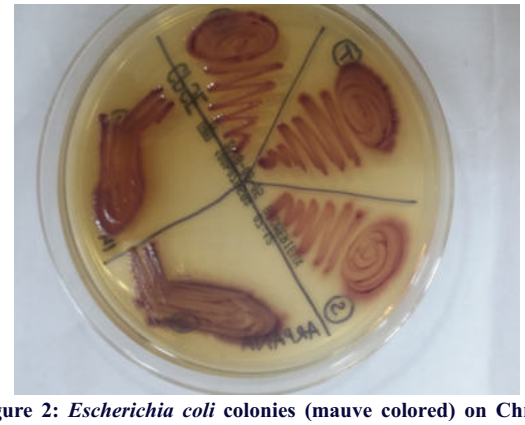

Figure 2: *Escherichia coli* colonies (mauve colored) on Chrom Agar

The confirmed *E. coli* were then subjected to polymerase chain reaction (PCR) for detection of Enteropathogenic *E.coli* (DEC).A total of 100 stool specimens were collected from patients of acute diarrhea. Median age of the patients is 17 years.

They were identified by amplification of virulence gene specific primers selected as per previous study (Taneja N et al 2012). EPEC was found in 12% of the analyzed samples . All EPEC pathotype possessed *bfpA* gene and was predominantly found in the adults.

B.Age group distribution of DEC(EPEC) molecular pathotypes

The study population was stratified into four various age groups to determine the high risk groups . The various age groups are : children 1-5 years (n= 22), adolescents 6- 17 years (n= 45), adult 18-65 years (n= 28)and elderly >65 years (n= 5) . Our study revealed EPEC Showed high incidence rates in children agegroup(27.3%,6/22) (Figure 3)

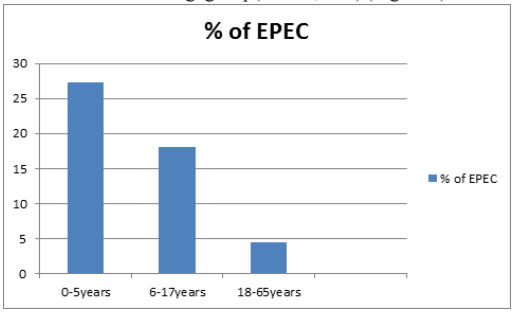

Figure3 : Age wise distribution of EPEC isolates

C. Antimicrobial resistance in EPEC pathotypes

EPEC pathotypes were also analysed for antibiogram patterns by antimicrobial susceptibility testing(Kirby- Bauer disk diffusion method) as per CLSI guidelines .AST showed that overall, majority of E.coli isolates were resistant to Cephalosporins and susceptible to meropenem. Significant levels of antibiotic resistance was found in antibiotics-ciprofloxacin, aztreonam, ceftazidime, piperacillin-tazobactam,ampicillin- sulbactam, gentamicin, cefepime.Multidrug resistance(resistance to more than three classes of antimicrobial drugs) was observed in 60% of the isolates. **Figure 4** shows antibiotic resistance pattern in EPEC .

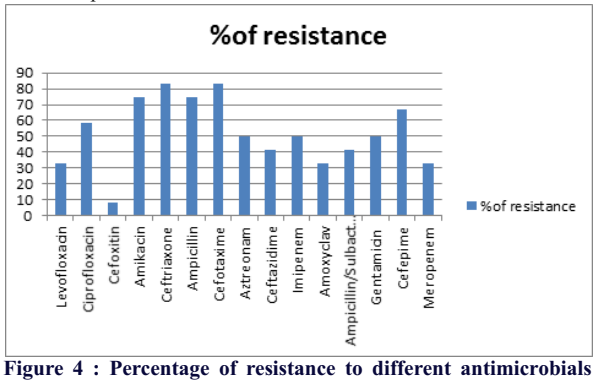

Figure 4 : Percentage of resistance to different antimicrobials among EPEC isolates

DISCUSSION

Diarrhea is a multifactorial illness caused by wide spectrum of pathogens including viruses, bacteria, and parasites .In India, diarrhea is still the main culprit for mortality among children under 5 years of age. Every year, an estimated 2.5 billion children die due to diarrhea; among them, 30% to 40% is contributed only to Diarrheagenic *Escherichia coli*(DEC).

Overall, the significance of EPEC as pathogens has declined in published literature of the last several decades It is unclear whether EPEC infections have declined due to interventions, particularly breastfeeding promotion, or whether earlier studies that based diagnosis on O- or O:H-typing overestimated the relative contribution of these organisms compared to recent studies in which EPEC identification was based on molecular methods and/or adherence assays[14]

Current findings strengthen evidences that EPEC is an emerging diarrheal agent in the South East Asian children population. These observations reinforce the fact that DEC pathotype is considerably responsible for severe gastrointestinal infections associated with childhood and adult diarrhea.

Antimicrobial resistance in enteric pathogens is of great importance in the developing world, where the rate of diarrhoeal diseases is highest. Though antibiotics have revolutionized the health sector but its abusive use has resulted in emergence of multidrug resistant isolates and certainly poses serious threat to the management of infectious diseases.[15]

Reports from other parts of India and neighboring countries showed 4-35% variation in DEC incidence rates [16,17,18]. Globally, prevalence of E. coli as an etiological agent of diarrhea is well reported between 30% and 40% cases[16]

By molecular identification approach, Enteropathogenic Escherichia coli (EPEC) is found to be a prevalent pathotype among all diarrheal patients.This enteropathogen EPEC possesses an innate propensity to persist longer in intestine than other pathotypes. [17].It may be emerging pathogen and its role in causing watery and chronic diarrhea is well documented. Various epidemiological studies from different parts of world showed similar observations which reported EPEC as the main DEC pathotype affecting children and adults with similar frequency([19,17]). These observations reinforce the fact that DEC pathotype is considerably responsible for severe gastrointestinal infections associated with childhood and adult diarrhea.

There has been an alarming increase in the antimicrobial resistant strains which threatens the effective prevention and treatment of

enteric infections caused by Gramnegative bacteria. E. coli, being notorious bacteria ,has become increasingly resistant to commonly used antibiotics in hospital and community settings and certainly poses serious threat to the management of infectious diseases.85In the present study, EPEC was found as most the resistant pathotype, and highest levels of antibiotic resistance were observed against Ampicillin (87.5%).In our study ,EPEC showed highest level of resistance to ampicillin (83.3%) andaztreonam (83.3%), followed by resistance to ceftriaxone and cefotaxime (75%).While it was found to be more susceptible to amikacin (91.7%),Levofloxacin and Ciprofloxacin (67% each).In EAEC strains , sensitivity was found to be more for meropenem (100%),amikacin (80%), cefepime (80%) followed by cefotaxime and ceftriaxone(60% each).

Mandal et al (2017) also reported similar pattern of resistance. The observed high resistance rates to antibiotics may be a result of misuse of antibiotics for treating infectious or extreme disease severity and persistence of infections90. Selective antibiotic pressure associated with inappropriate use of antibiotics leads to increased antibiotic resistance. Also , dissemination of transferable plasmids encoding MDR has been observed in DEC isolates from diarrheal stools. Canizalez- Roman et al conducted study in Mexico in 2016 reported >65% DEC strains were resistant to tetracycline, ampicillin and SMTX(sulpha-methoxazole) .In this study approximately 91% strains were resistant to atleast one antimicrobial agent. The observed high resistance rates to antibiotics may be a result of misuse of antibiotics for treating infectious or extreme disease severity and persistence of infections[18]. Selective antibiotic pressure associated with inappropriate use of antibiotics leads to increased antibiotic resistance. Also , dissemination of transferable plasmids encoding MDR has been observed in DEC isolates from diarrheal stools [21]. The problems associated with microbial resistance among enteric pathogens will continue to pose a challenge to public health workers.

Data generated from this study identified changes in the spectrum of antimicrobials in diarrhea related cases in India. These findings confirm the need to institute long-term surveillance programmes that are essential in identifying changes in the spectrum of antimicrobial patterns of EPEC in Rishikesh,Uttarakhand. The institution of such programmes would provide appropriate control measures for antimicrobial-resistant EPEC strains. In conclusion, the current study highlights the necessity for continuous monitoring of antibiotic resistance in EPEC and other bacterial pathogens.

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