FREQUENCY AND ANTIBIOGRAM OF ENTEROPATHOGENIC ESCHERICHIA COLI FROM A TERTIARY CARE HOSPITAL IN PAKISTAN

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ABSTRACT

Objective: To determine the frequency and antibiogram of Enteropathogenic *Escherichia coli* in children less than two years.

Study Design: Cross-sectional study.

Place and Duration of Study: The study was conducted at the department of microbiology, Army Medical College, Rawalpindi (NUMS) Pakistan, from Jul 2013 to Feb 2014.

Material and Methods: Stool samples collected from children with diarrhea who were below 2 years of age were included. *Escherichia.coli* isolates were identified by microscopy, culture and biochemical reactions (API 10S). Among the Escherichia coli isolates, EPEC isolates were identified by serogrouping. Antibiotic susceptibility of EPEC isolates was determined by modified Kirby-Bauer disk diffusion method according to CLSI guidelines.

Results: A total of 42 EPEC isolates were collected during the study period. None of the isolates were sensitive to ampicillin followed by ciprofloxacin (52.4%), ceftriaxone (7.3%), co-trimoxazole (12.5%), amikacin (87.5%), co-amoxiclav (9.5%), aztreonam (15%), meropenem (100%), sulbactum-cefoperazone (97.2%), piperacillin-tazobactam (89.5%), and gentamicin (63.4%). About 97% of the isolates were multidrug resistant.

Conclusion: EPEC is an important pathogen in pediatric diarrhea with very high rate of multi drug resistance.

Keywords: Drug resistance, Enteropathogenic Escherichia coli (EPEC), Microbial sensitivity tests.

INTRODUCTION

Diarrheal illness is an important public health problem globally. Diarrhea is caused by a variety of organisms like bacteria, viruses and even some parasites. In third world countries diarrheagenic *E. coli* is an important cause of gastroenteritis in children and is associated with high level antibiotic resistance[1]. The organism may spread to infants during delivery or by contaminated hands of the attendants. These may be a cause of significant morbidity and even mortality in these children. For the treatment of bacterial infection, antibiotics play an important role in reducing morbidity and mortality, however, their overuse and misuse could lead to the development of antibiotic resistance[2].

Enteropathogenic Escherichia coli (EPEC), one of the diarrheagenic *E. coli* pathotypes, along with *Enterotoxigenic Escherichia coli* (ETEC) and *Enteroaggregative Escherichia coli* (EAEC) are a significant cause of diarrhea in infants[3,4]. It is also one of the prime agents causing persistent diarrhea. It causes diarrhea by attachment of bacteria to enterocytes leading to its effacement known as attaching and effacing (A/E) lesion[5].

EPEC is no doubt a very important cause of persistent diarrhea. Currently the EPEC are divided into typical EPEC (tEPEC) and atypical EPEC (AEPEC). Typical EPEC are defined as "those isolates with the attaching and effacement (A/E) genotype (eae^+), which possess bfp A^+ and lack the stx^- genes are found strongly associated with diarrhoeal cases". Whereas the atypical EPEC isolates (aEPEC; $eae^+bfpA^-stx^-$), occur both in asymptomatic hosts as well as patients with diarrhea. Further studies are needed to determine their role in disease causation[6].

Early fluid and electrolyte replacement is the usual recommendation to be followed for the treatment of these patients and use of antibiotics

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in general is of minor importance and has been discouraged. Antimicrobials are usually not prescribed to these children on the grounds of toxicity of drugs and the risk of development of wide-spread antibiotic resistance[7]. However, use of antibiotics may sometimes become necessary in cases of severe diarrhea. Our country is a developing country and children who already are malnourished, when afflicted with diarrhea tend to become very critically ill. Sometimes this diarrhea may eventually lead to the death of these patients. Such conditions may necessitate the use of antibiotics to save the lives of children and we need to know the susceptibility pattern to prescribe the most effective antimicrobial. In addition due to emergence of resistance to routinely used antibiotic is a serious concern. As these resistant organism can transfer their genes for resistance to other organisms and both may eventually become very difficult to treat adversaries. Thus we need to keep an eye on the changes observed in the antibiogram of these frequently encountered pathogens, in order to monitor their upcoming pathogenic potential.

Frequency of EPEC varies from one area to another and multi-drug resistant EPEC strains are a usual in recent researches with worldwide spread. Even the more potent and newer antimicrobial agents are becoming ineffective against these pathogens. To select appropriate antibiotic for treatment of severe diarrheas knowledge of local antimicrobial sensitivity pattern plays an important role[8].

Keeping this in mind we planned a study to determine the frequency of EPEC as a cause of diarrhea in children younger than two of age, as well as to determine the resistance pattern of these isolates to commonly used antibiotics.

MATERIAL AND METHODS

This cross-sectional study was carried out at the department of Microbiology, Army Medical College (National University of Medical Sciences) Pakistan affiliated with Military Hospital, Rawalpindi, tertiary care hospital. The duration of study was seven months and it was carried out, from July 2013 to February 2014.

All the samples were collected by non-probability consecutive sampling.Stool samples that were received in the laboratory during the study period from children with diarrhea who were below 2 years of age were included in the study and their relevant clinical data was noted. Isolates other than Escherichia coli and duplicate samples of the same patient during the same period of illness were excluded from the study.

Stool samples collected during the study period were inoculated using a sterile loop on MacConkey agar (Oxoid, UK) and Xylose Lysine Deoxycholate agar (Oxoid, UK). These plates were incubated at 37°C for upto 48hours in ambient air. The plates were then observed for any growth. All the lactose fermenting colonies resembling those of Escherichia coli were subjected to further testing. The colonies were gram stained, checked for motility, oxidase and catalase production. A bacterial suspension equivalent to 0.5 McFarland turbidity standard was prepared of all catalase positive, oxidase negative and motile Gram negative rods. The suspensions were inoculated into Analytical Profile Index (API10S) (Biomerieux, France) and their sensitivity testing was setup. The results of API 10S were interpreted using manufacturer's guidelines. Next day the organism that were found to be Escherichia coli by API 10S were confirmed to be enteropathogenic Escherichia coli (EPEC) by serogrouping using antisera Escherichia coli polyvalent 2 types [O26 (b6), O55 (B5), O111 (B4), O119 (B14), O12 (B16)] and E. coli poly-valent 3 types [O86 (B7), O114:K90, O125 (B15), O127 (B8), O128 (B12)] (Remel, UK). Organism that showed agglutination with these antisera were confirmed as EPEC.

Antibiotic sensitivity to amikacin (30µg), ampicillin (10µg), co-amoxiclav (20/10µg), aztreonam (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), co-trimoxazole (1.25/23.75µg), gentamicin (10µg), meropenem (10µg), cefoperazone-sulbactam (105µg) and piperacillin-tazobactam

(100/10µg) was determined by Modified Kirby-Bauer disc diffusion method. The sensitivity to the antibiotics was determined by observing the zone of inhibition around each antibiotic disc using transmitted lightaccording to CLSI guide-lines[9]. The susceptibility profile of each isolate was noted.

To check for production of extended spectrum beta lactamase (ESBL) disk containing amoxicillin-clavulanate 20/10 µg (Oxoid, UK) was placed in the center of the Mueller-Hinton agar plate as the inhibitor of beta-lactamase and ceftriaxone 30 µg (Oxoid, UK) and aztreonam 30 µg (Oxoid, UK) disks were placed at 25 mm (center to center) from the amoxicillin-clavulanate disk. ESBL production was confirmed by noting an enhancement of inhibition zone around ceftriaxone and aztreonam in the presence of clavulanate. Production of ESBL by each isolate was also noted accordingly.

The number EPEC from the stool samples was noted and its percentage calculated. From the data collected we observed the male to female ratio and age wise distribution of EPEC. Anti-biotic sensitivity pattern to various commonly used antibiotics and the number of ESBL producing organisms was noted. Multi-drug resistance (MDR) was defined as resistance to at least three or more antibiotics[1]. The number of isolates that were found to have resistance to 3 or more antibiotics were taken as MDR EPEC and their number was also noted.

The Data was analyzed using Statistical Package for Social Sciences (SPSS) version 20.0. Qualitative variables like gender and suscep-tibility to antibiotics were measured by frequency and percentages.

RESULTS

From a total of 237 stool samples received during the study period, 42 (17.7%) EPEC were isolated. All the isolates were uniformally sensitive to meropenemn 42 (100%), followed by sulbactum – cefoperazonen 41 (97.2%), piperacillin-tazobactam 38 (90%), amikacin 37 (88%), gentamicin 27 (64%) and ciprofloxacin 22 (52.4%). Very low sensitivity rates to aztreonam, co-trimoxazole, co-amoxiclav and ceftriaxone, and aztreonam were observed with only 15%, 12.5%, 9.5% and 7.3% isolates being sensitive respectively. All the isolates were resistant to ampicillin. From a total of 42 isolates 22 (52.38%) were ESBL producers and 41 (97.6%) were MDR EPEC.

Of the EPEC isolates, most were isolated from males 26 (62%) while only 16 (38%) were from females. The male to female ratio was found to be 1.6:1.

Out of the total 42 EPEC isolated during the study period 17 (40.5%) were from children upto 6 months of age, 13 (31%) were from children aged between 7-12 months and the remaining 12 (28.5%) from children 1-2 years old. Among 26 (62% of total) isolates from males, 12 were from children aged less than 6 months, 7 were from children aged between 7-12 months and remaining 7 isolates were from 1 to 2 year old children. Among the 16 isolates (38%) from females, 5 were from patientsupto 6 months of age, 6 from children between 7 and 12 months and the remaining 5 from children between 1 to 2 years.

DISCUSSION

The frequency of EPEC in our study was 17.7% and about half of these were ESBL producers Study conducted in Iran at Kashan Shahid Beheshti Hospital during 2009-10 showed that 51 (28.6%) of 178 E. coli were positive for EPEC pathotype[10]. Tilak *et al*. 2012 found the incidence of EPEC to be 30% in the diarrheal group[11]. The study of Amela *et al* and Tawfeek *et al*. showed frequency of EPEC to be 54% and 13% respectively[8,12]. Cravioto *et al*. found the rate of diarrhea caused by EPEC in children less than one year to be 51.3%[13]. Studies in Brazil have reported that the prevalence of EPEC isolates were from 10.1 to 32.7%[14,15]. Another study by Dutta *et al*. showed that the prevalence EPEC was 1.8% and EPEC as compared to other DAE was more significant in children >2 year of age[16].

The male to female ratio in our study was 1.6:1 (n=26:16), showing slight male predominance. Mitra *et al*. found that there was female predominance i.e. female and male were 30 (58.8%) and 21 (41.2%), respectively[10].

A very high rate of multi-drug resistance (97%) was observed in our study. This alarming situation is most probably due to the injudicious use of antibiotics in our setup. Although only supportive therapy is recommended and antibiotics play a minor role in the treatment of diarrhea due to EPEC, but widespread indiscriminate prescription of antibiotics has possibly lead to the development of MDR strains. The MDR rate in EPEC was 70.6% in a study conducted by Mitra *et al*[8]. A study in Mexico noted that rate of MDR of EPEC pathotype in children less than five years old was 67%[17]. However in another study in Vietnam, on children, MDR rate of pathotype EPEC was 86%[18]. Ochoa *et al*. reported that MDR rate of EPEC pathotype in children at 2-12 months age was 47%[19]. In this most isolates were observed to be resistant to ampicillin, cephalexin, and ceftriaxone.

In our study more than 85% of the isolates were sensitive to meropenam, cefoperazone-sulbactam, piperacillin-tazobactam and amikacin.

Tilak *et al*. found that EPEC isolates showed most isolates were sensitive to Amikacin, followed by norfloxacin (46.6%), ciprofloxacin (36.7%), co-trimoxazole (34.4%), nalidixic acid (30%) and ampicillin (30%)[10]. Mitra *et al*. found that the rates of resitance were ampicillin (100%), cephalexin (84%), ceftriaxone (74.5%), nalidixic acid (62.7%), streptomycin (43.1%), ceftazidime (39.2%), ciprofloxacin (35.3%) and imipenem (0%)[11].

Antibiotics might not be needed routinely for treatment but knowledge of susceptibility pattern should however, be known and monitored continuously.

CONCLUSION

Enteropatogenic Escherichia coli were found to be important cause of diarrhea in children less than 2-years of age in our setup as they were isolated from 17.7% of the stool specimens received in our laboratory. Almost all of the isolates that were included in the study were multi drug resistant (MDR). Of these 52.34% were found to be ESBL producers. Meropenem was the only drug to which all isolates were sensitive, with amikacin, cefoperazone-sulbactam and piperacillin-tazoba-ctam showing good overall efficacy against Enterpathogenic Escherichia coli.

Ethical Approval

No human or animal testing was done in the study. So no ethical issue involved. The study was carried out on bacterial isolates from samples that were already sent to the laboratory.

CONFLICT OF INTEREST

All the authors declare that no conflict of interest involved in this study.

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