Original Research Paper

Volume - 13 | Issue - 06 | June - 2023 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Clinical Microbiology

ACCESS TO SAFE DRINKING WATER REMAINS NEED OF HOUR IN PREVENTING BACTERIAL AND PARASITIC DIARRHEA AMONGST UNDER 5 CHILDREN

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ABSTRACT In India, diarrhea prevalence among under 5 years children remains high and is associated with a wide range of bacteria, parasites, and viruses, transmitted through contaminated food and water. The present study aimed at the detection of enteric pathogens in children with diarrhea and its relationship to the source of drinking water. Fecal samples were collected from 157 children from July 2021 to July 2022. Among 157 children, parasitic and bacterial pathogens were detected in 4.5% (7/157). Parasites were detected in 2% (Giardia 67%, H nana in 33%) and bacteria in 2.5% (Ecoli O157 in 75% and salmonella in 25%) cases. Specific sources of drinking water more often associated with diarrhea were hand pumps (43%), house tap water (28.6%), bore well (14.2%), and well (14.2%) cases. Thus, demonstrating the persistence of significant pathogens and re-emphasizing that diarrheal illnesses are preventable through safe drinking water practices.

KEYWORDS : Diarrhea, bacteria, parasite, drinking water

Introduction:

Diarrhea is usually a symptom of an infection in the intestinal tract caused by a variety of bacterial, viral, and parasitic pathogens.[1]With a prevalence 9.2% in India,[2]childhood diarrhea is reported to be associated with a wide range of bacteria including Escherichia coli, Salmonella spp., Vibrio spp; parasites including Entamoeba *histolytica*, Giardia *lamblia*, Hymenolepis *nana*, Cryptosporidium *parvum* and viruses including rotaviruses and adenovirus.[3,4]Poor hygiene and improper sanitation increase the transmission of diarrhea in the community through fecal-oral routes by contaminated food and water, [5]Thus, the present study aims at the detection of enteric pathogens in children with infectious diarrhea.

Material and methods:

Study design

This study was conducted in the Department of Microbiology, tertiary care hospital, Sagar M.P. Fecal samples were collected from 157 children under 5 years from July 2021 to July 2022 presenting with acute diarrhea. WHO defined diarrhea as the passage of 3 or more loose or liquid stools per day (or more frequent passage than is normal for the individual).[1] The study was approved by the Institutional Ethics Committee. Informed consent was obtained from their parents/guardians evidenced by informed consent forms and patient datasheets were maintained for each participant.

Sample collection

Stool samples were collected using sterile plastic containers and labeled with the patient's details. Samples were transported to the lab and immediately processed in the Microbiology laboratory.[5]

Microscopy and culture

The direct wet mount was prepared using normal saline (0.85%) and Lugol's iodine. Microscopic examination was done for the detection of trophozoites, cysts of protozoa, and eggs of helminths. Stool samples were cultured on several media for maximal yield. The sample was inoculated directly in enrichment media of selenite F broth, Alkaline peptone water (APW), and standard media; McConkey agar and selective media; Xylose Lysine Deoxycholate agar (XLD), Deoxycholate Citrate Agar (DCA), and Bile salt agar. Sub-culture from Selenite fecal broth and APW was made onto Salmonella–Shigella agar (SSA) and Thiosulfate-citrate-bile salts-sucrose (TCBS), and incubated aerobically for 18-24 hr at 37°C.[6,7]The Suspected colonies were subjected to preliminary tests like Gram staining, hanging drop for motility, catalase, and oxidase tests. Their identity was established by biochemical tests including; Indole, Citrate, Urease, H2S on triple sugar iron agar, methyl red, Voges–Proskauer, nitrate test, lysine, ornithine decarboxylase tests, and arginine dihydrolase test, which were done to identify the organism causing infectious diarrhea.[3,6]

Antimicrobial susceptibility testing of bacterial isolates

Susceptibility testing was performed by disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2021.[8] Escherichia coli ATCC 25922 was used as a reference control strain. Antibiotics tested were ampicillin 10 µg, amoxycillin and clavulanic acid (Augmentin) 20/10 µg, erythromycin 15 µg, ciprofloxacin 100 µg, gentamicin 10 µg, cefotaxime 30 µg, co-trimoxazole 25 µg, chloramphenicol 30 µg and tetracycline 30 µg, doxycycline 30µg. According to the size of the zones of inhibition, the organisms were classified as susceptible, intermediate, or resistant to a specific antibiotic.[8]

Rapid confirmatory latex agglutination slide test

The stool sample was cultured on Sorbitol MacConkey Agar for the latex agglutination test. The test was done for the identification of E.coli serogroup O157 using HiE. coli O157™ Latex test kit. E.coli O157 strains are sorbitol non-fermenters and give colorless colonies on Sorbitol MacConkey Agar. Non-fermenting colonies are then tested with the latex reagents, to identify whether the isolate belongs to the O157 serogroup. The majority of non-pathogenic E.coli isolates do ferment sorbitol sugar and give characteristic pink-colored colonies. Sorbitol MacConkey Agar was used as primary screening test.[9] For the identification of salmonella spp.,a HiSalmonella™ Latex test kit was used and samples from a Selenite F broth 18 to 24 hours old culture or a suspension of bacteria from solid agar were taken to form a homogenous suspension with sample diluent and reacted with salmonella latex reagent, coated with antibodies to salmonella antigens and forms agglutination with salmonella antigen which can be read by the naked eye and simple to perform.[10]

Multiplex RT-PCR

Detection of enteric viruses was done by multiplex real-time one-step RT-PCR on 150 stool specimens which were negative for both microscopy and culture. RNA extraction was carried out using MagMax™ viral pathogen Nucleic Acid Isolation kit.[11] The reaction tubes were placed in a thermal cycler for 50°C for 20 min and 95°C for 15 min followed by 45 cycles of 95°C for 10 min, 60°C for 1 min, and 72°C for 30 sec, and the result was obtained as an exponential fluorescence graph.[12]

Result:

Out of 157 samples, parasitic and bacterial enteric pathogens were detected in 4.5% [7/157] cases. On routine microscopy, parasitic infections were detected in 2%[3/7] of acute diarrheal cases. Among these, Giardia spp. was identified in 28.6%[2/7] and H nana in 14.3%[1/7] cases. Bacterial enteropathogens were isolated by bacteriological culture, identified by biochemical tests, and confirmed by latex agglutination slide test. The overall bacterial infection was present in 2.5%[4/7] of cases and among them, Ecoli O157 was present in 42.8%[3/7], and salmonella in 14.3%[1/7] was responsible for childhood diarrhea. Table 1 shows the detection of enteric pathogens associated with diarrhea (p-value 0.000055).

Out of 150 samples negative for both microscopy and culture negative, viruses causing diarrhea were detected in 17.8% [28/157] samples. Single viral infection was seen in 57% whereas mixed viral infection was in 43% of cases. Rotavirus was most common at 57.1%, followed by Adenovirus 53.6%, Norovirus 28.6%, Enterovirus 21.4%, and Astrovirus in 14.3% cases.

Our study indicates the source of water supply can be another reason for the occurrence of bacterial and parasitic diarrhea. The maximum cases of diarrhea were seen with patients consuming handpump water 43% [3/7], followed by house tap water 28.6%[2/7], borewell 14.2%[1/7], well 14.2%[1/7], and no cases seen while using RO water. Figure 1 shows the distribution of total and positive cases based on source of drinking water.

Discussion:

It was observed that the bacteria E.coli spp and Salmonella spp were significantly associated with diarrhea with 75.0% [3/4] and 25.0% [1/4] respectively. The sample was found to be positive with parasite Giardia spp. 66.0% [2/3] and H.*nana* 33.0% [1/3]. Intestinal parasites may be associated with serious clinical diseases and mortality and can cause malnutrition leading to impairment of physical and mental development in children. Thus, it is necessary to have an accurate description of the situation to target interventions in affected areas.[3,5]In our study, most of the bacterial pathogens isolated were resistant to ampicillin, followed by ciprofloxacin, and ceftriaxone. The rate of resistance among diarrheagenic E.coli to 1st-line, 2nd-line, and 3rd-line of therapeutic drugs was high. The majority of the isolates were sensitive to amikacin, imipenem, and tetracycline. In the current study, the Salmonella isolates exhibited resistance to ciprofloxacin but were susceptible to ceftriaxone. Most diarrheal infections can be effectively treated by fluid and electrolyte replacement, particularly in the case of viruses and some bacteria. In bacterial diarrhea antibiotic therapy shortens the duration of diarrhea and limits the shedding of the organisms in the environment, thus preventing the further risk of transmission of infections.[3,5] This highlights the necessity for continuous monitoring of antibiotic resistance in diarrhea-related bacterial pathogens along with the conventional culture techniques and improves the quality of detection of bacterial enteropathogens.

According to Shrivastava et al., bacterial pathogens were isolated from 581 (8.90%) of the total of 6527 stool samples. These included diarrheagenic E. coli, which were identified by serotyping in children below five years of age. Parasites were identified in 312 (4.78%) patients as the aetiological agent of diarrhea. Giardia *intestinalis*, Ascaris *lumbricoides*, and Entamoeba *histolytica* comprised the major parasitic pathogens with 2.27%, 1.15%, and 0.64% respectively.[5] According to Ashie et al. stool parasites identified were Giardia species, Ancylostoma spp., Strongyloides spp., and bacteria Salmonella spp. or Shigella spp. were significantly associated with diarrhea. According to Rauthaur et al. study which aimed at determining the frequency of various bacterial enteropathogens causing acute childhood diarrhea showed Salmonella and Shigella were major contributors to severe diarrhea accounting for 28.2% and 13.2% respectively.[6]

According to Kaur R et al, intestinal helminths or protozoa were found in 59[46.5%] cases including Giardia *intestinalis* and Entamoeba *histolytica* in 14 (11%) cases each, Balantidium coli in 3 (2.4%) cases and Cryptosporidium spp in 24 (18.9%) patients.[13] According to Farid H., Elamreen A et al, bacterial enteropathogens were detected in 26 (17%) cases. Shigella spp. was found in 6 (4%) by bacteriological culture Salmonella spp. was found in 3 (2%) by bacteriological culture. E. coli O157:H7 was found in 6 (4%) by bacteriological culture. Microscopy identified parasites in 23 cases (15%, Entamoeba (*histolytica/dispar*), Giardia *intestinalis* in 2 cases, and Strongyloides *stercoralis* in 1 case. The Salmonella and Shigella isolates were frequently resistant to doxycycline (89%).[14]Earlier studies carried out in different parts of the world also showed the emergence of MDR among enteric pathogens According to a few studies, E. coli O157:H7 is not routinely found but has been associated with 10–15% of cases of bloody diarrhea. Children with gastrointestinal infections caused by E. coli O157:H7 are at risk for a hemolytic-uremic syndrome which can be fatal because it may lead to acute kidney failure. Some studies state that morbidity due to intestinal parasites has always been an important public health problem in the tropics, but the incidence and severity may vary depending on the location and period (Sethi et al, 1999), moreover, supposed differences in the rate of prevalence may be due to the use of different diagnostic methods and the difficulties involved in the identification of certain parasites.[13,14,15]

According to a study done by Nair GB et al. Vibrio *cholerae* O1 was isolated in (26%) followed by EAEC (6.3%), Shigella spp (6.1%), C. jejuni (4.7%), and ETEC (4.5%). Among parasitic infections, G. *lamblia* was most predominant in 281 (11.2%) cases followed by Cryptosporidium sp. (6.3%) and E. histolytica (3.3%).[16] According to Langendorf C et al. EPEC, Salmonella spp., and Campylobacter spp. were frequent in children with watery and bloody diarrhea and Shigella spp. were the most frequent among children with bloody diarrhea.[17] According to Mukherjee AK et al., 413 (10.2%) cases tested positive for G. duodenalis.[18] According to Okada K et al. The pathogen detection rates of bacteria and parasites in diarrhea cases were 84.9%, and 3.2%, respectively.[19] According to the Platts-Mills JA et al. study, EAEC, Giardia, and atypical were frequently detected pathogens associated with diarrhea.[20]

Our study also showed the prevalence of infectious diarrhea present more frequently in cases consuming public tapwater of 57% [4/7] compared to house tapwater of 43%[3/7}. Similarly, other studies also showed the presence of diarrhea using public and house tap and well water in 89 and 67 diarrhea cases respectively.[21] According to Stanly M. et al study, diarrhea was present in 101 cases using public tap and well, whereas only 14 diarrheal cases used house tap and well water.[22] Also according to Shrivastav S. et al., those living in rural areas, are more likely to suffer from diarrhea using unimproved drinking water, sanitation facility, and low access to healthcare facilities in rural areas associated with high prevalence of diarrhea.[23]Thus identified risk factors that can predispose a child to diarrhea includes lack of potable water supply, poor hand hygiene in both child and caregiver as the transmission of infectious diarrhea is usually fecal-oral route.[24]

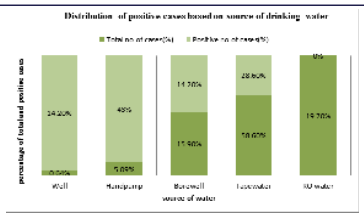
Conclusion:

Our current study found that in our district bacteria and parasites are an important cause of diarrhea in children under 5 years. A higher incidence was noted in the rural population usually lacking access to safe drinking water versus the urban population. Usual infections were parasitic diarrhea, which might not be life-threatening but impair the normal growth and development of these children. Further, these intestinal pathogens may increase susceptibility to infection with other pathogens. It is recommended that routine analysis of stool samples should include elaborate parasitological investigation, bacterial culture for enteropathogens along with continuous monitoring of antibiotic resistance in diarrhea-related bacterial pathogens. This will help limit the indiscriminate use of antimicrobials, aiding prevention of resistance to these. Maintenance of sanitation and hygiene and access to safe drinking water remains the key in checking the transmission of enteric pathogens.

Table 1: Detection of pathogens associated with diarrhea

Enteric pathogens	No.of cases	Percentage	Chi-square value	Degree of Freedom	P value
Escherichia coli	3	42.8%	22.3512	3	0.00005512 (< 0.005)
Salmonella spp	1	14.3%			
Giardia	2	28.6%			
H. nana	1	14.3%			
Total	7	100%			

Figure 1: Distribution of total and positive cases based on source of drinking water



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