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Bacteriological Study of Drinking Water from Different Sources and Associated Flood Diseases

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Abstract

Background: The drinking water problem is a globally WHO-accepted concern problem. As per WHO data 80% of the diseases are due to sanitation issues, unsafe drinking water issues, or non-availability of drinking water worldwide. This information from WHO itself indicates the importance of this study. **Purpose:** The main aim of the study is to find out the bacterial health impact of flooding. The study results help to know the association between floods, water contamination, diseases, etc. **Method:** Water samples were collected by aseptic techniques from different flood-affected areas of Nalbari District of Assam, India. The study samples were collected from January 2022 to December 2024. Collected samples were processed for bacteriological tests. **Results:** Test results showed maximum positive cases during flood season. This proves that flood water contaminates the drinking sources. Different disease-causing bacteria were isolated from the sources. Out of all positive samples, the most isolated bacteria were Escherichia coli (E. coli). The distribution is Escherichia coli (50%), Salmonella (3.12%), Campylobacter (3.12%), Vibrio cholerae (0%), Streptococcus (18.75%), Staphylococcus (18.75%), Klebsiella (3.12%), and Pseudomonas (3.12%). Nalbari district of India disease patterns showed that diarrheal diseases, typhoid, UTI, etc. were commonly found in Nalbari. The isolated organism and disease pattern were matched. These indicate that contaminated water was the cause. **Conclusion**: Bacteria can easily transmit with water. To minimize the water-borne diseases living behavior, hygiene maintenance, safe drinking, etc. are the most important points. Special care is required during flood season. Community health awareness on safe drinking water, communicable diseases, etc. will play an important role in preventing these diseases or reducing the number of cases.

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KEYWORDS: Diarrhea, Flood, Water, Bacteria, Contamination

1. INTRODUCTION

Nalbari district is one of the most common flood-affected districts in Assam. Every year, lots of lives are lost during the flood season. From 1998 to 2005, about 22.54 hectares of land came under flood in Assam, which is 28.75% of the total area [1]. In this situation, humans, animals, birds, and all other lives come together in one shelter. This enhances disease transmission. Drinking water crises lead to contamination, which may be the source of different diseases. In the year 2022, a total seven numbers of revenue circles were affected by a flood in Nalbari district of Assam, India. This covered 425 villages with 499090 numbers of affected populations [2].

Nalbari District of Assam is surrounded by Kamrup (rural) district, Bajali district, Barpeta district, Baksa district, and the Brahmaputra River (Figure: 1). Waterborne communicable diseases like typhoid, diarrhea, dysentery, and hepatitis A and E are all common in Assam. Nalbari and its surrounding districts are already predisposed to different communicable diseases [3, 4, 5, & 6]. In 2013-14, Nalbari district reported 31.4% communicable diseases out of all reported hospital cases as per the Health Management Information System report (HMIS) [7]. Where 25.7% are noncommunicable diseases, 4.3% are injuries, and others are 38.7%, this data itself indicates the importance of communicable diseases. The communicable diseases are diarrhea, typhoid, tuberculosis, vector-borne diseases, viral hepatitis, etc. As these diseases are directly or indirectly related to water contamination, we have chosen the subject water to study in this specific district, Nalbari, of Assam, India. There are various data and studies in Assam already providing water chemical contamination information [14]. These data also raise the question of bacterial contamination, protozoan contamination, viral contamination, etc. If a chemical could contaminate the water, microorganisms could also do the same as per the prevalence.

Bacteriological drinking water contamination is a major problem worldwide. As per WHO concern, 1.1 billion people are drinking contaminated or unsafe water, and 88% of diarrheal cases are due to the cause of drinking water (WHO 2003) [16]. The World Health Organization also says that out of all sicknesses or diseases, 80% of the sicknesses or diseases are caused by contaminated water, unavailability of water, or inadequate sanitation [17]. These are the data of WHO itself, saying how important the study or research of drinking water is. That is why we have chosen this topic to study the flood-affected district of Nalbari, India.

2. OBJECTIVES

To find the association between floods, diseases, and water contamination.

3. MATERIALS AND METHODS

The study tries to cover all the areas of Nalbari district. The study was conducted from January 2022 to December 2024 at the District Public Health Laboratory of Nalbari district. All drinking water sources were included in the study. Water samples were collected by Multi-Purpose Workers (MPWs) or Laboratory Technicians in the presence of a Microbiologist to avoid contamination in a sterile water collecting container from different areas of Nalbari district, namely Tihu, Makhibaha, Haribhanga, Sialmari, Jalkhana, Gobradol, Niz-Namati, Chandakuchi, Jagra, Dharmapur, Boritopa, Chamata, Bornaddi, Kakaya, Piplibari, Bolowa, Bhadra, Solmara, Belsor, Bahjani, Barbila, Kalag, Nankarbhaira, Burinagar, Banekuchi, Marowa, Kamarkuchi, Karia, Nilpur, Balitara, Dhamdhama, Khatikuchi, Barkhala, Ghagrapar, Alliya, Silpotabari, Sanekuchi, Adabari, Mugdi, Lowtola, Larkuchi, Mukalmua, and Bortola (Figure 1).

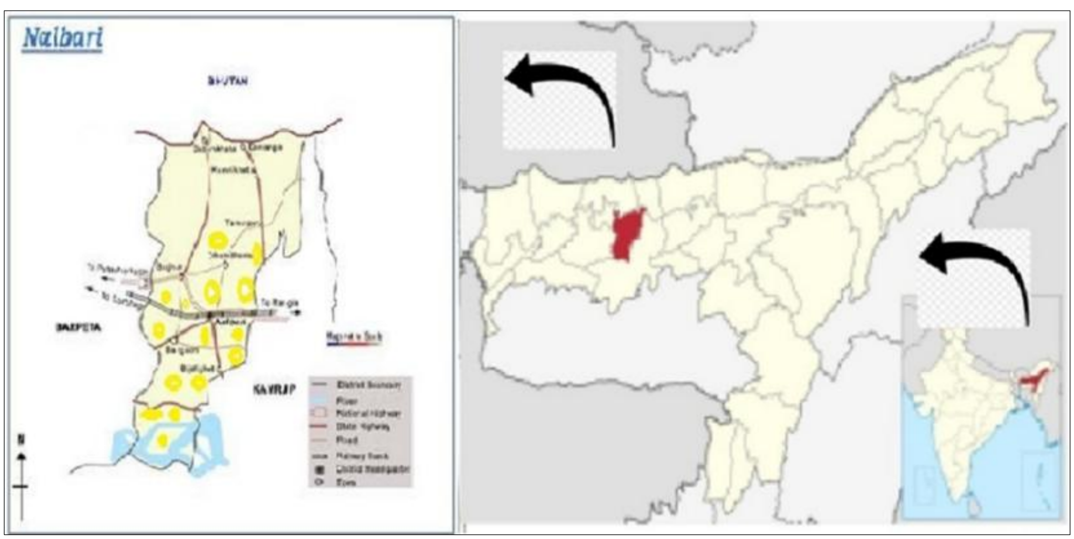

Figure 1: Nalbari district of Assam India with water collection area with yellow marks

The samples were collected during flood and pre-post-flood season. Water sources are mainly tube wells, wells, and running tape as per the source availability during the time of collection.

Based on source collection procedures were different to avoid the risk of sample contamination.

Water from the tube well

It was observed that all tube wells are mainly made up of iron (Fe). That is why the mouth of the tube well, from where water comes out of the tube well, was flamed for 20 minutes; after that, water was allowed to run out for 20-30 minutes. After 30 minutes of passing water, the samples were collected in a sterile container.

Water from running tape

Flamming was not done in running tape as the maximum running tape mouths are made of plastic materials. 30-40 minutes of water was allowed to come run by opening the tape after that samples were collected in a sterile container.

Water from well

The well-collecting pot/bucket was allowed to go down to the maximum level for collection and wait till the water wave stopped. After that, the bucket is filled with water, and the sample is transferred to the sample collection container.

The collected samples were processed with an H_2S strip test, an MPN (most probable number) test, and inoculation in different Chromogenic agar and selective agar media. Isolated organisms were finally confirmed with Gram stain microscopy, followed by different biochemical tests, namely the Triple Sugar Ion (TSI) test, Methyl Red (MR) test, Catalase test, Oxidase test, nitrate reduction test, Indole test, Citrate test, Voges-Proskauer (VP) test, urease test, H_2S production test, Coagulase test, etc., as per growth characteristics and gram stain microscopy nature.

The results were analyzed and compared with other bacteriological diseases which were tested viz- Widal, Blood culture, Typhidot, Stool culture, Urine culture, Viral hepatitis A & E at DPHL, Nalbari during the study period as well as with flood season.

Collection of human biological samples: Based on the clinical condition of patients and as per treating physicians' advice, human biological samples were collected and tested at DPHL, Nalbari. Patients or attendees were informed about the study.

Blood Sample

Venepuncture was done to collect blood samples. Gloves were worn before starting the procedure. The selected site was cleaned with a spirit swab, as well a tourniquet was used to choose the site. 5 ml blood samples were collected from each patient for widal, typhidot, viral hepatitis A, and viral hepatitis E. Serum was obtained after centrifugation from the collected samples to perform the tests. For blood culture, 10 ml of blood samples were collected at BHI broth (brain heart infusion broth) for adults and 5 ml for children at BHI broth.

Stool Sample

To collect stool samples, patients or attendants were advised to take care during collection to avoid contamination of samples with urine, water, and soil. Clean, sterile containers were used to collect stool samples.

Urine sample

Midstream morning urine samples were proffered to perform the tests. Patients were advised to clean their genitals with soap and water before voiding the samples in the sterile container.

Test Procedure

Following probable waterborne diseases tests were included in our study as per test protocol.

Typhoid

To diagnose typhoid fever, the Typhidot kit, Widal kit, and blood culture were included. For Typhidot and Widal, Recombinant Laboratories Pvt. Ltd. manufactured kits were utilized, and for blood culture, Himedia Brain Heart Infusion Broth was used. The blood culture bottles were prepared in the laboratory itself. The sensitivity and specificity of Typhidot were 96% & 99.5%, respectively, for IgM and 98% & 99.5%, for IgG. Whereas for Widal kits, only those above a 1:80 titer was considered positive as per the kit literature, i.e., 1:80 was negative. Blood culture bottles (BHI) were incubated for seven (7) days to declare as negative. Positive blood culture bottles were subcultured on XLD (*Xylose Lysine Deoxycholate)* agar media and SS (Salmonella-Shigella) agar media, followed by gram staining and biochemical tests and sensitivity.

Diarrhea/Dysentery

Stool samples were inoculated at SS agar, TCBS agar, blood agar, and XLD agar and incubated at 37°C. If no growth was recorded till 48 hours it was considered as negative. Isolated organisms were confirmed by different biochemical tests, viz., indole, oxidase, MR, VP, H_2S, TSI, catalase, coagulase, etc., after Gram stain microscopy.

Urinary tract infection (UTI)

Urine samples were inoculated on UTI agar & CLED agar at 37°C for 48 hours if no growth was observed within 24 hours. Isolated organisms were confirmed after Gram stain microscopy followed by different biochemical tests, viz., indole, oxidase, MR, VP, H_2S, TSI, catalase, coagulase, etc.

Viral hepatitis A & E

IgM antibody detection ELISA tests were performed to diagnose viral hepatitis A & E. Qingdao High-top Biotech Co Ltd Hepatitis an ELISA and hepatitis E ELISA kits were used. The sensitivity of the kits was 95% and specificity was 94.7%.

4. RESULTS

The test results showed that 9.83% (41/417) of water samples were found positive for different bacteria. This one also observed that the year-wise positivity rate was decreasing, i.e., the highest number of positive cases was in the year 2022, and lowest positive cases were found in the year 2024. In the year 2023, positive cases are lower in comparison with the year 2022 (Fig. 2). If we analyzed the data month-wise, the highest positive case was in July, which is the flood peak time or post-flood time of Assam every year (Fig. 2).

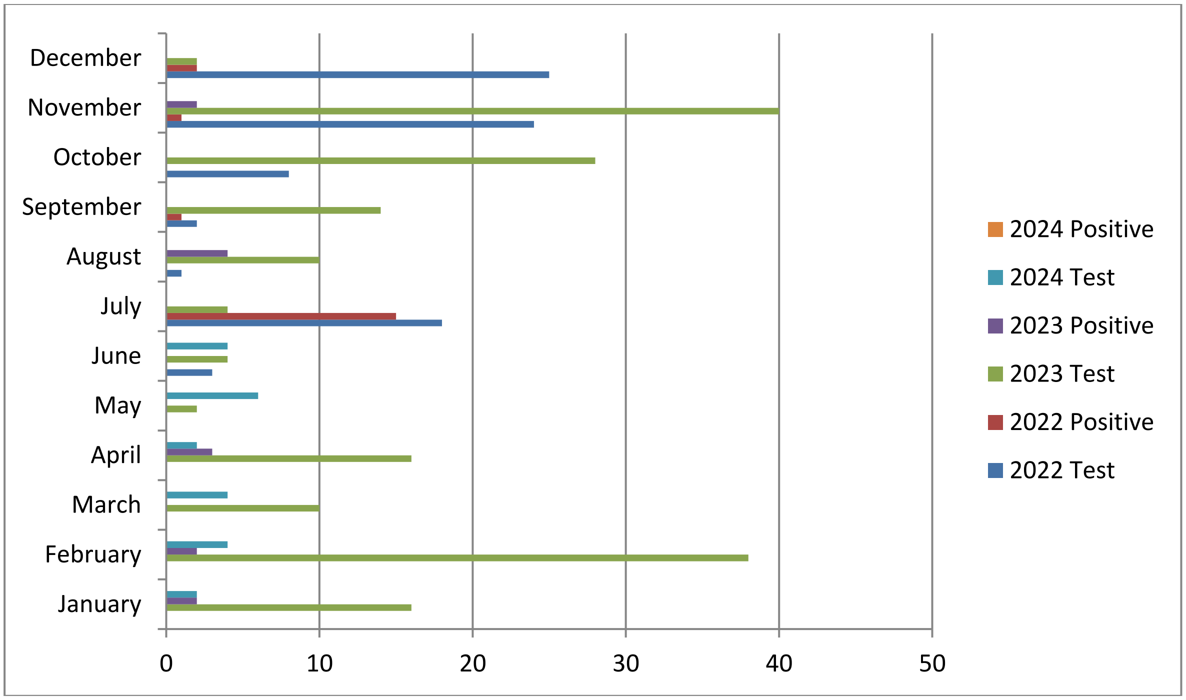

Figure 2: Month-wise water positivity rate

We could not collect the water sample during starting i.e. pre-flood season of 2022. The bacteriological etiology of positive samples showed maximum cases were positive for the bacteria E. coli, followed by Streptococcus, Staphylococcus, Klebsiella, Pseudomonas, Campylobacter, and Salmonella. No sample was found positive for vibrio cholera (Fig 3).

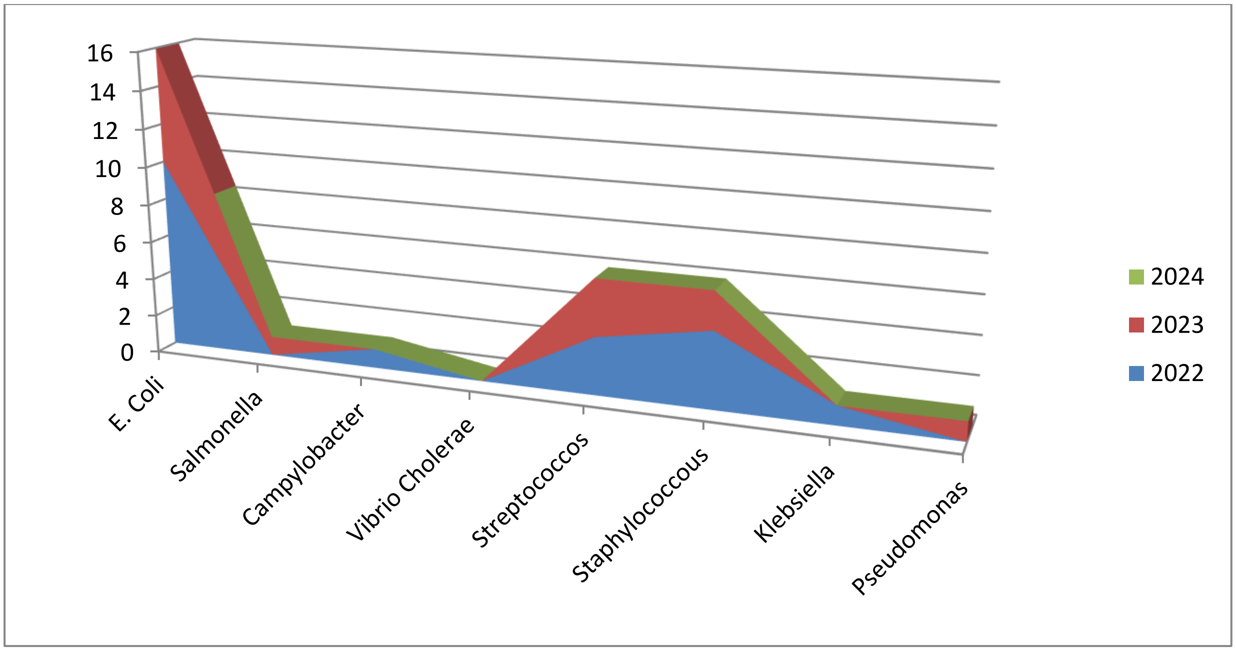

Figure 3: Year-wise water bacterial etiology

The biological samples showed a positivity rate year wise as in 2022 widal 8.92% (55/616), Typhidot 0.39% (2/513), Blood CS 11.12% (3/27), HAV 8.82% (3/34), HEV 0% (0/34), Stool CS 16.13% (5/31) and UTI 39.73% (116/292). Whereas in 2023 positivity rate of widal was 10.71% (39/364), Typhidot 6.20% (43/693), Blood CS 3.70% (1/27), HAV 1.35% (1/74), HEV 0% (0/74), Stool CS 70.83% (34/48) and UTI 15.69% (94/599). Again in 2024, widal positivity rate was 12.59% (57/643), Typhidot 2.16% (46/2120), Blood CS 3.44% (1/29), HAV 3.26% (3/92), HEV 0% (0/92), Stool CS 50.31% (80/159), UTI 11.0% (217/1971). If we compare biological sample data with flood, cases are higher during flood (May-June-July-August) and post-flood (September-October-November-December) in comparison with pre-flood timing (January-February-March-April) except in one or two cases (Table 1). In the same way the bacterial-contaminated water samples are higher during flood and post-flood.

Table 1: Year-wise biological samples test with results

Year-wise Number of human biological samples tested																		
	Year 2022						**Year 2023**						**Year 2024**					
	Pre-Flood Period January to April)		**Flood Period (May to August)**		**Post Flood Period (September to December)**		**Pre-Flood Period January to April)**		**Flood Period (May to August)**		**Post Flood Period (September to December)**		**Pre-Flood Period January to April)**		**Flood Period (May to August)**		**Post Flood Period (September to December)**	
	Test n %	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**
Widal	49	9	252	32	315	14	127	11	129	17	108	11	142	12	272	27	229	18
Typhidot	53	0	292	1	168	1	62	12	225	14	406	17	639	10	765	19	716	17
Blood CS	2	0	5	0	20	3	13	0	6	0	8	1	5	0	21	1	3	0
HAV	7	0	8	0	19	3	35	0	16	1	23	0	32	1	35	2	25	0
HEV	7	0	8	0	19	0	35	0	16	0	23	0	32	0	35	0	25	0
Stool CS	4	0	14	2	13	3	10	8	8	7	30	19	32	6	72	45	55	29
UTI	14	2	120	50	158	64	94	27	79	17	426	50	815	78	690	76	466	63

Table 2: Year-wise water tests bacterial isolation results

Year-wise Number of isolated bacteria from water samples																		
	Year 2022						**Year 2023**						**Year 2024**					
	Pre Flood Period January to April)		**Flood Period (May to August)**		**Post Flood Period (September to December)**		**Pre Flood Period January to April)**		**Flood Period (May to August)**		**Post Flood Period (September to December)**		**Pre Flood Period January to April)**		**Flood Period (May to August)**		**Post Flood Period (September to December)**	
	Test n %	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**	**Test n %**	**Positive n %**
E coil	0	0	81	5	59	5	80	1	20	4	84	1	12	0	26	6	114	1
Salmonella	0	0		0		0		0		1		0		0		1		0
Campylobacter	0	0		1		0		0		0		0		0		0		0
Viberio cholerae	0	0		0		0		0		0		0		0		0		0
Streptococus	0	0		2		1		0		2		1		0		0		0
Staphylococus	0	0		3		1		0		2		0		0		0		1
Klebsiella	0	0		1		1		0		0		0		0		0		0
Pseudomonas	0	0		0		0		0		2		0		0		0		0

5. DISCUSSION

SKShahina *et al.* reported 11 (eleven) different species of bacteria in 20 (twenty) water samples, namely Staphylococcus aureus, CoNS, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Enterobacteraerogenes, Pseudomonas aeruginosa, Vibrio cholera, Salmonella typhi, Aeromonashydrophila, and Citrobacterfreundii. Our study reports 7 (seven) numbers of different bacteria, which are higher samples. Their study samples were groundwater in Chennai, and our study samples were from drinking sources either during or post-flood in Assam [8]. Choudhury M *et al.* 2021 found E. coli as the most common isolated bacteria in the Goalpara district of Assam, which is found in our study too [9]. In our study, it was observed that 50%, i.e., half of the isolated organisms, were E. coli out of all isolated bacteria. In a study of microbial pollution by S. Some, et al. in 2021, Escherichia spp., Klebsiella spp., Enterobacter spp., Shigella spp., Serratia spp., Proteus, Salmonella spp., Arizona spp., Citrobacter spp., and Pseudomonas spp. are microbial water pollutant organisms [10]. In our study, we did not find Shigella, Proteus, Serratia, Citrobacter, etc. from the positive samples or pollutant water. A study was conducted at Guwahati of Kamrup-Metro district, which is a neighboring district of Nalbari. The people of Nalbari district have very close contact with Guwahati as Guwahati is the main city of the state of Assam. In this study at Guwahati by Barman RS et al. in 2014, they counted maximum coliform bacteria in the monsoon season [11]. Another study by Yadav N et al. in 2019 at Delhi, the metropolis of the country, also found maximum bacteria in the monsoon season [12]. This is similar to the Guwahati study. In our study too, we isolated maximum bacteria in July. From Hojai College, a study was conducted at KarbiAnglong district of Assam. This is a hilly district of Assam, where Nalbari district is a completely plain area district, but Goalpara and Kamrup-Metro, both districts of Assam, are occupied by hilly as well as plain areas. The study of Karbi Anglong district was conducted by Sarkar S *et al.* in 2012, and as per their report, contaminated drinking water bacterial etiologies are Escherichia coli (85%), Citrobacterfreundir (3.5%), Enterobacter cloacae (17%), Klebsiellaoxyloea (0.8%), Citrobacterdiversus (0.8%), and 5.2% unidentified [13]. In our case it was not exactly similar. All isolated bacteria were identified by our study. No unidentified positive sample was kept by us during the study. Ahmed MR *et al.* (2015) studied in Gwalior city of Madhya Pradesh, India, and correlated the water results with human diseases [15]. They discussed the increasing disease risks like diarrhea, cholera, gastrointestinal diseases, and other disease risks. We have also analyzed our data with the Nalbari district's disease report for 2013-14 [7]. It was also observed that diseases like diarrhea, typhoid, etc. also increased in July or during flood season as per the data of Nalbari district's disease report. Joydev Dutta *et al.* (2010) studied in the plain district of Assam, India, which is Golaghat. They found in their study that Coli forms bacteria from drinking water isolated maximum from that area where maximum diarrheal cases were recorded [18]. Shokri A. *et al.* (2020) and Espana JDA *et al.* (2024) described in their article different diseases due to flood in flood-affected areas [19, 20]. Our study also reveals the same after dividing the samples into the flood season (January-April), flood season (May-September), and post-flood season (October-December) (Table 1). Typhoid cases are higher during a flood in all three years i.e. 2022, 2023, and 2024. In the case of viral hepatitis, A, which is a fecal-contaminated disease in 2022 no positive cases were reported but in 2023 and 2024 cases were reported during or after the flood. Viral hepatitis E was not found as prevalent during our study. In the case of diarrhoea, the maximum cases were reported either during flood or post-flood. Urinary tract infection (UTI) also shows the same trends i.e. higher either during flood or post-flood. Yard EE et al. (2014) studied during and after flood-reported contamination of water [21]. Again, another study by Dzodzomenyo M *et al.* (2022) recorded contamination of drinking water due to flood and its impact on health [22]. In our study too both cases, diseases, and water contamination increase during flood and post-flood. These disease data, water contamination data also indicate the association of flood with diseases. Serotyping and molecular level study of isolated organisms will help to specify the disease burden during the season of every year. This limitation of the study is stated for further studies. The community in question received the test results. Health awareness regarding bacteriological water contamination, probable diseases, preventive measures, etc. given. The authors accept that this is not the only solution because every year floods, water contamination, and sufferers are seen in this district.

6. CONCLUSION

To prevent waterborne diseases, saving drink and hygiene must be a priority. Every person must be aware of it. The government may share awareness messages on every individual's mobile phone. Especially during the flood and post-flood seasons. Home department, Flood and River Erosion Management Agency of Assam, public health engineering department, health department, media house, irrigation department, nongovernment organization, and community leaders all must come together to solve and reduce the cases. People in the community must be aware of all communicable diseases and safe drinking water to prevent the diseases.

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