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Original Research Article

A PRELIMINARY STUDY ON BACTERIAL, PARASITICAL AND ROTA-VIRUS BURDEN IN STOOLS OF UNDER FIVE CHILDREN HAVING DIARRHOEA FROM INDORE REGION OF MADHYA PRADESH

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ABSTRACT-

Introduction: Children's diarrheal illnesses have a variety of aetiologies and risk factors linked with them. Paucity of data from Indore region of Madhya Pradesh state encouraged us to conduct this research study. **Objectives:** This study aims to assess various diarrheal aetiologies in children under the age of five years. **Methodology:** Stools of 105 children with diarrhoea were sampled in order to identify the bacterial, viral and parasitic cases. The samples were processed in the laboratory along with culture through the use of gram staining and biochemical processes, the isolates were identified. The antibiotic susceptibility of the notable isolates was then evaluated. **Results:** Bacterial aetiology was seen among 57/105 subjects (54.28%), followed by virus among 28/105 subjects (26.7 %) and parasitic aetiology in 20/105 (19%). Amongst bacterial isolate, Diarrheagenic Escherichia coli 34/57 (59.64%) was the most common bacteria followed by Klebsiella species 14/57 (24.56%). Klebsiella isolates were sensitive to gentamycin and ciprofloxacin. **Conclusion:** There are numerous, avoidable microorganisms that can cause diarrhoea in children. Raising awareness and educating people, particularly mothers, about the illness, clean drinking water, healthy food consumption, and good sanitation are crucial in achieving reduction in the causes of children's diarrheal illnesses.

Keywords: stool, diarrhoea, under five children, bacteria, Rota-virus

INTRODUCTION

Human rotavirus is still an important pathogen causing acute diarrhoea in a subset of population under the age of five years [1]. Infections with the rotavirus are common, with high rates of morbidity in industrialised nations and mortality in underdeveloped nations [2]. Around the world, rotavirus is responsible for 125 million cases of diarrhoea and more than 600,000 child fatalities each year. India spends between 3-4 billion Indian Rupees to treat rotavirus diarrhoea in children under the age of five

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

every year [3].

Various kind of microorganisms are the main cause of diarrheal diseases in Indian scenario. Such microorganisms include mainly some specific kind of bacteria and viruses. These microorganisms are all spread through tainted food and water. The precise infectious dose required to produce the sickness varies amongst them [4].

There are few research that concentrate on the many aetiologies of childhood diarrhoea, and the aetiology differs from place to region [5,6]. We conducted this study due to the dearth of data from the Indore region of the Madhya Pradesh state.

OBJECTIVES

The study aims to determine the many diarrhoreal aetiologies in children below five years of age.

MATERIALS AND METHODS

The present study was cross-sectional in design, conducted at department of microbiology a tertiary care teaching institute (Index Medical College) situated in Indore region of Madhya Pradesh state for a duration of one year (August 2021 to July 2022). 105 children with diarrhoea had stools taken in order to identify the bacterial, viral, and parasite causes. The samples were examined using Lugol's iodine mount first. After that samples were tested by saline wet mount. Modified acid fast staining was performed thereafter, and finally culture was done. Through the use of gram's staining and biochemical processes, the isolates were identified.

Standard bacteriological methods were used to determine the bacterial aetiology. Polymerase chain reaction (PCR) with gene specificity was used to identify diarrheagenic E. coli (DEC). Rotavirus antigen was detected with the help of enzyme immunoassay. Wet mount preparation was used to identify parasites, Sabouraud's Dextrose Agar culture and microscopy were used to determine the fungus that caused the infection.

The samples showing presence of rota virus were subsequently subjected to RTPCR to characterise rota virus strains. For determination of presence of rota virus in the specimen, VP6-specific monoclonal antibodies in the Antigen capture ELISA, were used in this study.

Antimicrobial susceptibility testing was conducted for positive isolates. This testing was done by the Kirby-Bauer disc diffusion method for the antibiotics, cefotaxime 30 g, ampicillin 10 g ciprofloxacin 5 g, co-trimoxazole T1.25/S23.75 g, amoxy clavulanic acid 20 g, gentamicin 10 g, chloramphenicol 30 g and tetracycline 30 g.

Lawn culture was made from Mueller-Hinton agar plate inoculation. Discs were applied to the agar surface with sterile forceps and incubated at 37°C for whole night. According to Clinical Laboratory Standards Institute (CLSI)guidelines, the data were interpreted.

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

All tests utilised a specific strain of E. coli ATCC 25922 for quality check.

Identifying the bacterial cause

MacConkey agar, Columbia blood agar medium, deoxycholate citrate agar (DCA), and Thiosulphate-citrate bile salts-sucrose agar (TCBS) were directly inoculated with the stool samples followed by incubation at 37 degree Centigrade and processed in accordance with conventional bacteriological procedure. The colony appearances, common biochemical assays, motility test, andagglutination with certain antisera were used to identify the isolated colonies.

Identification of the parasitic cause

Macroscopical examination of the stool samples to find out the parasites followed by wet mount, microscopic examination to check for the existence of ovum, cysts and other stages by low-power (10X) and high-power (40X) microscopy.

Determination of the cause of a virus

Enzyme Immuno-Assay (EIA) kit was used to detect rotavirus antigen in all of the samples. (Premier Rota clone meridian Bioscience). The VP6 antigen specific monoclonal antibodies are used in this test [7].

The investigation was started after receiving ethical approval from the institute. The data collected was coded appropriately on MS Excel spreadsheet. Data was checked for any potential errors. Statistical software was used for analyzing the data.

RESULTS AND DISCUSSION

Bacterial aetiology was seen among 57/105 subjects (54.28%), followed by virus among 28/105 subjects (26.7 %) and parasitic aetiology in 20/105 (19%). (Table 1)

Table 1: Causative agents of diarrhoea in study subjects (n=105)

Microbial etiology	Number	Percentage
Bacterial	57	54.28
Viral (Rotavirus)	28	26.7
Parasitic	20	19.0

Same results have been reported by Saeed et al [8]. Authors of a different study from the state of Odisha examined 130 samples of faeces. The aetiologies of viruses, bacteria, and parasites were examined in the samples. Escherichia coli, Rotavirus, Shigella, Adenovirus, Cryptosporidium, and Giardia were found to be the predominant etiological agents, respectively, accounting for 30.07%, 26.15%, 23.84%, 3.07%, and 0.77 percent of cases, respectively [9]. A few studies have also emphasised Shigella's significance as an etiological factor in diarrheal illness [10,11].

Amongst bacterial isolate, Diarrheagenic Escherichia coli 34/57 (59.64%) was the most common bacteria followed by Klebsiella species 14/57 (24.56%). Shigella and Pseudomonas species were

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

seen in 2/57 (3.50%) subjects each. Amongst parasitic agents, Giardia lamblia 7/20 (35%) was the most prevalent parasite observed, followed by Hymenolepsis nana 5/20 (25%) and Ascaris lumbricoides 4/20 (20%). (Table 2)

Table 2: Bacterial, Parasitic and Viral Isolates found in stool samples of study subjects

Bacterial and Parasitic Isolates		Number	Percentage
Bacterial isolate	Diarrheagenic Escherichia coli	34	59.64
	Klebsiella species	14	24.56
	Shigella species	2	3.5
	Pseudomonas species	2	3.5
	Staphylococcus aureus	1	1.75
	Enterococci species	1	1.75
	Proteus species	1	1.75
	Salmonella species	1	1.75
	Vibrio cholerae O1Eltor	1	1.75
Parasitic agents	Giardia lamblia	7	35.0
	Hymenolepsis nana	5	25.0
	Ascaris lumbricoides	4	20.0
	Cryptosporidium parvum	2	10.0
	Entamoeba histolytica cyst	2	10.0

In India, it was discovered that Giardia and Cryptosporidium can harm children under the age of five [12]. In this investigation, we found that 35% of diarrheal cases were caused by Giardia and 10% cases caused by cryptosporidium . In a study from Bangladesh, giardia lamblia was not substantially linkedto diarrhoea [13].

Klebsiella isolates were observed to be sensitive to ciprofloxacin and gentamycin. Shigella isolates were found to be sensitive to ciprofloxacin, cefotaxime, gentamycin, and amoxy clavulanic acid.Vibrio was found to be sensitive to Ampicillin and Chloramphenicol. (Table 3)

Table 3: Antimicrobial susceptibility of Klebsiella (n=12), Shigella (n=2) and Vibrio (n=1) isolates

Antimicrobial susceptibility to	Sensitive to number of Klebsiella isolates	Percentage	Sensitiveto number of Shigella isolates	Percentage	Sensitive to number of Vibrio isolates	Percentage
Ampicillin	2	16.7	1	50	1	100

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

10 mcg						
Cotrimoxazole 1.25/3.75 mcg	2	16.7	1	50	0	0
Ciprofloxacin 5 mcg	12	100	2	100	-	-
Cefotaxime 30 mcg	6	50.0	2	100	-	-
Gentamycin 10 mcg	12	100	2	100	-	-
Amoxyclav 20/10 mcg	6	50.0	2	100	-	-
Chloramphenicol 30 mcg	-	-	-	-	1	100
Tetracycline 30 mcg	-	-	-	-	-	-

We discovered that the Klebsiella isolates in our investigation were susceptible to ciprofloxacin and gentamycin. The antibiotics ciprofloxacin, cefotaxime, gentamycin, and amoxy clavulanic acid were found to be effective against Shigella isolates. Concerns are raised and a more limited usage of these antibiotics is required in order to prevent further increases in resistance due to very low sensitivity to amoxicillin and nalidixic acid as well as increasing resistance to third generation cephalosporins and ciprofloxacin [14].

Ampicillin and chloramphenicol were proven to be effective against Vibrio in our investigation. According to Uppal B et al., Vibrio was mostly susceptible to the antibiotics ciprofloxacin, amikacin, gentamicin, and cefotaxime while being resistant to the drugs nalidixic acid and amoxicillin [14]. Similar findings were reported in earlier Delhi-based research [15].

There are limited estimates of the burden of diarrhoea caused by certain pathogens in the community, and the majority of studies on the causes of diarrhoea in under developed and developing countries have focused on cases of severe disease in patients seeking medical attention. Another multisite birth cohort study observed magnitude of diarrhoea that was pathogen-specific. Additionally, age, geography, season, vaccination use, and symptoms being the key factors [16].

One of the main risk factors for childhood diarrhoea is malnutrition. A significant risk factor for diarrheal illnesses is socioeconomic status [17]. Wang et al., from Beijing, found a similar finding. They demonstrated that children from less affluent provinces who had lower environmental and health circumstances were more vulnerable to diarrheal infections [18]. Therefore, it is essential to provide basic amenities like a clean water supply and sanitation in order to improve community cleanliness [19].

ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

The main risk factors for diarrhoea have been identified as being water-borne and faecal-oral infections, unsanitary behaviours, subpar sanitation, and a lack of knowledge about treatable diarrheal disorders [20]. The prevention and treatment of diarrhoea in children may benefit from the identification of the etiological agents and risk factors linked to it. Since polluted water and food are the primary means of transmission, raising knowledge and educating people, mainly mothers, about the disease and the importance of safe drinking water, a healthy diet, and good hygiene are essential to battling this virus.

CONCLUSION

There are numerous, avoidable microorganisms that can cause diarrhoea in children. Raising awareness and educating people, particularly mothers, about the illness, clean drinking water, healthy food consumption, and good sanitation are crucial in achieving reduction in the causes of children's diarrheal illnesses.

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None

Conflicts of Interest

None

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ISSN:0975 -3583,0976-2833 VOL 15, ISSUE 01, 2024

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