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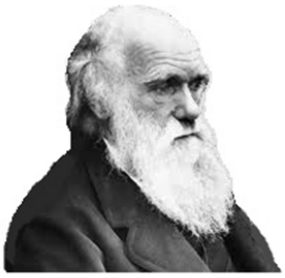

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Assessment of Bacterial Contamination of Source of Drinking Water During Flooding in Yenagoa Metropolis, Bayelsa State, Nigeria

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ABSTRACT

Flooding is a recurrent event in Nigeria and its impact is usually devastating in Bayelsa due to the low topography. One effect of the recurrent flooding events is bacterial contamination of drinking water. Therefore, the bacterial contamination of sources of drinking water during flooding was investigated in the Yenagoa metropolis. Triplicate tap and flood water samples were collected at three different locations in the communities in Yenagoa metropolis and their bacterial content including bacterial load and culturable bacterial species were assessed. The results revealed the presence of feacal coliform, enterococci and total coliform, which were indications of possible bacterial contamination of tap water. Colony Forming Units of bacteria in samples of tap water collected after flooding (CFU-AF) reduced significantly when compared to those collected during flooding (CFU-DF). The diversity of bacterial species in the tap water also reduced from three to one during and after flooding, respectively.

***Keywords*:** Bacterial load, flooding, bacterial contamination

Abbreviation: CFU-AF: colony forming units in tap water after flooding. CFU-DF: colony forming units in tap water during flooding, CFU-FLW: colony forming units in flood waters

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1. INTRODUCTION

Water is of fundamental importance to life on earth. The synthesis of metabolites, constituents of cell structure and transport of nutrients into cells, as well as other metabolic activities of organisms depend on water. Contaminants present in water can disturb the spontaneity of its usage, resulting in long or short-term diseases (Zhang *et al*., 2011). Adequate supply of clean and safe drinking water is a very demanding task, as water is essential in major sectors of the economy: agriculture, health, industry, etc. Contaminants make the availability of safe drinking water difficult, as they add materials to water sources that make them harmful or hazardous to life. The global rise in climate and its consequences, which include flooding, hurricane, tsunami etc., hinders ecological provision of safe drinking water. This is because natural disasters dislodge harmful substances from their natural reservoir into sources of drinking water thereby resulting in water pollution (Levy *et al*., 2016).

Water contaminants can be of chemical or biological form. The chemical pollutants in water include: organic pollutants, endocrine disrupting compounds and mutagenic disinfectants. While microbial contaminants in drinking water are the emerging waterborne pathogens consisting of protozoa, viruses and bacteria, such as *Escherichia coli* O157:H7, *Cryptosporidium parvum*, *Giardia lamblia (*Phanuwan *et al*., 2006). Microbial contamination of drinking water can cause both acute and chronic diseases to human health. Pathogenic microbes cause intestinal track disorders like vomiting, diarrhea and other infectious diseases such as cholera. All forms of water borne diseases require anti-microbials for treatment and this cost a lot of money. Leakage of microbes from untreated water, sewage and poor handling of refuse also increases the epidemiological risk of drinking water (Wardrop *et al*., 2018). When bacteria come in contact with other strains of bacteria that have developed some form of resistance as a result of exposure to antibiotics, they in turn pick-up these resistant gene thereby becoming drug resistant themselves. This has led to antibiotic failure, giving credence to the adage; “prevention is better than cure”. It is therefore pertinent to elucidate the effects of floods on sources of drinking water and proffer solution to it, as the floods in 2022 resulted in the death of over 10million poultry birds and several human lives by water borne disease in Yenegoa Metropolis alone. Interestingly, biological contaminants do not usually change the physicochemical characteristics of drinking water they contaminate (WHO/UNICEF., 2019). For example, the colour and odour may remain unchanged if drinking water contains a waterborne pathogen. As a result, microbial contamination is very dangerous, since in most cases, it can only be confirmed when the severe consequence such as death occur. It is therefore, not only the responsibility of Government organizations and other regulatory authorities to investigate microbial contamination of sources of drinking water during and after floods, but also every individual to learn about microbial water pollution and take necessary steps for its reduction (WHO, 2021).

2. MATERIALS AND METHODS

Study area

The geographical location of Yenagoa is in the north and east hemisphere. Yenagoa lies between the coordinates of latitude 04°15’ North and latitude 05°23’ South and longitude 05°22’ West and 06°45’ East.

Sample collection

The water samples were collected from three sites of each community in Yenegoa metropolis: Agudama, Akenfa, Okaki, Igbogene, Akenpai, Edepie, Tombia, Okutukutu, Etegwe, Biogbolo, Yenezuegene, Kpansia, Okaka, Amarata, Swali, and Obele. Three tap and flood water samples were for each site following the WHO recommended procedures (WHO, 1984). Briefly, the water tap was first wiped, using a clean cloth. Then the tap was turned on at maximum flow rate and allowed to flow for 2 minutes. The interior of the tap was sterilized using alcohol and a gas burner. Then 500-ml of water sample was aseptically collected in sterile plastic bottles. Sampling from the surface run-off flood water was done by immersing the sterile bottle into the pool of water to a depth of about 10 cm with the mouth facing slightly upwards and two litres of water samples were collected and taken to the Microbiology Laboratory of the University of Africa, Toru-orua for bacteriological examinations.

Sample culturing

Serial dilution was carried out for each water sample by adding 1 ml of the water sample to 9 ml of distilled water in a test tube. Using a different sterile pipette, 1ml from the first test tube was pipetted into the second test tube already containing 9 ml of diluted normal saline, this continued following the same procedure till the last dilution. Using the spread plate method for all prepared media, 0.1ml of each sample unit from the test tubes was pipetted into the sterile Petri dishes containing sterilized and solidified growth media (Nutrient Agar, MacConkey Agar, Plate count Agar, Thiosulfate-Citrate-Bile-Salts-Sucrose Agar, and Salmonella Shigella Agar). The plates were incubated at 37 °C for 24 hours and subculturing was done from plates where bacterial growth was observed.

Bacterial isolate identification

Identification of isolates were based on culture media, morphological and biochemical characteristics following standard methods of gram staining and tests for presence of oxidase, catalase, coagulase, indole, citrate, as well as tests for sugar fermentation. The gram staining was done by adding a smear of bacteria onto a slide, which was air dried and the smear heat-fixed on it by flaming. Five drops of Hucker's Crystal Violet were added to the smeared and heat-fixed samples on the slides and allowed undisturbed for one minute during which purple staining occurred. The excess stain was washed and shaken off and five drops of iodine solution was added. Thereafter, decolorization with acetone-alcohol solution was done and it was washed immediately (within 5 seconds) with water and excess shaken off. Five drops of Safranine O stain were added and the slide was examined under the microscope at both 400x and 1,000x oil immersion.

For oxidase test, 0.2 ml of 1% α-naphthol and 0.3 ml of 1% p-amino dimethylaniline oxalate (Gaby and Hadley reagents) were used. The culture containing the α-naphthol and oxalate was vigorously shaken for proper oxygenation and a part of the bacterial colony was picked by dipping cotton bud to check for colour changes on the area with the picked organisms. An observation of deep blue colour was recorded as positive for presence of oxidase and observation of no change in colour was recorded as negative for presence of oxidase.

The catalase test was conducted with hydrogen peroxide in which 2 ml of it was poured into a test tube and several bacterial colonies were collected from the subcultured plates that were incubated for 18 to 24 hours and immersed in the hydrogen peroxide solution.

The culture was observed for bubbling and the presence of bubbles within 3-15 seconds was taken as positive for presence of catalase and negative was taken as negative for absence of catalase.

A suspension of the test organism was made on a clear glass slide and a drop of fresh human plasma was added and mixed, and then it was observed for clumping, which indicates a positive result.

The indole test was done with peptone water solution by growing pure bacterial culture in sterile tryptone broth overnight. Thereafter, 1.0ml of chloroform was added to the broth and shaken gently. Five drops of P-Dimenthyl-aminbenzaldehyde [KPVAC'S reagent], was added to the broth culture and the occurrence of red color on the surface layer of the broth indicated positive while yellow indicated negative indole tests, respectively.

Using simmon's citrate agar, the ability of the organisms to use citrate for energy was assessed. 15 ml of the citrate agar was dispensed into test-tubes and slanted before solidification to obtain slanted medium. The slanted cultures were inoculated with the test organisms and incubated at 37 °C for four days. The change in colour of the medium from green to blue was an indication of use of citrate and the absence of change in colour was recorded as lack of use of citrate.

For sugar fermentation test, 15 ml of diluted and sterilized TSIA was dispensed into test tubes to produce slanted medium that was inoculated with the test organism and incubated at 37 °C for 24 hours. Color change on butt and slant was observed to know if the organism has glucose, sucrose, or lactose. The broth was observed for black coloration along the stabbed line to know if the organism has hydrogen sulphide (H_2S) and the broth was also observed for bubble/crack for indication of present or absence of the gas.

3. RESULTS AND DISCUSSION

Figure 1 shows the number of Colony Forming Units in samples of tap water during (CFU-DF) and after flooding (CFU-AF) and Figure 2 shows the Colony Forming Unit in flood water (CFU- FLW) at sites close to water taps.

There were significant differences between tap waters during the flood with a few having no significant difference and a mostly no significant difference after flooding except in a few communities as shown in Figure 1. In addition, the bacterial colony forming unit in flood water was significantly different among the sites in the communities where samples were collected (see Figure 2).

The highest colony forming unit of bacteria in tap water during flood was recorded in Swali and Tombia and the lowest was in Yenezuegene and Amarata. Similarly, the highest CFU of bacteria in tap water after the flood event was obtained in tap water samples collected from Swali and Tombia but the lowest was observed in the other communities. Furthermore, the results revealed a positive correlation (Figure 3) between colony forming unit in flood water and colony forming unit of bacteria in tap water during flood.

Table 1 shows the morphological characteristic of bacteria isolated from water samples. It was observed that bacteria species with three different morphological forms were isolated from the samples.

Isolate A was entire, raised, circular and opaque while isolate B was filiform, flat, filamentous and non- opaque and isolate C was undulate, flat, irregular and non- opaque.

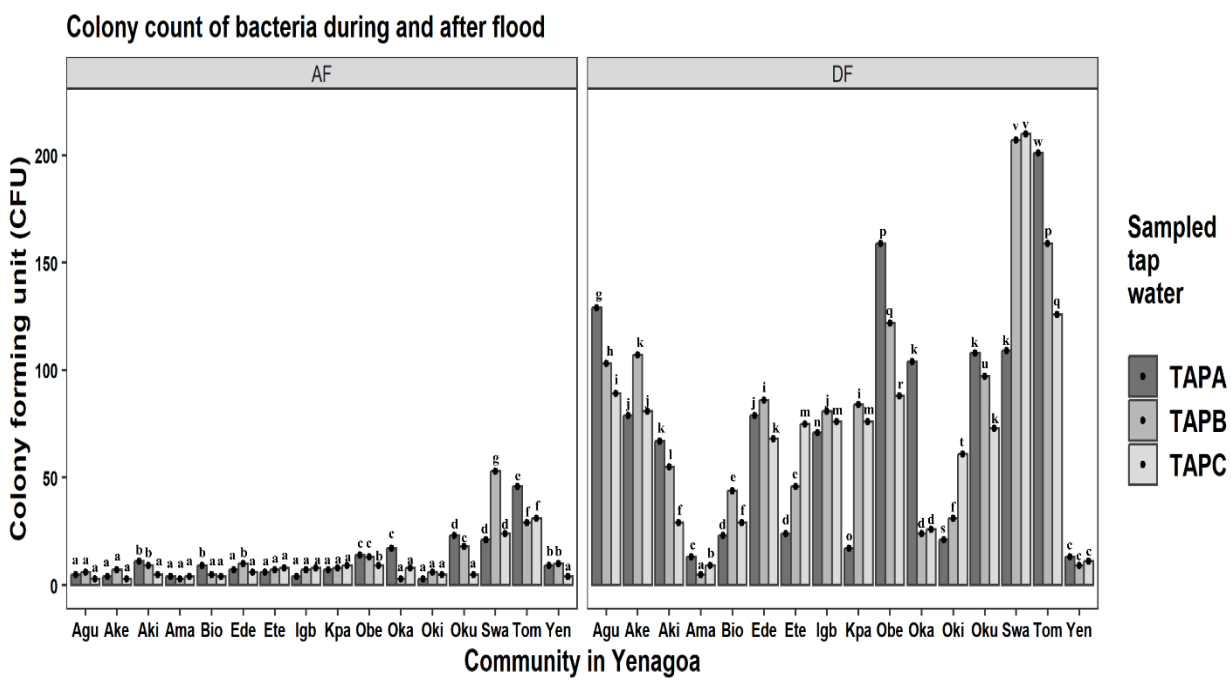

Figure 1. Shows microbial load in Tap and Flood water in the different study sites

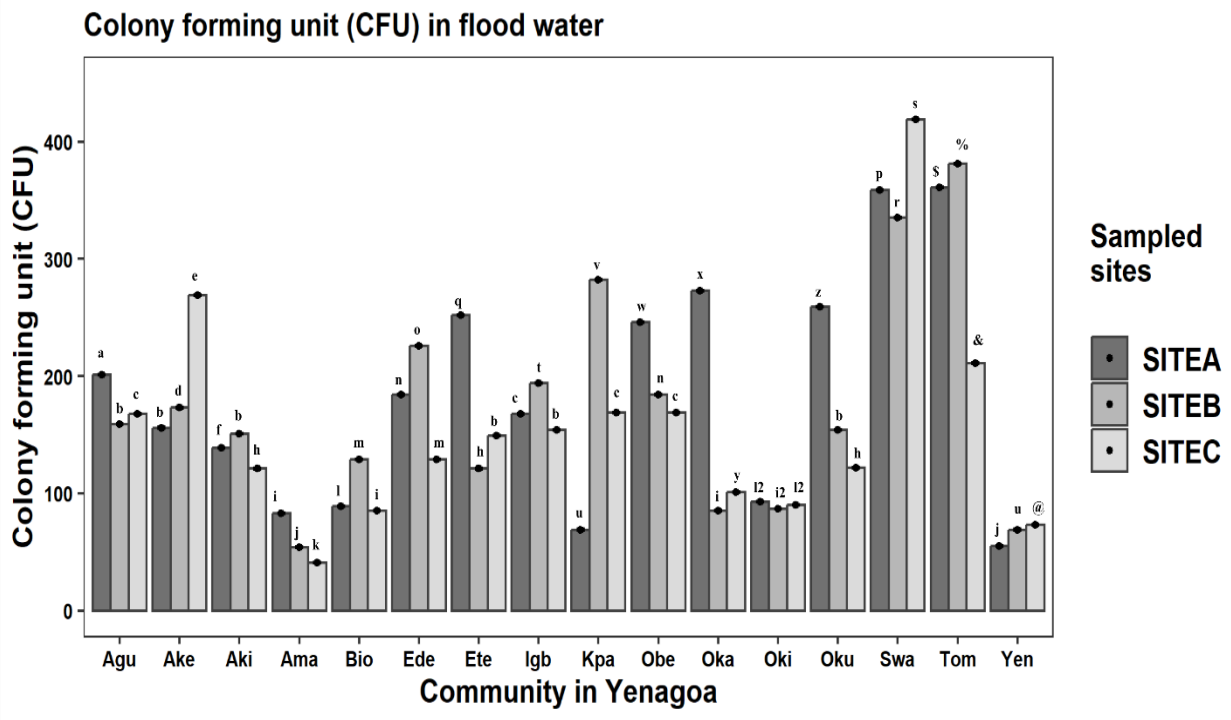

Figure 2. Colony forming unit of bacteria in flood water

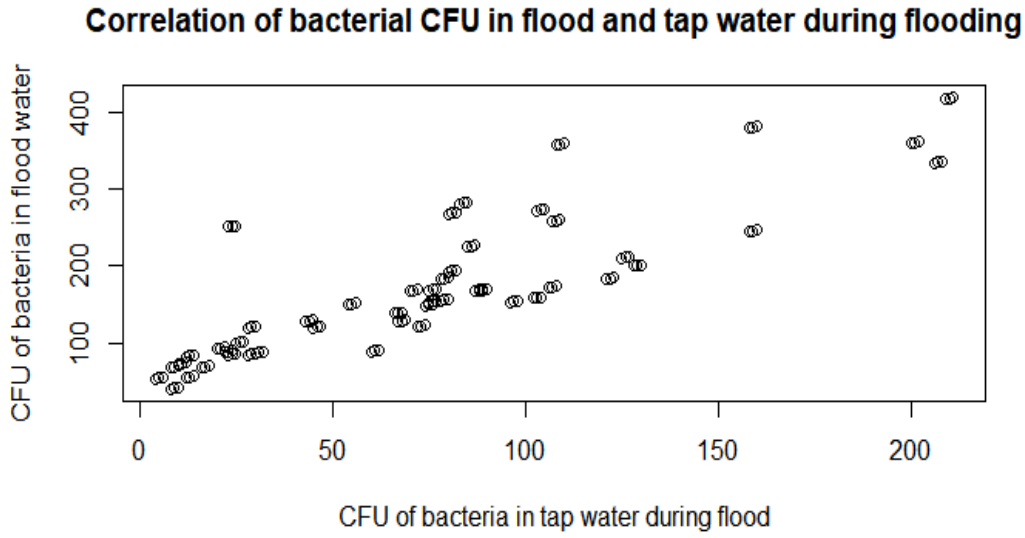

Figure 3. Correlation of bacterial colony forming units in flood and tap waters during flooding

Table 1. Morphological traits of bacterial strains isolated from water samples

Community	**Isolate**	**Morphology**			
		Margin	**Elevation**	**Form**	**Opacity**
Agudama	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Akenfa	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Okaki	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Igbogene	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Akenpai	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Adepie	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Tombia	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Okutuku	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Etegwe	A	Entire	Raised	Circular	v

	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Biogbolo	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Yenezuegene	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Kpansia	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Okaka	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Amarata	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
swali	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x
Obele	A	Entire	Raised	Circular	v
	B	Filiform	flat	filamentous	x
	C	Undulate	flat	irregular	x

Table 2 shows the diversity of bacteria isolated from the water samples. Bacteria species isolated were faecal *coliform*, *enterococci* and *E. coli* indicating presence of pathogenic microbes in water samples collected. This indicated that the drinking water and the flood water samples have been contaminated with faeces. Besides *E. coli, fecal coliform* and *enterococci* are indicator organisms for bacterial water contamination.

Table 2. Bacteria isolated from different water samples

Bacterial isolate	**Water samples**	
	Tap	**Flood**
Escerishia coli	+	+
Enterococci	+	+
Faecal coliform	+	+

Key: + = Present - = Absent

Table 3. Biochemical identification of isolated microorganisms

Isolate	Oxidase	Catalase	Coagulase	Indole	Glucose	Lactose	Sucrose	H_2S	Gas	Citrate	Gram Reaction
A	-	+	+	-	+	+	+	N	N	-	+

B	+	+	+	-	+	-	-	N	N	+	+
C	-	+	+	-	+	+	+	N	N	-	+

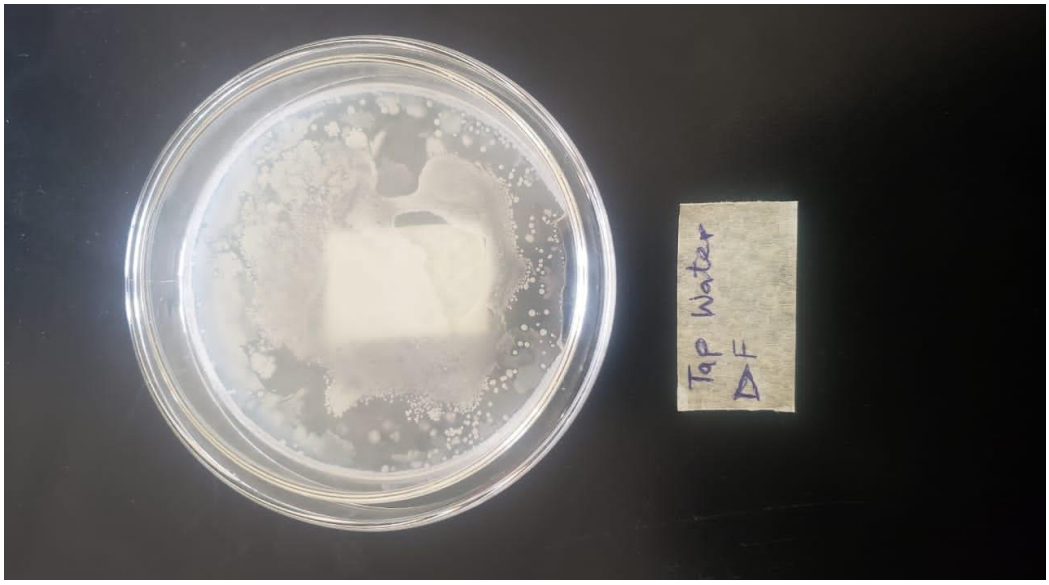

Plate 1. Petri-dish showing bacterial species isolated from tap water during floods.

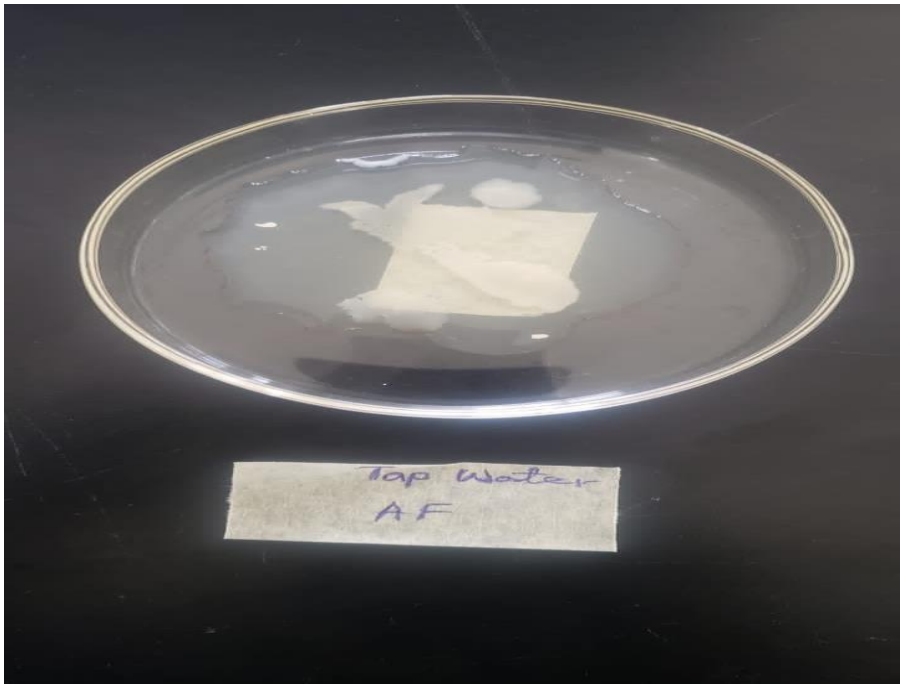

Plate 2. Petri-dish showing bacterial species isolated from tap water after floods.

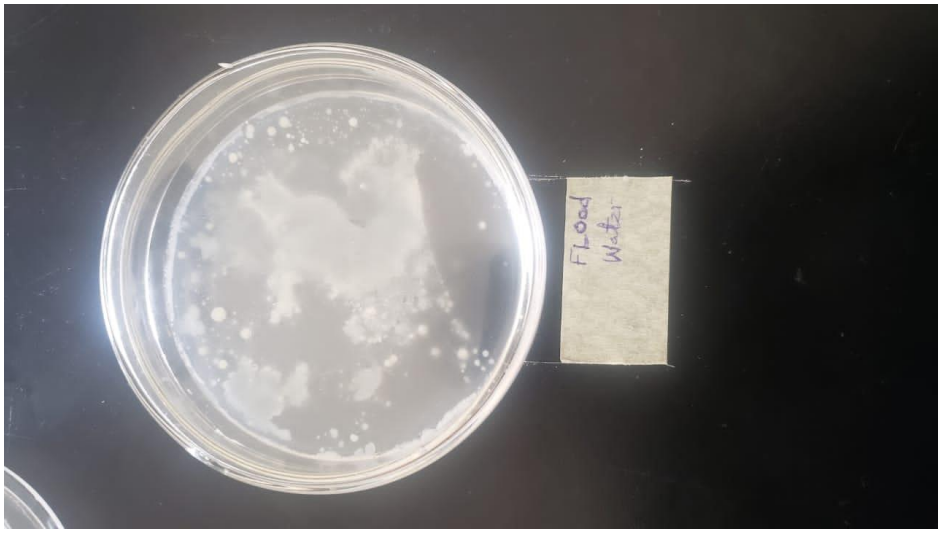

Plate 3. Petri-dish showing bacterial species isolated from flood water

Samples of water collected for both flood and tap were observed to contain same species of bacteria. After rainfall or snowmelt, there are often massive increases in turbidity in flooding creeks in mountain ranges, resulting in microbial contamination of household water sources. In one study the microbial loads of three tributaries of different drinking water reservoirs were investigated for the bacteriological colonies (*Escherichia coli*, *coliform*, *fecal streptococcal*, and *Clostridium perfringens* counts) and it was observed to have highly increased during extreme runoff events (Sophia *et al*., 2022). If relevant sources of parasitic contamination occurred in catchment areas, the concentrations of *Giardia and Cryptosporidium* rose significantly during flooding events. In addition, during the 2004 flood, a high incidence of diarrhea owing to enterotoxigenic *Escherichia coli* (ETEC) and *Vibrio cholerae* O1 was observed in flood affected areas of Dhaka (Firdausi *et al.* 2005). The disease pattern was quite similar to that of the 1988 and 1998 floods in Bangladesh (Naomi *et al.* 2020). Previous studies elsewhere also suggested that several epidemics have been caused by the contamination of drinking water.

The most recognized and useful reference enteric bacteria in developed regions are *Salmonella enterica*, *Campylobacter jejuni*, and *E. coli* O157:H7, each containing human pathogenic strains that vary by fecal source (Hrudey *et al*., 2002). Flood waters contaminated by sewage with *E. coli* bacteria can cause serious gastrointestinal illness. After Hurricane Katrina, surveys identified cases of *Vibrio* illness, a bacterial illness classically associated with exposure to saltwater or brackish water. This illness led to a handful of fatalities.

Higher values of *E. coli* counts are usually observed in samples from open wells and streams compared to protected springs. The protected springs had a spring box with an overflow pipe but with no fence around the spring. According to Berendes *et al*., 2017, water source protection status, location with respect to latrine, proximity to open defecation and unhygienic practices are significant determinants of variability in *E. Coli* counts in different samples. WHO recommends the health risk categories of *E. Coli* counts in drinking water to be 0 cfu/100 ml (conformity), 1–10 cfu/100 ml (low risk), 10–100 cfu/100 ml (intermediate risk), 100–1000 cfu/100 ml (high risk), and > 1000 cfu/100 ml (very high risk) (Biag *et al*., 2012). Higher

E. coli counts could therefore indicate a greater risk of water consumers developing diarrhea disease infections through drinking the untreated water.

4. CONCLUSION AND RECOMMENDATIONS

Flood waters contaminate sources of drinking water by leaching and spreading of faecal matter from human septic tanks, sewage drainage and suck-away; it is therefore advisable to apply more stringent methods for treating drinking water during floods. In addition, households should construct alternative plumbing lines with pipes above flood level to avoid recontamination of drinking water after treatment. Food items should also be properly washed and/or cooked before consumption. Plumbing pipes with leakage, sand beds and other water purification materials in household water purification and treatment process should be changed after floods to control bacterial biofilm formation. Bathing, washing, and swimming in flood waters should also be avoided as pathogenic bacteria could make their way to our body systems through openings such as: mouth, ear, nose, anus and sweat pores. Locals should avoid open defecation to prevent spread of infectious bacteria by flood water. There should be dissemination of information on the dangers of consuming contaminated drinking water to counter the mis-conception that germs do not kill Africans.

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