Field application of the Micro Biological Survey method for the assessment of the microbiological safety of different water sources in Tanzania

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Abstract

Access to safe water is stated within human rights as essential for life, as water can be a source of severe enteric infections threatening human health, in particular children from Developing Countries. Along with reference methods, need is pressing for alternative methods to flank reference ones to improve water safety on-site monitoring and in the absence of scientific facilities or even electricity supply. The Micro Biological Survey (MBS) method has already been successfully applied to water safety assessment in Developing Countries. A total of 18 water samples were collected from different sources (rivers, dug wells, tap water) within the Rukwa Region, Tanzania, and underwent analysis for Total Coliforms following the MBS method. Globally, rivers showed more frequently contamination, followed by dug wells, tap water and tanks. Results demonstrate the need for continuous monitoring of water sources, even in difficult frameworks lacking electric supply, to help improve control over water quality, possibly using alternative methods to simplify existing protocols.

Introduction

Water plays a crucial role in human life, as it is vital for survival and is also a cornerstone in the progress of society. Distribution and availability of freshwater resources depend mainly on seasonal precipitations; their variations are affected by climate change and deep anthropic alterations of ecosystems, which lead to a decline in water quality and availability.[1] Access to safe water is stated within human rights as essential for life, as water can be a source of infection threatening human health.[2] Water safety is hence a critical topic, especially affecting Low-income and Developing Countries, where international safety standards are more hardly met. A meta-analysis showed that the country income level is significantly related to water quality, highlighting how Low-income Countries are more than twice prone to drinking-water contamination; moreover, rural areas display comparable higher contamination from fecal indicator bacteria than urban areas.[3] Major microbiological water contaminants belong to the Enterobacteriaceae family, causing alterations of physiological intestinal balance and fever, ultimately leading to death especially in childhood. As indeed stated by WHO, *diarrheal diseases are one of the main contributors to global child mortality, causing 20% of all deaths in children under five years"*, with fecal-oral pathogens accounting for most cases due to either poor hygiene or lack of sanitation practices of both house environment and sewage system.[4,5] A reduction in diarrheal morbidity and other water-borne diseases could be achieved through improved water quality and personal hygiene practices, despite many people only have access to improved drinking-water sources, which are not necessarily safe and could still cause harm. It has been estimated that many (34%) diarrheal cases in Low and Medium-income Countries are indeed related to inadequate drinking-water.[4]

Microbial quality of drinking-water may change a lot and in a short time according to environmental conditions, possibly triggering outbreaks of water-borne diseases when pathogens are involved.[2] In order to minimize people's exposition to unsafe water, microbial monitoring should not be performed on end-products only and should allow fast and reliable results. Moreover, analysis should be repeated during events that may affect water safety, such as floods, epidemics and interruption of supply.[2] As stated by WHO, *frequent examination by a simple method is more valuable than less frequent examination by a complex test or series of tests.*[2]

Microbial water quality assessment can be performed based on fecal indicator microorganisms; in particular, *Escherichia coli* is considered the main representative of fecal contaminants, providing information on recent fecal pollution and hence guiding downstream corrective actions. Guidelines state that such bacteria should not be present in drinking water. Additional indicators may be useful in specific cases.[2]

Reference methods for water microbiological quality assessment specified by international guidelines rely on vital count of bacteria through either plate count or multiple tube techniques. Such traditional methods are not always suitable to ensure wide and adequate control: due to high costs and the need for equipped facilities supplied with electricity along with trained personnel, analysis in low-resource settings may be difficult following these methods, not to mention complications associated to shipping of samples to external laboratories.

For these reasons, the need is pressing for alternative methods to flank reference methods to improve monitoring of water safety on site and in the absence of scientific facilities or even electricity supply, in order to ensure a foreseeable improvement of wellbeing in communities.

Taking into account these still unmet needs, the Micro Biological Survey method (MBS srl, Rome, Italy) could be a valuable tool to help implementing water monitoring worldwide. This is a culture-based colorimetric method which allows both detection and evaluation of microorganisms in various samples (food, water, surfaces and biological samples). The MBS method is based on the measurement of metabolic activity of bacteria, thanks to redox indicators that

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change color following bacterial growth in the medium. Viable bacterial concentration in the sample can be estimated using specific tables that correlate this parameter with time required for color change of vials following inverse correlation: the higher the bacterial load in samples, the shorter the time required for color change; this means highly contaminated samples yield results in a shorter time, ensuring fast intervention in the most severe situations. The MBS method has already been successfully applied to water safety assessment even in Developing Countries;[6-8] among its features, cost-effectiveness, user-friendliness and portability play a key role in simplifying microbiological analysis, making it feasible in low-resource settings, while maintaining high accuracy, reproducibility and repeatability,[9] thanks to straightforward analytical procedure and reduced labor.

Tanzania is a Country located in East Africa still having extensive geographic areas where the recent and ongoing economic development has not benefitted significantly all social groups, with poverty and hunger still posing a crucial issue. Conditions in rural and urban areas differ in terms of both access to safe water and sanitation.

Urban areas can rely on more than 30% safely managed drinking-water and more than 40% on basic service, while no such estimate is available in rural areas, in which use of surface water accounts for almost 20% of cases. A national estimate shows that half of drinking-water is available with basic service, even though almost 40% of water should be considered unsafe. Sanitation also shows some flaws in that on a national scale a basic service only accounts for 24%, while more than 50% of cases show unimproved sanitation; open defecation is still carried out especially in rural areas, where it accounts for 16% of cases. Nevertheless, both basic drinking water and sanitation services displayed an improvement from 2000 to 2015,[10] though not reaching 2015 Millennium Development Goal to improve access to safe water.[11,12]

In addition, it was reported that up to 30% of bacteria recovered from water sources in Tanzania displayed resistance against first-line antibiotics (ampicillin, tetracycline, trimethoprim, sulfamethoxazole and streptomycin), meaning not only people, but also livestock may be exposed to multi-resistant microorganisms, with no effective treatment options possibly available in this framework. Therefore, extensive monitoring of water sources should be performed, even in remote areas, to reduce the propagation of both pathogens and antibiotic resistance and ultimately improve communities' health.[13]

The aim of this study was to further investigate the performance of MBS method in a particularly difficult framework and confirm the feasibility of field application of the method for on-site evaluation of water microbiological safety.

Total coliforms concentration was the microbiological parameter considered in this work, as it is one of the main parameters referred to in international guidelines to assess safety of water samples. Fecal coliforms are indeed considered indicators of fecal contamination, reflecting harmful contamination upstream.[2,14]

Materials and Methods

Study area

The Rukwa Region is one of the 31 administrative Regions of Tanzania; it is located in the Southwest of the Country, between Ruwa and Tanganika lakes. The Region is divided in four Districts; the Mvimwa Abbey, a Benedictine monastic community founded at the end of the seventies, is located in Nkasi District. The plateau (1600 m above sea level) enjoys favorable climatic conditions, with availability of abundant water and good soil fertility. This area is characterized by two seasons, a dry season from May to October and a rainy season from November to April, with mean annual maximum temperature being 24°C and 27°C respectively and minimum temperatures not lower than 5-8°C (as retrieved from *www.rukwa.go.tz* and monks' personal communication). The area is in the Southern Agricultural Growth Corridor of Tanzania (see *www.sagcot.com*). In the plateau is located a Benedictine Abbey around which 10 villages have developed, displaying a population of about 20,000 inhabitants. Each village is governed by a chief, elected for a 4-year term by the inhabitants, who manages the ordinary life of the village.

Despite the favorable pedo-climatic conditions, the area is one of the poorest of the Country, as in the whole Rukwa Region only one of the 10 villages (Kate) has electricity. The Abbey itself use the electricity autonomously produced by a biogas generator, which allows the monks to have electricity for 5-6 hours a day. In the villages, very few buildings (the Abbey, dispensaries and few schools) are provided with running water. The whole population takes water from wells and public cisterns and, in some cases, from sources or streams. The containers used for collecting water commonly are 20 liters plastic buckets that girls and women bring on their head from the water collection points to their house, sometimes even for 1-4 km according to information given by the chief and the inhabitants of the villages.

Figure 1 provides an overview of the area under study. Sampling points were grouped as following: 3 points in Ntemba, 2 in China, 1 in Kitosi, 2 in Miangaluwa, 1 in Ntengamwa (fraction of Miangaluwa), 3 in Nkata, 4 in Kate, and 2 in Nchenje.

Sample collection

A total of 18 water samples were collected from sampling points above mentioned over a period of 20 days (from August 10th to 30th) and analyzed on-site. Different water sources were taken into account to ensure a wider and more complete screening of water quality in the area: 7 samples came from rivers, 5 from dug wells, 3 from public tanks, all located at different distance from corresponding built-up areas within 4km range, and 3 were tap water coming from public distribution system. Water samples were collected using sterile tubes, then stored within a thermal bag and underwent analysis following the MBS method within 2 hours.

Microbiological safety assessment on water samples following the Micro Biological Survey method

Safety assessment of water samples using the MBS method was performed using specific vials for the quantification of total coliforms. Such vials are sterile and disposable, already pre-filled with an original selective medium specifically developed for the enumeration of coliforms. All vials were produced by MBS srl, Rome, Italy. MBS vials were filled with 10 mL of sterile distilled water (provided within the analytical kit) and approximately 1 mL of each water sample was added to each vial. Analysis were performed in duplicate on 1 mL samples in accordance to results obtained by Arienzo *et al,* 2015, which show that analysis for total coliforms performed in 1 mL of sample is comparable to analysis on 100 mL required by regulations. After inoculation, vials were incubated for up to 72 hours at room temperature (22±1°C, constantly measured through a thermometer), recording by periodic visual inspection the time required for color change of vials. Indeed, in the presence of viable coliform bacteria in the inoculated sample, vials' color changes from red to yellow, providing a positive result. In case no color change occurs during time set for analysis, a negative result is yielded, meaning absence or very low concentration of coliforms in the sample. Results obtained in

terms of time required for color change of vials can be then easily converted into results in terms of bacterial concentration (CFU/mL) thanks to a specific conversion tables provided (Table 1). Depending on temperature of analysis, a specific conversion table must be performed for the MBS method, drawing for each temperature a conversion table based on experimental data. Best results can be yielded in terms of time, resources and accuracy coupling naturally and artificially contaminated samples. The conversion table at 22°C, needed for this work, was obtained in the Department of Sciences, Roma Tre University, Italy. The conversion table was based on artificially contaminated samples using an ATCC *Escherichia coli* strain (ATCC 25992), this being the main representative of coliforms. No samples from Tanzania could be shipped to Italy for further analysis due to prohibitive conditions.

Results and Discussion

Samplings and ensuing analyses were performed in August 2017, namely during the dry period. Due to lack of electricity system in almost all sites, no electrically driven equipment could be used in such framework.

Among the screened water sources, none was used for agricultural purposes: only the Abbey was provided with drip irrigation, other villages did not generally use water for irrigation, relying on rain, as it is a precious good also taking into account the effort for carrying it to the village. Hence, water from all sources examined was exclusively intended for drinking and personal hygiene.

No disinfection practice was applied to water except for sampling point F4 (Kate), where water underwent boiling before consumption.

The different water sources were tested for coliforms contamination following the MBS method, highlighting different conditions: results showed different contamination levels, ranging from 10^3 CFU/mL to < 10 CFU/mL. Therefore, results were sorted into three categories: high contamination (≥10^3 CFU/mL, 48 hours required for MBS vials color change or less), medium contamination (between 10^3 and 10 CFU/mL, time required for MBS vials color change between 48 and 65 hours) and low contamination (<10 CFU/mL, more than 65 hours required for MBS vials color change) (Table 2). Samples from rivers and dug wells showed an overall higher contamination compared to samples from tanks and tap water; tanks yielded best results, showing only low contamination, possibly because they collect deep ground water, whereas dug wells were mainly highly contaminated (Figure 2). Globally, rivers showed more frequently at least medium contamination (4/18, 22%), followed by dug wells (3/18, 16%), tap water (1/18, 6%) and tanks (0/18, 0%). No drilled wells could be built in the

Figure 1. Map of area under study (red inst in Rukwa Region, Tanzania, Africa). A1: fraction of Ntemba (7° 47' 40.7" S 31° 06' 36.5" E); A2: Ntemba (7° 47' 27.9" S 31° 06' 20.8" E); A3: Ntemba (7° 46' 30.3" S 31° 05' 45.5" E); B1: China (7° 45' 15.6" S 31° 03' 43.6" E); B2: China (7° 46' 01.9" S 31° 05' 01.6" E); C1: Kitosi (7° 44' 58.1" S 31° 09' 19.4" E); D1: Miangaluwa (7° 49' 56.7" S 31° 07' 41.6" E); D2: Miangaluwa (7° 50' 13.9" S 31° 07' 37.3" E); D3: Ntengamwa (7° 51' 00.6" S 31° 08' 35.9" E); E1: Nkata (7° 52' 13.3" S 31° 07' 37.7" E); E2: Nkata (7° 52' 07.2" S 31° 07' 40.1" E); E3: school in Nkata (7° 51' 56.8" S 31° 07' 38.8" E); F1: Kate (7° 51' 07.0" S 31° 10' 44.4" E); F2: Kate (7° 51' 21.1" S 31° 10' 41.5" E); F3: school in Kate (7° 50' 54.7" S 31° 12' 38.2" E); F4: nuns' residence in Kate (7° 51' 39.7" S 31° 10' 32.5" E); G1: Nchenje (7° 47' 07.3" S 31° 13' 22.2" E); G2: Nchenje (7° 47' 31.8" S 31° 13' 29.8" E). Blue points correspond to water samples from rivers; green points correspond to water samples from dug wells; red points correspond to water samples from tank; yellow points correspond to tap water samples.

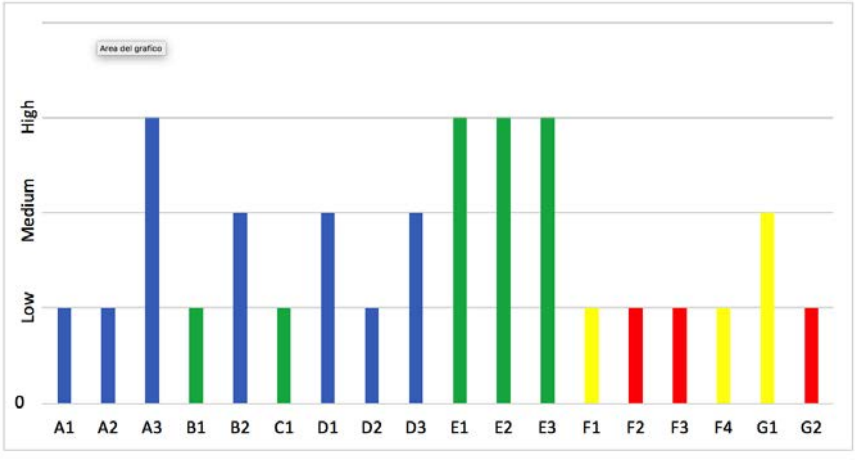

Figure 2. Contamination levels (low meaning <101 CFU/mL, medium meaning 101 – 103 CFU/mL, high meaning ≥103 CFU/mL) of sampling points under study associated with source type. Blue bars correspond to water samples from rivers; green bars correspond to water samples from dug wells; red bars correspond to water samples from tank; yellow bars correspond to tap water samples

area due to absence of power supply, so only dug wells are available in this area, exploiting superficial groundwater, which are more prone to harmful contamination from nearby activities due to a shallow dig and the absence of lining.

It is worth noting that both wild animals and free livestock use some of the water sources under study, mainly rivers; hence, drinking animals may constitute contamination sources for people and villages downstream. Moreover, the use of animal sewage for fertilizing cultivations may cause downstream water contamination after through leaching by rainwater. Animal excreta can thus directly contaminate either soil or surface water used for drinking and recreational purposes, carrying fecal bacteria, eventually causing enteric and diarrheal diseases, and other pathogens.[2,4]

The same problem may arise from contamination through human excreta when open defecation is practiced, as it is estimated to be in 16% of cases in rural Tanzania.[10]

Surface water, such as rivers, is therefore more prone to such contamination, and thus may cause diarrheal waterborne diseases, especially in children, as they are more vulnerable.[15]

Failure to ensure drinking-water safety may expose the community to the risk of outbreaks of intestinal and other infectious diseases. Outbreaks of waterborne disease are particularly to be avoided because of their capacity to result in the simultaneous infection of a large number of persons and potentially a high proportion of the community. Thus, a rapid evaluation of drinking-water quality throughout collection and storage could help detect corruption at different moments and identify sources of contamination; this would thus guide interventions to either increase water quality (*e.g.* increasing awareness about point-of-use disinfection practices such as boiling) or reduce contacts with unsafe water.[14-17]

Our experience in these Rukwa villages highlighted how inadequate hygienic conditions, especially related to foodstuffs storage and even more to water supply, are one of the main cause of diseases. An epidemiological study on health and eating habits carried out in the villages around the Abbey during August 2017 showed an inverse correlation between health and water consumption,[18] inferring consumption of unsafe water participates into a general decrease in health. In addition, the author also estimated water consumption of the inhabitants of the area to be much lower than that suggested by the international guidelines, with about 80% of the interviewed population consuming less 500 mL of water per day versus the over 2000 mL suggested.[19-21]

Therefore, it is essential to develop screening tools for the assessment of the microbiological safety of water that feature both portability and feasibility in difficult contexts, in order to effectively improve people's access to safe water sources and ensure higher wellness standards.

The results of this work confirm that the MBS method is applicable independently from instruments and conditions, even in areas lacking power supply, not to mention dedicate facilities for microbiological analysis. The use of such approach, exploiting ready-to-use portable devices, is strong-

Table 1. Correlation table for MBS total coliforms vials at 22°C between time required for vials color change (in terms of hours) and bacterial concentration in the sample (in terms of CFU/mL).

Time required for MBS vials color change (hours)	Coliform concentration in water samples (CFU/mL)
<48	$\geq 10^3$
48-65	$10^3 - 10^1$
>65	$<10^1$

Table 2. Correlation table for MBS total coliforms vials (22°C) between time required for color change (hours) and sample contamination (CFU/mL). In the presence of viable coliforms in samples, vials' color changes from red to yellow; if no contamination from coliforms is detected, no color change occurs within analytical timeframe (80h).

Location	Sampling site code	Water source	Distance from build-up area (km)	Total coliforms contamination range (CFU/mL)	Time required for color change of MBS vials (hours)
Fraction of Ntemba	A1	River	0,2	Low	No color change
Ntemba	A2	River	3	Low	72
Ntemba	A3	River	2,5	High	43
China	B1	Water pump/dug well	0,5	Low	No color change
China	B2	River	2	Medium	65
Kitosi	C1	Water pump/dug well	1	Low	No color change
Miangaluwa	D1	River	0,3	Medium	51
Miangaluwa	D2	River	0,8	Low	74,5
Ntengamwa (fraction of Miangaluwa)	D3	River	0,1	Medium	66,5
Nkata	E1	Dug well	0,2	High	43,5
Nkata	E2	Dug well	0,5	High	43,5
Nkata	E3	Dug well	0,9	High	43,5
Kate	F1	Tap water	0	Low	No color change
Kate	F2	Tank	0,5	Low	No color change
Kate	F3	Tank	3,5	Low	No color change
Kate	F4	Tap water	1	Low	No color change
Nchenje	G1	Tap water	0	Medium	54,5
Nchenje	G2	Tank	0,8	Low	No color change

ly recommended to non-governmental organizations in Low-income and Developing Countries worldwide. This could fall within a multidisciplinary strategy to improve health in such rural areas, being easily coupled with educational contacts with village chiefs and people from the community, to increase awareness about safety of available water sources, which could be then sorted for proper usage (drinking, cleaning, animals, irrigation) or receive proper treatment (*e.g.* boiling, chlorination).

Conclusions

In conclusion, microbiological analysis of both water sources and water samples is important to obtain correct evaluation of water safety, as geographic conditions and types of water sources (river, tank, pump) cannot exclude contamination, therefore water safety assessment is crucial in each case. The MBS method has proved useful in difficult frameworks even lacking electric supply to help improve control over water quality. Such method can give essential information on water safety though simplifying existing protocols, hence its broader application could provide a more capillary monitoring of water sources safety ultimately helping to move towards consumers' wellness.

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