South Asian Journal of Research in Microbiology

6(3): 9-16, 2020; Article no.SAJRM.57764
ISSN: 2582-1989

Assessment of Coliforms Bacteria Contamination in Lake Tanganyika as Bioindicators of Recreational and Drinking Water Quality

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Authors' contributions

This research work was conducted in collaboration among all the authors. The Corresponding author LN designed the research topic, collected the field water samples, wrote the protocol, performed the laboratory analyzes and wrote the first draft and the latest version of the article. Authors AG and MN carried out documentary research, suggested the possible improvements to the manuscript. All authors have read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2020/v6i330150
Editor(s):
(1) Dr. Ravi Raghavbhai Sonani , Bhabha Atomic Research Centre,India.
Reviewers:
(1) Samuel Antwi-Akomeah , Forensic Science Laboratory, Ghana.
(2) Oluwasanmi Anuoluwapo Adeyemi , University of Ilorin, Nigeria.
Complete Peer review History: http://www.sdiarticle4.com/review-history/57764

Original Research Article

Received 24 March 2020
Accepted 29 May 2020
Published 03 June 2020

ABSTRACT

Worldwide coliform bacteria are used as indicators of environmental and fecal contamination and hence, the possible presence of pathogenic organisms. As most people living on the shores of Lake Tanganyika use its water for cooking, drinking and washing; the monitoring of organisms indicating water pollution is more predictive of the presence of certain pathogens to protect public health. This study was carried out along the Burundian coast at 4 sampling sites (Kajaga, Nyamugari, Rumonge and Mvugo) in the months of January, February and March 2018, to assess quantitatively the presence of coliform bacteria in comparison to the standards recommended by BIS-10500 (1991, 2012) and WWF-Pakistan (2007) for drinking and recreational water quality and to sensitize the populace using the untreated water about the potential health risks. The ColonyForming Unit (CFU) method was used and the results showed that total coliform

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bacteriaobtained was in the range of 9000 to 60000 CFU/100 mLand are indicative of environmental contamination of all sampling stations with an average of 33250 CFU/100 mL. Fecal coliform bacteria ranged from 0 to 5000 CFU/100 mL with an overall average of 2000 CFU/100 m Land Kajaga site appeared free of contamination as fecal coliform count there was nil. The *Escherichia coli* count recorded ranged from 0 to 3000 CFU/100 mL with an average of 1350 CFU/100 mL. At Kajaga stations, *Escherichia coli* count was 0 and therefore there is no evidence of recent fecal contamination. Thus, if only fecal contamination is taken into account, the water from Kajaga station can be considered as safe for drinking and bathing purposes but incidentally total coliforms were found at Kajagastation. The water from all sampling stations require treatment before any use.

Keywords: Coliforms Bacteria; water quality indicators; Lake Tanganyika.

1. INTRODUCTION

Stream and lake monitoring involve assessing the microbial quality of water and the risk of possible transmission of waterborne infectious diseases. The protection of public and environmental health needs drinking water without pathogenic bacteria. Coliform bacteria have been widely used as water quality indicators and have historically resulted in the concept of public health safeguard. The Indicator bacteria levels in swimming water have been found to correlate with the incidence of disease among swimmers in California, Santa Monica Bay [1]. Today, the most commonly measured bacterial indicators are the coliform group used to determine the bacteriological characteristics of natural waters [2]. The term coliform bacteria refers to gram-negative rod-shaped bacteria able to grow in the presence of bile salts or other surfactants having similar growth inhibiting properties and capable of fermenting lactose at 35^0 C to 37^0 C with the production of acid, gas and aldehyde within 24-48 hours [3]. They are also oxidase-negative and non-spore-forming and display *β*-galactosidase activity [4]. MacConkey [5] defined 128 coliform types and this number has increased to 256 by a system developed by Bergey and Deehan (1908) [6]. However, as defined by modern taxonomic methods, the group is heterogeneous consisting of as many as 32 genera [7]. Coliform bacteria are present in the feces of all warm-blooded animals and humans and in the environment (nutrient-rich waters, soil and decaying plant material) as well as in drinking water with relatively high concentration. Coliform bacteria can be indicators of potential pathogens responsible for various waterborne diseases [8]. They include three different groups of bacteria (total coliform, fecal coliform and *Escherichia coli*) and they serve as indicators of drinking and recreational water quality with different risk level (Fig. 1). Total coliform bacteria are usually found in the environment and are in general inoffensive. If only total coliform bacteria are discovered in drinking water, the source is possibly environmental. Fecal coliform bacteria are a sub-group of total coliform bacteria appearing in great quantities in the intestines and feces of human and animals. The presence of fecal coliform in water sample often indicates recent fecal contamination, meaning that there is a greater risk that pathogens are present than if only total coliforms bacteria are detected [9]. *Escherichia coli* are a sub-group of fecal coliforms. Most of *Escherichia coli* are harmless and are found in large amounts in the intestines of people and warm-blooded animals. However, some strains can cause illnesses and therefore, the presence of *Escherichia coli* in water samples almost always indicates contamination of such water with fecal matter and the possible presence of pathogenic organisms of human origin [10].

The use of the coliform group as an indicator of the possible presence of enteric pathogens in aquatic systems has been a subject of debate for many years. Most authors have reported the outbreaks of waterborne illnesses in water that meet coliform regulations [11,12,13,14]. Their presence in drinking water should at least be seen as a probable threat or indicator of deterioration of microbiological water quality. In general, it is not feasible to test water for all known waterborne pathogens in an attempt to assess whether it is safe for drinking [15,16,7,17]. We rely on tests that reflect the presence of commensal bacteria of intestinal origin such as those of the coliform group, which are more numerous, more easily tested and are the most reliable indicators of fecal pollution [18]. In the present study, it was revealed that most of the people living along the shoreline of Lake Tanganyika collect water from the lake for

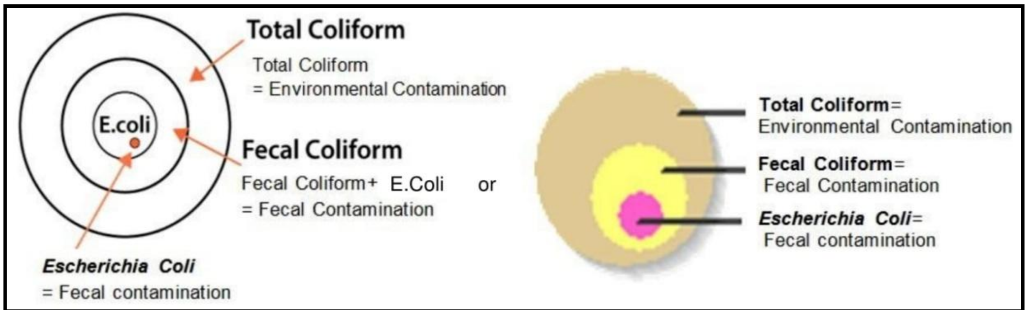


Fig.1. Diagrams showing different groups of Coliform bacteria
Source: https://www.doh.wa.gov/portals/1/images/4200/coliform.png

cooking, drinking and washing; hence the purpose of the present study was to quantify the coliform bacteria occurring in 4sampling stations of Lake Tanganyika and compare to standards recommended by BIS-10500 [19], WWF-Pakistan [20] and BIS-10500 [21] for drinking and recreational water quality (Table 2).

2. MATERIALS AND METHODS

2.1 Study Area

Water sample for bacteriological analysis was collected early in the morning from 4 sampling sites (Kajaga, Nyamugari, Rumonge and Mvugo) along the Burundian coast. The field data collection lasted 3 months (January, February and March, 2018). Table 1 and Fig. 2 show the geographical location of the study areas:

2.2 Determination of Coliforms Bacteria

The Colony Forming Unit (CFU) method was used. This method counts colonies unit formed from appropriate serial decimal dilutions of the water sample (Fig. 3). The Buffered Peptone water (BPW) was used as reagent and Chromocult Coliform Agar (CCA) was used as culture medium.

Procedure: (Fig. 3) 1 mL of the water sample was added to a tube containing 9 mL of Buffered Peptone water (BPW) and this resulted in a dilution of 10^{-1}. Using separate sterile pipettes, decimal dilutions of 10^{-2}, 10^{-3}, 10^{-4}, etc were prepared by transferring 1 mL of the previous dilutions to 9 mL of diluents (Peptone water). 15-18 mL of the molten sterilized PCA medium (agar cooled to 44°C - 47°C) was poured into each Petri plate and 1mL of the diluted water sample was inoculated into sterile petri plates. After complete solidification, the prepared plates were inverted and incubated under different temperatures according to the targeted bacteria (35±0.5°C for 24±2h for Total Coliform; 44±0.2°C for 24±2 h for fecal coliform and 37°C for 21±3h for *Escherichia coli*). The number of microorganisms per milliliter in sample was calculated from the number of colonies obtained on PCA plate from selected dilutions. It was assumed that each visible colony was the result of multiplications of a single cell on the agar surface [21].

2.3 Calculation and Expression of Results

$$\text{CFU/mL/plate} = \frac{\text{Number of Colonies x dilution factor}}{\text{Volume of culture plate}}$$

Case 1: Plates having microbial count between 10 and 300CFU

$$\mathbf{N} = \frac{\text{Colonies Plate 1+Colonies Plate 2}}{2}$$

Table 1.Geographical location of the study sites

Study sites	Geographical location				
	Province	**Commune**	**Longitude-East**	**Latitude-South**	**Altitude**
Kajaga	Bujumbura Rural	Buterere	029°17’ 56’’	03°20’ 55’’	783 m
Nyamugari	Bujumbura Rural	Kabezi	029°20’ 24’’	03°30’ 27’’	776 m
Rumonge	Rumonge	Rumonge	029°26’ 03’’	03°58’ 23’’	767 m
Mvugo	Makamba	Nyanza-Lac	029°34’ 06’’	04° 7’ 42’’	810 m

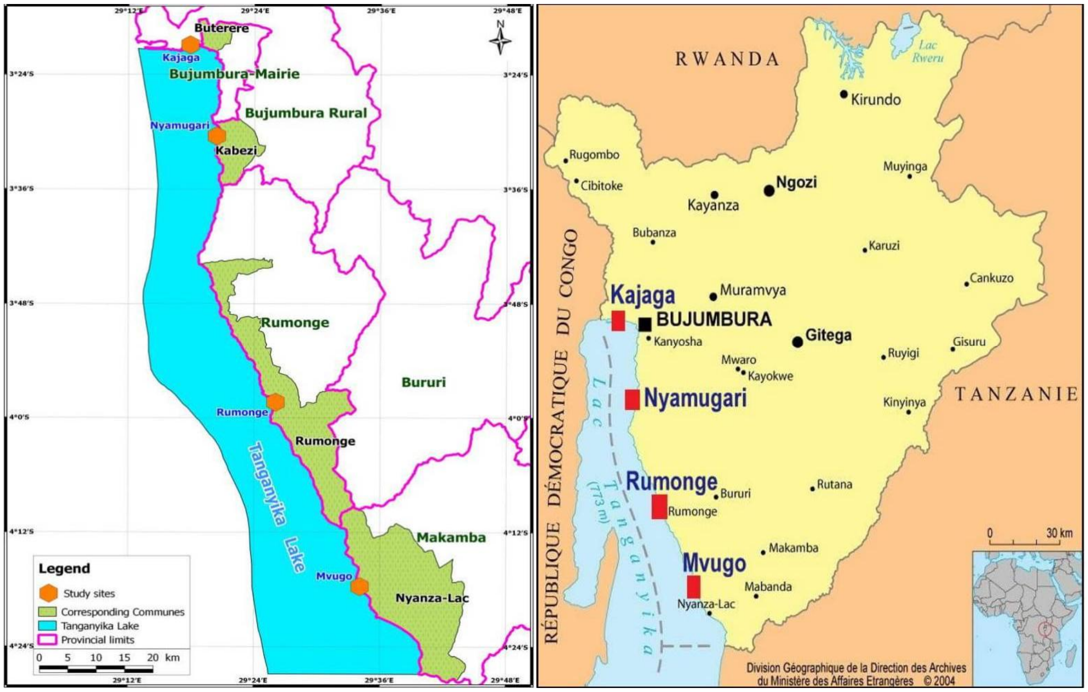


Fig.2. Map of the study area showing sampling sites

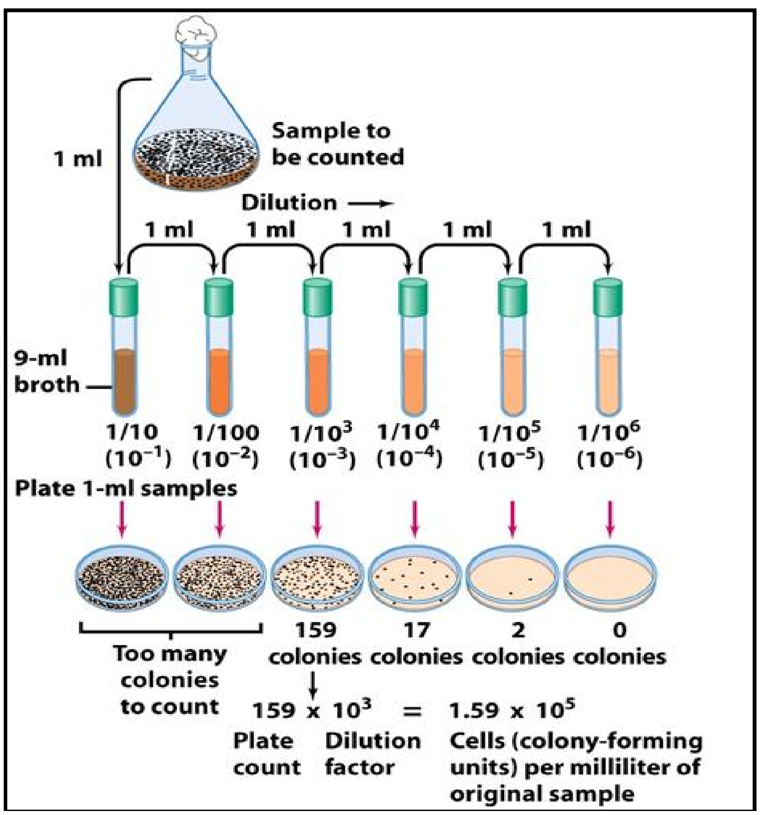


Fig.3. Microorganisms counting process

Source: https://nptel.ac.in/courses/102103015/module5/lec1/images/3.png

Case 2: Plates having microbial count less than 10CFU but at least 4, Calculate the results as given in Case 1.

Case 3: If microbial load is from 3 to 1 then reporting of results shall be: "Microorganisms are present, but, less than 4 per mL".

Case 4: When the test samples/plates contain no colonies then reporting of results shall be: Less than 1 CFU/mL".

3. RESULTS

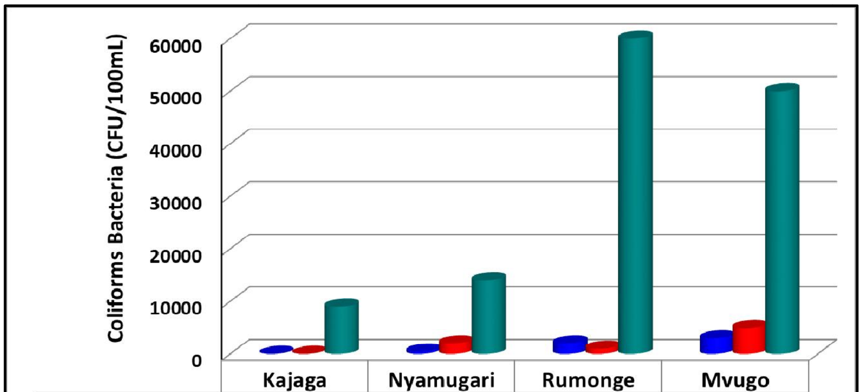
Total coliform bacteria comprise fecal coliform and *Escherichia coli*. The presence ofonly total coliformin water sample indicates the environmental contamination. According to the World Wide Fund-Pakistan [19], total coliform counts of less than or equal to 1000 CFU in 100mL is acceptable for recreational water quality whereas counts of less than or equal to 20 CFU in 100 mL is acceptable for drinking water. As per the Bureau of Indian Standards [20], total coliform counts of less than or equal to 10 CFU in 100 mL is acceptable for drinking water quality. In the present study, total coliforms obtained were in the range of 9000 to 60000 CFU/100 mL (Table 2). Rumonge site was found to have the maximum value while the minimum value was recorded at Kajaga site. Considering all study sites, mean value was 33250 CFU/100 mL and the results obtained from all stations were not in accordance with the standard limits for drinking and recreational water recommended by both the World Wide Fund-Pakistan [19] and the Bureau of Indian Standards [20]. The presence of fecal coliform in water sample is a good indication of recent fecal contamination. In the present study, the fecal coliforms counts ranged from 0 to 5000 CFU/100 mL (Table 2) with an average value of 2000 CFU/100 mL considering all the stations. Kajaga site appeared not contaminated as fecal coliform numbers were zero. According to the standards for recreational and drinking water quality defined by the World Wide Fund-Pakistan [19] and the Bureau of Indian Standards [20] reported in Table 2, it was revealed that only the waters from Kajaga station were safe for both bathing and drinking during the investigation period. The detection of *Escherichia coli* in water sample was the indisputable evidence of the occurrence of recent fecal contamination and was indicative of potential presence of enteric pathogens [22,7,23,24].

During the study, the counts of *Escherichia coli* recorded ranged from 0 to 3000 CFU/100 mL (Table 2) with 1350 CFU/100 mL as average. At Kajaga station, *Escherichia coli count was* 0, reflecting the absence of bacterial contamination at this station. According to the World Wide Fund-Pakistan [19] and the Bureau of Indian Standards [21], the standards defined for bathing and drinking water quality reported in Table 2 above, show that only the waters from Kajaga station were suitable for drinking and recreational purposes during the investigation period but, since total coliforms were incidentally found at Kajaga station, the waters from that station cannot be considered as safe for the intended purposes. The spatial variation of coliforms bacteria amount is presented in Fig. 4 while the colonies shape of the different coliforms bacteria types are presented in Fig. 5.

Table 2. Mean data per sampling stations in comparison to the Standards of Water quality required for recreational and drinking (CFU/100 mL)

Bacteria	Mean data per sampling stations (CFU/100 mL)				Standards of water quality suitable for bathing and drinking (CFU/100 mL)	
	Kajaga	**Nyamugari**	**Rumonge**	**Mvugo**	**Bathing**	**Drinking**
Total Coliforms	9000	14000	60000	50000	≤1000 [19]	≤20 [19] ≤10 [20]
Fecal Coliforms	0	2000	1000	5000	≤200 [19]	≤20 [19] ≤10 [20]
Escherichia Coli	0	400	2000	3000	—	Must not be detectable in any 100 ml of sample [21]

CFU: Colony Forming Unit



	Kajaga	Nyamugari	Rumonge	Mvugo
Escherichia Coli (CFU/100ml)	0	400	2000	3000
Fecal Coliforms (CFU/100ml)	0	2000	1000	5000
Total Coliforms (CFU/100ml)	9000	14000	60000	50000

Fig. 4. Spatial variation of coliforms bacteria amount

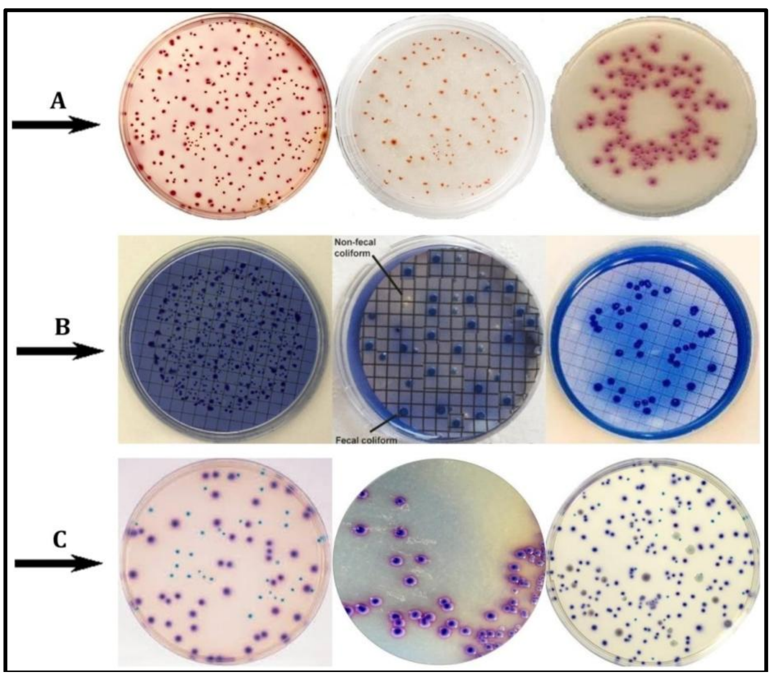


Fig. 5. Different types of coliforms bacteria grown on Chromocult Coliform Agar (CCA): Total Coliforms (A), Fecal Coliforms (B) and Escherichia Coli (C)

4. DISCUSSION

Coliform bacteria are organisms occurring in the environment and in the feces of all warm-blooded animals and humans. In the present study, both fecal coliforms and *Escherichia coli* which are good indicators of fecal contamination were absent at Kajaga site but were detected in counts ranging from 400 to 5000CFU/100mL at Nyamugari, Rumonge and Mvugo stations (Table 2 and Fig. 4). The minimum value was recorded at Nyamugari site whereas the maximum was recorded at Mvugo site. The presence of this fecal contamination is attributed in part to the nocturnal fishing activity leading fishermen to defecate in the lake while they are fishing. Besides, Rumonge and Mvugo stations are close to human settlements contributing to the release of fecal coliforms into the lake through the raw sewage or partially treated sewage being discharged into the lake as well as the runoff and subsurface flow from the urban area. Local communities interviewed on spot reported a water-borne cholera outbreak during the rainy season in populations living around and using the water of Lake Tanganyika for domestic purposes, which was also the evidence of fecal contamination. The presence of fecal coliforms and *Escherichia coli* at Nyamugari station where there are no human settlements was also due to feces released by nocturnal fishermen who defecate on spot while they are fishing. In addition, field observation revealed also that women and young people cooking for fishermen spend many hours collecting firewood and fishermen can themselves defecate anywhere around Nyamugari station during the day time, because sanitation facilities are not available there. The total absence of fecal coliforms and *Escherichia coli* at Kajaga site during the study does not necessarily indicate the no contamination and the good quality of the water at this station because these bacteria are generally more sensitive to disinfection of laboratory equipment than pathogens more resistant to chlorine such as viruses [25] and *Cryptosporidium oocysts* like *Cryptosporidium spp.*[14]. Total coliforms were detected in all sampling stations and ranged from 9000 to 60000 CFU/100 mL. Minimum numbers were recorded at Kajaga site while maximum numbers were recorded at Rumonge station. The presence of total coliforms indicated both environmental and fecal contaminations which were mainly due to diffuse pollution from runoff, shortcomings in land management of the catchment, human activities and settlements, household sewage, livestock dung and open defecation.

5. CONCLUSION

The results regarding bacteriological community revealed the presence of total coliforms in large numbers in all sampling stations which reflect environmental contamination. The fecal coliforms and *Escherichia coli* were not detected at Kajaga site, showing theoretically that the water at Kajaga station was suitable for drinking and swimming if only fecal contamination was considered. At Nyamugari, Rumonge and Mvugo stations, the presence of fecal coliforms and *Escherichia coli* were due to open defecation in these areas. That is why the people using the waters from all the sampling stations must be aware of the health risks that may occur and therefore the waters must be treated before any domestic use to avoid contracting waterborne diseases.

ACKNOWLEDGEMENT

The authors express their heartfelt gratitude to the Ministry of Higher Education and Scientific Research of the Government of Burundi and to the Indian Council for Cultural relations (ICCR) of the Government of India for providing financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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