Enteric pathogens and their resistance pattern in paediatric diarrhoea in A.P

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ABSTRACT

Diarrhoeal diseases remain one of the most prevalent public health problems of today. It is estimated that over 1.3 billion cases of diarrhoeal illness occur each year resulting in 5 million deaths. Progressive increase of antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern. The present study was therefore conducted to study the bacterial pathogens in paediatric diarrhoeas and their antibiotic resistance pattern in Hyderabad, A.P, India. Prospective study of 100 faecal samples from diarrhoeal cases and 30 age matched controls was performed for a period of 6 months. The isolates were identified by standard biochemical tests and antibiotic resistance patterns of the isolates was determined as per CLSI guidelines. Esch. Coli was the predominant isolate in both the study and control groups. The next common isolate was Klebsiella in both the study and the control groups. More than 50% of the isolates were below 1 year of age. Slight male preponderance of the isolates was observed in the study group. Mild to Moderate dehydration was seen in 60% of cases. High degree of resistance was observed to Nalidixic acid, Ampicillin and Cotrimoxazole in the study group. The isolates showed maximum sensitivity to Amikacin followed by Norfloxacin. High level resistance to first line antimicrobials is due to unselected use of these drugs in low risk patients without complications. Periodic monitoring of drug resistance in enteric pathogens in each geographical area helps in choosing the appropriate antimicrobial agent for empiric therapy.

Keywords: Diarrhoea, Escherichia coli, Antimicrobial resistance.

INTRODUCTION

Diarrhoeal diseases have been recognised since the beginning of civilization and remain one of the most prevalent public health problems of today. About two-thirds of the world population live in areas regarded as underdeveloped and it is estimated that over 1.3 billion cases of diarrhoeal illness occur each year in the underdeveloped countries. Of these over 2.7 million deaths occur in children [1]. The evolution of antibiotic resistance in bacteria is a topic of major medical importance.[2] Antimicrobial Resistance in enteric pathogens is of great importance in the developing world where the rate of diarrhoeal diseases is highest. The progressive increase in antimicrobial resistance among enteric pathogens in developing countries is becoming a critical area of concern.[3]. The resistance of enteropathogenic bacteria to commonly prescribed antibiotics is increasing both in developing as well as in developed countries. Resistance has emerged even to newer, more potent antimicrobial agents. [4]. Hence the present study was conducted to know the prevalence of diarrhoeal pathogens, identify the bacterial agents causing diarrhoea and to identify the current antibiotic resistance pattern of the isolated organisms in children below 5 years of age in Hyderabad city, A.P
A total of 100 faecal samples were collected from children below 5 years of age suffering from acute diarrhoea admitted to Gandhi and Niloufer hospitals during the period May to November 2009. 30 samples were collected from nondiarrhoeal age matched children to serve as the control group. The history of diarrhoea was elicited from the patients and details regarding age, sex, number of stools passed per day, previous medication taken for diarrhoea, passing of blood, pus, mucus and other relevant details like history of fever, vomiting, presence of dehydration, source of water and sibling history were also taken. Stool specimens were collected in sterile leakproof widemouthed container before administration of antibiotics. Naked eye examination of collected specimens was done and the colour, odour, presence of mucus and blood and consistency of stool samples were noted down. Normal saline mount was done and the preparation was examined for the presence of faecal leucocytes, RBC, Ova and cysts. A loopful of faeces was inoculated on Mac Conkey agar, Wilson & Blair brilliant green bismuth sulphite medium and Thiosulphate citrate bile sucrose agar and incubated at 37°C aerobically overnight. 2-3 loopfuls of faeces were inoculated into enrichment media i.e. Selenite F broth and Alkaline peptone water. After incubation for about 5hrs, a loopful of culture from Selenite-F broth was inoculated on Mac Conkey agar, Wilson & Blair media. A loopful of culture from Alkaline peptone water was inoculated on Mac Conkey agar and TCBS media and after 18-24 hrs, a loopful of culture from Selenite-F broth was inoculated on Mac Conkey agar and Wilson & Blair media. Mac Conkey agar plates were incubated overnight at 37°C and examined the next day. Wilson and Blair and TCBS media were incubated for 48 hrs at 37°C. After incubation the colony characters were observed and the organisms were identified by Grams stain, hanging drop and other standard biochemical reactions like catalase, oxidase, Indole, methyl red, vogesproskauer, citrate, urease, sugar fermentation tests, nitrate reduction and ammoin acid decarboxylase tests as per Mackie Mc Cartney Practical Medical Microbiology (14 th Edition). Antibiotic susceptibility testing of the isolates was done by Kirby Bauer disc diffusion method on Mueller Hinton agar with Ampicillin (10mic g), Amikacin (30 mic g), Cotrimoxazole (1.25mic.g trimethoprim + 23.75 mic g sulphamethoxazole), Nalidixic acid (30mic.g), Norfloxacin (30 mic g) and Ciprofloxacin (30 mic g) discs as per CLSI guidelines.

RESULTS

A total number of 100 acute diarrhoeal cases from children below 5 years were studied to identify the bacterial etiology. Stool samples from 30 nondiarrhoeal cases of the same age group served as control group. More than 50 % were less than 1 year of age in both the test and the control groups. There was a slight male preponderance of cases in the study group. There was no difference in the sex distribution in the controls. Loose stools was seen in 100 % of cases. Fever was seen in 90% and Vomiting was seen in 62% of cases. Mild to Moderate dehydration was seen in 60% of diarrhoeal cases. In the control group, mild dehydration was seen in only one case. Faecal leucocytes were seen in 67% of the isolates and no RBC were observed in microscopy in the diarrhoeal group. Analysis of 100 stool samples from acute diarrhoeal cases showed Escherichia coli (82%), Klebsiella (8%), Proteus (5%), Citrobacter (3%), Enterococci (2%) and in the nondiarrhoeal cases showed Escherichia coli (60%), Klebsiella (30%), Proteus (3.3% and Enterococci (6.7%). Escherichia coli was the most common isolate in both study and control groups followed by Klebsiella, Proteus, and Citrobacter in the study group whereas in the control group, Klebsiella was the next common pathogen followed by enterococci. Antimicrobial susceptibility of the isolates showed highest degree of resistance to Nalidixic acid (87%) and Ampicillin (81%) followed by Cotrimoxazole (79%) and Ciprofloxacin (65%). They showed 100% sensitivity to Amikacin. Antibiogram of the predominant isolate Escherichia coli showed highest resistance to Nalidixic acid (84.1%) and Ampicillin (84.1%) followed by Cotrimoxazole (78.3%) and Ciprofloxacin (69.6%). They showed 100% sensitivity to Amikacin followed by 35.3% sensitivity to Norfloxacin.

DISCUSSION

In the present study, 100 diarrhoeal cases of either sex, below 5 years of age and 30 age matched controls were studied. 58% of cases were of the age group below 1 year, which correlated with the previous studies [7,8] which showed that 50 -74% of cases belonged to below 1 year age group. The male : female ratio was 53% : 47% in our study indicating only a slight male preponderence which is in agreement with the previous workers [7]. In our study, 51% of all cases showed mild to moderate dehydration. Similar incidence has been reported in other studies [5,6]. Malnutrition would have contributed to an increased incidence of diarrhoea in the present studies because malnutrition causes increased activity of disaccharides, villous atrophy and depresses immunity. In the study group, the isolation of Escherichia coli was 82%, Klebsiella 8%, Proteus 5%, Citrobacter 3% and Enterococci 2%. In a study [7], 73% isolation of Escherichia coli, 4.6% Klebsiella, 0.8% Enterococci was reported which correlates well with our study. Another study [8] showed an isolation of Escherichia coli 21.1%, Proteus (1.4%), Klebsiella (2.8%). Escherichia coli isolation rate in the present study was 82%. This is in contrast to the isolation rate seen in [10]. Our isolation rate correlated with another study [10]. Other organisms isolated in our study were Klebsiella 8...
The isolation of these doubtful pathogens in a study [11] was 23%. The pathogenicity of organisms other than Escherichia coli isolated in the present study is controversial. However, when these suspected pathogens were isolated in pure culture or in significant numbers and in the absence of other definite pathogens, their presence cannot be ignored. All these organisms were isolated in pure culture. There was a high degree of resistance to Nalidixic acid (87%), Ampicillin (81%), Cotrimoxazole (79%), Ciprofloxacin (65%), and Norfloxacin (59%) exhibited by enteric pathogens in our study. A study [12] showed 65% resistance to Cotrimoxazole, 75% resistance to Ampicillin, and no resistance to Ciprofloxacin. Another study [6] showed 63.6-89.5% resistance to Nalidixic acid and 58.1% -77.8% resistance to Cotrimoxazole and 56% resistance to Ciprofloxacin which correlated with our study. Escherichia coli isolates showed highest resistance to Nalidixic acid (84.1%), and Ampicillin (84.1%) followed by Cotrimoxazole (78.3%), Ciprofloxacin (69.6%), Norfloxacin (64.7%), and Amikacin (0%) in the present study. In one study [13], the highest resistance rates to Ampicillin (95%), Cotrimoxazole (84%), and Amikacin (0.5%) were seen that correlated with our study. The Ciprofloxacin resistance (18%) was less when compared to our study. In yet another study [14], high resistance to Cotrimoxazole (88.6%) and Ampicillin (72.7%) was observed which correlated well with our study. In our study, low resistance reported in his study to Nalidixic acid (8%) did not match our study. In yet another study [15], 1.4% of the Escherichia coli isolates showed resistance to all the antimicrobials tested. In our study, none of the isolates showed resistance to all the drugs tested.

CONCLUSION

High level resistance to first line antimicrobials in diarrhoeal cases is due to unselected use of these drugs in patients with a mild presentation with low risk for complications or who would recover well without antibiotics. The choice of antimicrobial agent has to be made empirically, it should consist of the narrowest antimicrobial spectrum that covers the most likely pathogens. Also, routine use of antibiotics for infectious diarrhoea in children must be avoided as it brings little benefit in most cases. Further, periodic monitoring of drug resistance in enteric pathogens should be carried out in each geographical area so that an appropriate agent can be chosen for empiric therapy. This could lead to not only control of drug resistance but also decrease the financial burden on the community.

REFERENCES