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Original Article

Bacterial etiology among diarrheal cases

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ABSTRACT

Objectives: The main objective of this article was to check the bacterial etiology to find out the routes of infection, which might help with control strategies. As Nalbari is one of the most common flood-affected districts in Assam, India, we chose Nalbari district as our study area. The socioeconomic factor enhances the transmission of bacteria easily, as every year during floods, pictures of water from neighboring hilly states or countries (e.g., Bhutan)—rain, waterlogging, and more—are seen.

Material and Methods: Stool samples or rectal swabs were collected from the patients who had reported to the District Public Health Laboratory(D-PHL) with acute diarrheal disease in Nalbari. Patients or their attendant's consent was collected prior to collecting samples. Samples were processed for culture, gram stain microscopy, and biochemical tests to isolate the organism.

Results: Test results showed *Escherichia coli (E. coli)* 40.95%, *Salmonella* 2.86%, *Shigella* 1.42%, and *Campylobacter* 0.48%. No other bacteria were isolated during the study period. Maximum cases were reported during the rainy season.

Conclusion: *E. coli* can transmit through fecal-oral means; *Salmonella* through contaminated food, water, contact with infected people/animals, and so on; *Shigella* through fecal-oral, contaminated food, water, oral-anal sexual routes, and more; and *Campylobacter* through contaminated or raw food, milk, water, and so on. This study concludes that water can easily transmit bacteria to other people. Health hygiene, economic condition, living behavior, awareness, and administration's activities all might play an important role in minimizing this burden. Making plans prior to flood by administration and more research studies can help in controlling this disease burden.

Key words: Water, Flood, Culture, Bacteria, Hygiene, Awareness

INTRODUCTION

Diarrhea is a common high-risk disease in developing countries. Every year, worldwide, nearly 1.6 million death occurs only due to diarrheal diseases in developing countries.[1] Both the adult and child populations are equally at risk for diarrhea. Most of the diarrhea cases and diarrheal deaths (89.37%) are reported from South Asia and sub-Saharan Africa.[2] In India, 120,000 children die due to diarrheal diseases, which is a huge contribution to global—half million—diarrheal child deaths.[3] From 2011 to 2020, diarrhea outbreaks were reported from Tamil Nadu, Maharashtra, West Bengal, Punjab, Karnataka, and Madhya Pradesh in India.[4] In 2000 and 2001, a rural remote village in the Sivsagar district of Assam reported 1077 and 1719 diarrhea cases, respectively[5] (this is the data of only one village in a district of Assam, India). The data itself reveal how many cases report from a block that includes many villages, from a district that includes many blocks, from a zone that includes many districts, or finally from the state of Assam. From the Tinsukia district of Assam, India, a big diarrhea outbreak was reported where 11 people died within ten days in May 2024.[6] From time to time, these diarrhea outbreak cases are reported from various districts of Assam, India. Assam is a flood-affected state where people are demanding to declare this center as a national issue because it's affecting people's lifestyle, living behavior, and so on. During flood season, bacteria may transmit easily to the community, leading to diarrhea cases in the locality. There could also be some other factors that may need to be studied. Limited study in etiology, affecting factors, treatment patterns, and more, may hinder control of the disease.

The main aim of this study was to check the bacterial etiology and correlate it with the rainfall or flood.

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Study area

This study was conducted in the Nalbari district of Assam, India, after ethical approval. Nalbari is one of the common flood-affected districts, with Baralia River, Pagladiya River, and Brahmaputra River the three main rivers of this District. Water from Bhutan plays a major role during floods, which is only 67.6 km from Guwahati, the capital city of Assam.

MATERIAL AND METHODS

All acute diarrheal cases reported to the District Public Health Laboratory(DPHL), Nalbari, were included in the study. All age groups and both sexes were included in the study who reported to the DPHL with acute diarrheal disease or loose, watery stool. Stool samples or rectal swabs were collected as per the patient's clinical condition. Sample collection was preferred prior to antibiotics, but patients's samples who reported to the DPHL with antibiotics were also collected.

Stool sample collection: Instructions were given to the patient or attendees to prevent contamination of the sample with water, urine, or soil. To prevent contamination, sterile bags were used; and the patients, attendees, or collectors were instructed to wash their hands before collection. Sterile, clean disposable bags were placed in the toilet before passing the stool [Image 1a]. Clean, sterile stool collection containers [Image 1b] were given for sample collection. After passing the stool, it was collected in a container with proper gloves and mask to prevent contamination. Samples were inoculated in different culture media within one hour of collection.

Rectal swab collection: Rectal swabs were collected in Cary Blair (CB) transport media [Image 2] with the help of a sterile swab stick. Samples were processed within one to two hours after collection. Before collection, hands were washed carefully with soap. Instructions were given to the patients to sleep sideways and comfortably in bed. Sterile swab sticks were moistened with the CB transport media, and then they were inserted into the rectum of the patients, about 1–2 inches

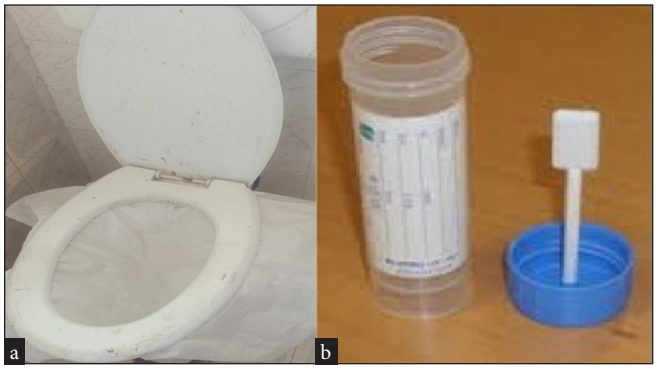

Image 1: (a) Sample collection bag on bowl. (b) Sample collection container.

Image 2: Cary Blair transport media without rectal swab and with rectal swab.

(3–5 cm), and gently rotated clockwise for five to ten seconds. Swabs were withdrawn without touching the skin and placed in CB transport media. The outer wooden parts of swab sticks were broken and recapped into the CB media after collection [Image 2]. The collected rectal swab samples were placed at 2–8°C till inoculation.

Culture media were prepared in the DPHL and stored in the refrigerator. Before inoculation of samples in the culture plates, plates were allowed to dry in an incubator **at 37°C.** XLD (Xylose-Lysine-Deoxycholate) Agar, BA (Blood Agar), MAC (Macconkey) Agar, TCBS (Thiosulphate-Citrate-Bile-Salt Sucrose) Agar, SS (Salmonella-Shigella) Agar, and MH (Mueller-Hinton) Agar were used for inoculation and isolation of bacteria. Isolated bacteria were confirmed with Gram Stain and microscopy with different biochemical tests. The common biochemical tests used for confirmation are the Indole test, Oxidase test, Methyl Red test, Voges-proskauer test, Citrate Utilization test, Urease test, Triple Sugar Ion (TSI) test, H_2S test, Coagulase test, Catalase test, Oxidase test, Methyl Red test, Voges-proskauer test, Citrate Utilization test, Urease test, Triple Sugar Ion (TSI) test, H_2S test, Coagulase test, Catalase test, and more.

After clarification of bacteria, whether gram positive or gram negative, morphology (Coccoi, Bacilli, or other) cluster or single and more by gram stain and microscopy, further biochemical tests were chosen. The comparison of biochemical tests results, growth characteristics, and gram stain microscopy altogether helps to identify bacteria. Indole test shows positive by *E. coli, Proteus, Flavobacterium sp., Hemophilus influenza*, and more. Oxidase test shows positive by *Vibrio cholerae, Campylobacter jejuni, Helicobacter pylori, Pseudomonas aeruginosa* etc. Methyl Red test shows positive by *E. coli*, *Proteus vulgaris*, *Salmonella*, *Shigella*, and so on. Voges-proskauer test shows positive by *Enterobacter, Klebsiella, Serratia, Vibrio cholerae*, and so forth. Citrate

Utilization test shows positive by *Proteus mirabilis, Klebsiella, Salmonella, Citrobacter*, and more. Urease test shows positive by *Proteus, Klebsiella, Helicobacter pylori, Staphylococcous*, and so on. Triple Sugar Ion test shows positive by *E. coli, Salmonella, Shigella*, and so on, but with different color pigmentation. H_2S test shows positive by *Campylobacter, Citrobacter, E. coli, Salmonella*, and so on Coagulase test shows positive by *Staphylococcus*. Catalase test shows positive by *Staphylococcus* and *Micrococcous*.

RESULTS

A total of 210 patients were enrolled in the study, from January 2022 to July 2024. It was observed after analysis of the data that maximum diarrhea positive culture cases were isolated with *E. Coli* 40.95% (86/210), followed by *Salmonella* 2.86% (6/210), *Shigella* 1.42% (3/210), and *Campylobacter* 0.48% (1/210). No other bacteria like *Vibrio cholera, Yersinia species, Aeromonas species, Klebsiella or Enterobacter species, Pseudomonas, Proteus, Citrobactor and* so on were isolated during the study period. The highest numbers of bacteria were isolated in 2024 which is 40.62% (39/96), followed by 2023 which is 35.42% (34/96), and 2022 which is 23.96% (23/96). Year wise graphical analyses are shown in Figure 1. If we analyzed the data month wise, the highest positive cases were reported in July, followed by June [Figure 2]. Nalbari district's highest rainfall season is the monsoon season, which is June to October.[7] If we compare the data with rainfall, the maximum cases reported were from May to September. If we analyze the data sex and year wise, it was observed that both sex groups were equally affected in those three years [Figure 3]. Blockwise analysis showed that maximum cases were reporting from Ghograpar Block, followed by Chamata Block [Figure 4]. Ghograpar Block is the neighboring block of Baksa district of Assam and Bhutan.

DISCUSSION

In a study by Alzaher *et al*., (2022), the maximum stool-isolated organism was *salmonella* followed by *Shigella*, but according to the study, the maximum isolated organism was *E. Coli* followed by *salmonella, Shigella*, and so on.[8] They had isolated some other organisms too not found by us during our study, like *Vibrio cholera, proteus* and more. Okada *et al*. (2020) reported that in Thailand, *E. Coli* was the most common isolated bacteria from stool samples of diarrhea patients, which was similar to our studies.[9] Another study by Ballal M *et al*. (2014) also found *E. Coli* as the most common isolated bacteria from stool samples in India.[10] They have isolated 46.3% *E. coli* from positive stool culture, followed

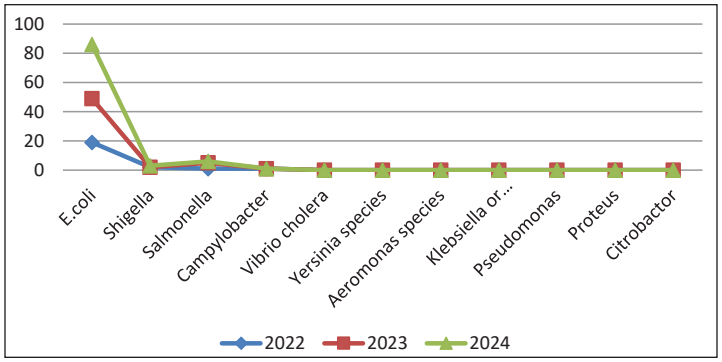

Figure 1: Year wise bacterial etiology.

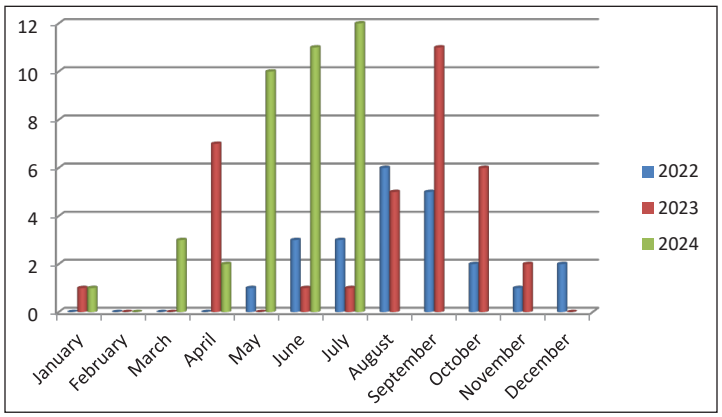

Figure 2: Month wise analysis of the positive cases.

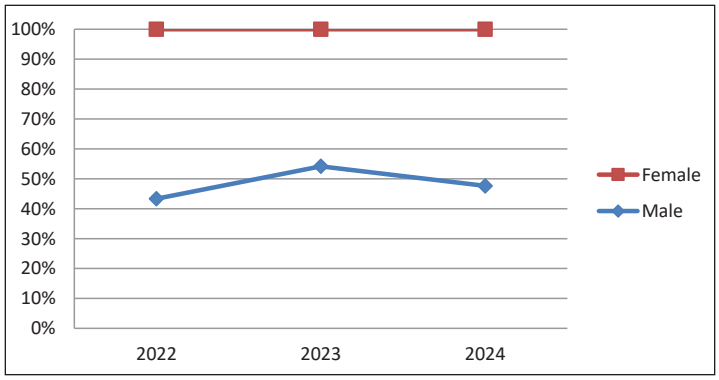

Figure 3: Acute Diarrheal Disease cases sex and year wise.

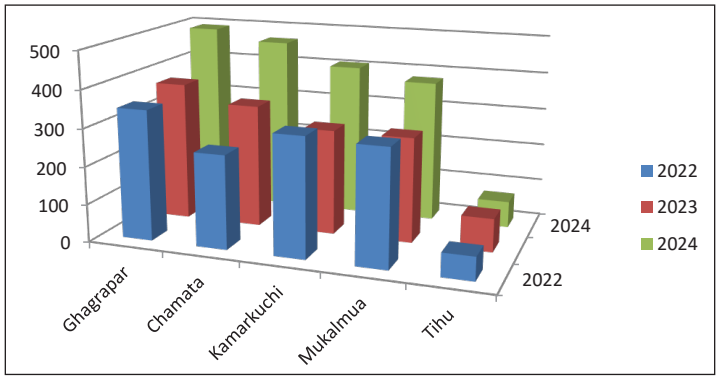

Figure 4: Block wise affected cases.

by *salmonella* and *Shigella*. In our study, it was 40.95% for *E. Coli*, followed by *Salmonella* and *Shigella*. Rathur *et al*. (2014) studied childhood diarrhea and found that *E. Coli* was 44.2%, *Shigella* 28.2%, *Salmonella* 13.6%, *Klebsiella* 7.8%, and *Campylobacter* 6.1%.[11] In our study, *Klebsiella* was not isolated, and the isolate highest after *E. Coli* was *salmonella*.

Moharana *et al*. (2019) studied childhood diarrhea and found maximum cases in the month of September; in our study, it was July. The study by Moharana *et al*. was held in Eastern Odisha, India.[12] The rainfall rate of Odisha in the month of September is 242 mm, as per different studies.[13] Pokharel *et al*. (2009) isolated the highest bacteria from stool specimens of diarrhea cases in the month of July, followed by June.[14] The study was done in Kathmandu, Nepal, which is similar to our studies. But this study included child groups below 12 years only, whereas we have included all age groups in our study. In a study from 2003 to 2013 by Sharma *et al*., Assam also found maximum diarrhea cases during the rainy season as per their month wise table.[15] The study was about *Vibrio cholerae* serotype isolation. This included Dhubri, Barpeta, Nalbari, Kamrup-Metro, Kamrup-Rural, Sonitpur, Nagaon, Golaghat, DimaHasao, Chachar, and Karbialong districts of Assam. Though the cases increased in the rainy season in our study too, no *Vibrio cholerae* were isolated in our study.

Different studies have already proved that water is one of the main causes of diarrhea or other similar diseases, though other factors are also equally responsible, like hygiene, awareness, living behavior, immunity, nutrition, age factors, and so on.[16,17] Our study analyzed the diarrhea causing bacterial etiology and relation with flood, rain, or water. But diarrhea due to parasites, viruses, and more is also equally important.[18,19]

CONCLUSION

Diarrhoea is a common burden for every district of Assam. Bacteria, viruses, parasites all may be the causing agents for this disease. Much more research is required to know all details, including control and treatment strategy. Health departments must get help from other departments to control the burden, like meteorology, irrigation, agriculture, Flood Control Board, media houses, district administration, food and supply, public health, and engineering . Public awareness might play a very important role to minimize the disease risk and transmission.

Authors' contributions

Each author has equal contribution.

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Ethical approval

The research/study is approved by the Institutional Ethics Committee at District Health Society, IDSP; Nalbari, number: IDSP/NLB/DPHL/SW/2022/13, dated 22nd December 2022.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent.

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Nil.

Conflicts of interest

There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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