

Full Length Research Paper

Identification and tracking of microorganisms from the biofilms of container walls used for water storage: Case of rural communities in Burkina Faso

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Safe drinking water is an important necessity for humans. In rural communities of Burkina Faso, many households use local containers for storing drinking water. During water storage, some microorganisms get attached to the surface walls of the containers to form a biofilm which can deteriorate drinking water quality over time. This study aimed at evaluating the attachment of indicator microorganisms to containers walls during drinking water storage. Raw drinking water from wells and drilling were stored in three different containers: earthenware jar, polyethylene and galvanized steel for a period of 48 h. During the experiment, attached total coliform, *Escherichia coli*, Enterococci, *Clostridium perfringens*, somatic and F-specific coliphages were measured according to standard methods. Bacteria were enumerated by using conventional membrane filtration procedure and coliphages were done using double layer plaque assays. The results showed that, the adhesion of indicator microorganisms on the surface of earthenware jar, polyethylene and galvanized steel containers was detected and this adhesion was correlated to different parameters, such as temperature, pH, turbidity, concentration of organic nutrients and indigenous microorganism communities. The survival and regrowth of indicator microorganisms on the container walls was due to the quality of raw drinking water before storage. Clay-based material was subjected to more attachment of indicator microorganisms than that of plastic-based polyethylene and metal-based galvanized steel. The lowest yield of biofilm formation by indicator microorganisms was *Clostridium perfringens* (<1 cfu.cm⁻²) while the highest was total coliform (355 cfu.cm⁻²). However, the persistence of indicator microorganisms on container walls during drinking water storage deteriorates the water quality more. To meet national guidelines of drinking water quality, it is important to conduct simple water treatment regime such as chlorination before and during water storage in containers.

Key words: Attachment, indicator microorganisms, biofilm, water containers, drinking water, rural communities.

INTRODUCTION

In the next decades, global population growth will place an increasing pressure on the environment, and thereby

threaten key resources, such as water. Effects of the current water crisis are already noticeable: more than a

billion of people have no access to safe drinking water (WHO/UNICEF, 2015). As a result, millions of people, mostly young children die every year from water-related diseases, mainly in lower- and middle-income countries. Water-borne diseases are any illness caused by drinking water contaminated by human or animal feces, which contained pathogenic microorganisms (Ashbolt, 2015). These diseases are usually infectious diarrhea such as cholera and typhoid among others. Whilst the detection of microbial pathogens has largely improved, several waterborne microorganisms may persist in so-called "safe" drinking water (Richards et al., 2015). Therefore, the issue of water quality in the transmission of diseases in humans remains a matter of great concern.

In Burkina Faso, 21% of the population has no access to adequate supply and potable water, especially in rural area, where most communities use raw groundwaters for drinking (WHO/UNICEF, 2015). Populations in rural environment are confronted with the optimal management of water supply points (Boubacar et al., 2013).

To manage the increase in water demand, measures undertaken generally focused on the quantitative aspect to meet the needs of the populations. However, the issue of water quality, responsible for diarrheal disease and other diseases associated with microorganisms are the leading cause of infant mortality in Burkina Faso (Some et al., 2014). Thus, the quality of water consumed by rural populations in Burkina Faso is a concern because of the traditional water sources competition, the lack of maintenance of hydraulic structures and the lack of appropriate disinfection methods at house level (Dianou et al., 2011).

In Burkina Faso, water from wells and drilling as ground-water sources are still used and are remained the major drinking water in rural areas for human consumption (Boubacar et al., 2013). From these water source points, the water is only provided at a certain time interval during the day. Although, connected to a supply system, the user still has to store water to have a sufficient amount available for non-supply periods. In addition, because of the long distance between drinking water sources and households, containers are used for the storage of drinking water. Water storage is therefore a necessity both for those who are connected to a non-continuous water supply system and those who depend on drinking water sources (Günther and Schipper, 2013). Under hot climatic conditions in Burkina Faso, households usually store water in local containers: earthenware jar, polyethylene bucket and galvanized steel. During the storage of water in the containers, it is well known that some microorganisms can get attached to the surface walls of the containers and form a biofilm (Hamsch et al., 2013). Attached bacteria can detach

from the surface walls and this leads to continuous recontamination of the water (Mathieu et al., 2014). Besides, there is a problem of bacterial regrowth which is related to the increase in bacteria contained in the water (Ikonen et al., 2013). Factors known to affect recontamination of water during storage at home are size of the storage vessel mouth, transfer of water between containers from collection to storage, hand contact and dipping of utensils (Singh et al., 2013), but also bacterial regrowth within the storage container (Machdar et al., 2013) and prospering of organisms in biofilms of containers (Ahmed et al., 2013). A large variety of different heterotrophic bacteria from pathogenic to non-pathogenic ones have been isolated from drinking water biofilm (Richards et al., 2015). So, during storage, water can be deteriorated to a quality often not safe for human consumption.

As a result of the increasing number of households using these containers for storing drinking water in rural areas, it is essential to assess the impact of biofilm formation on water quality to prevent the microbial survival and regrowth in water. Moreover, the type of container material may considerably influence the density of biofilm formation (Waines et al., 2011). Momba and Kaleni (2002) conducted a similar study based on plastic and metal material, but the storage containers made with clay material have not been investigated. Therefore, this study aimed at evaluating the attachment of indicator microorganisms to different water containers used in household for storage of drinking water by rural communities in Burkina Faso.

MATERIALS AND METHODS

Study area

The study covered a rural community named "Ziniare" village (250 000 habitants) which is 35 km from Ouagadougou, the capital city of Burkina Faso. Their main occupation is agriculture and their income is from crops selling. The lack and/or inadequacy of drinking water remained the main problem faced by the inhabitants. Therefore, the rural community of "Ziniare" uses well water and drilling water (boreholes) for drinking in their households. This study was realized focused because of the failure of several project for water supply and particular water quality. A questionnaire related to the type of containers and the duration for water storage was administered to 50 households of the village.

Filling of containers and sampling of stored waters

Two pilot families were chosen for this experiment. These two families were selected as representative of a family in "Ziniare". Each family constituted of a father, mother and three children and having access to both well water and drilling water. During three

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Table 1. Physico-chemical quality of raw waters from well and drilling.

Parameter	Well water			Drilling water		
	EJ	PE	GS	EJ	PE	GS
Temperature (°C)	26.5±1.15	30.1±1.45	30.6±1.22	25.4±1.26	29.1±1.31	29.8±1.39
pH	7.57±0.16	7.59±0.16	7.55±0.16	7.18±0.16	7.21±0.16	7.17±0.27
Turbidity (NTU)	32.01±4.46	27.60±3.67	33.24±5.21	9.24±1.73	9.32±1.26	9.19±1.02
DOC (mg l ⁻¹)	21.54±2.91	17.69±3.01	14.73±2.72	19.35±2.23	15.18±1.98	12.64±1.69

weeks, raw waters (12 samples) for the experiment were collected once a week from well and drilling in the two households. After cleaning with soap water, the earthenware jar (EJ), polyethylene (PE) and galvanized steel (GS) were filled with 25 L of tested waters and transported to the laboratory to be store at ambient temperature as the community normally does (without any disinfection and covered with a lid). A 25 L sample drinking water was collected as control and stored in a sterile bottle at 4°C.

Physico-chemical analysis

Temperature and pH were measured using a hand-held multi-parameter Hanna Instruments (HI-98129, Inc., Woonsocket, Rhode Island, USA). Turbidity was measured using a hand-held turbidity meter Hanna Instruments (HI-93102, Keysborough, Australia). The glassware receiving the water samples for the dissolved organic carbon (DOC) analysis was muffled at 500°C for 4 h after cleaning. DOC concentrations were measured using a total organic carbon analyzer (Dohrmann DC-180, Sigma-Aldrich, Belgium) which uses UV-promoted persulfate oxidation. Samples were previously filtered on carbon free borosilicate 0.7 µm pore-size filter to remove particulate organic carbon.

Detachment of microorganisms from EJ, PE and GS container walls

The PE and GS containers were bought at the local market and have the shape of a bucket. The EJ containers were made especially for the experiment by local manufacturer and also have the shape of a bucket. For the detachment of microorganisms from EJ, PE and GS surfaces, some slides were considered. Therefore, 8 slides per sampling were aseptically removed from each container after 24 and 48 h and transferred into sterile plastic bottles containing 100 ml saline. To release attached microorganisms into the saline, the contents were mixed for 5 min using a vortex mixer.

Microorganism's enumeration

Indicator bacteria were enumerated by using conventional membrane filtration procedure according to International Standards Organization (ISO) protocols. The ISO 9308-1 (2000), ISO 7899-2 (2000) and ISO 17994 (2014) were used for the detection of total coliforms, *E. coli*, Enterococci and *Clostridium perfringens*, respectively. Water samples, of 100 ml were filtered through hydrophilic mixed cellulose esters membranes (Pall Corporation) of 0.45 µm pore size and 47 mm diameter. Sterile Petri dishes were filled with selective media: chromocult coliform agar ES (Merck, Germany) was used for simultaneous enumeration of total coliform and *E. coli*, chromocult Enterococci agar (Merck, Germany) was

used for Enterococci and tryptose sulphite cycloserine agar (Difco, Detroit USA) was used for *C. perfringens*. After filtration, membranes were placed in each Petri dish and incubated at 37°C for 24 h, at 44.5°C for 24 h, at 37°C for 48 h and 44°C for 24 to 48 h, for each media and temperature, respectively.

The detection of F-specific and somatic coliphages was carried using double layer plaque assays according to the ISO 10705-1 (1995) and ISO 10705-2 (2000) standards, respectively. The host bacterial strains WG49 and WG5 were exposed to the eluates to culture the F-specific and somatic coliphages, respectively. In the presence of both F-specific and somatic coliphages, plaques could be enumerated after overnight culture. All analyses were done in triplicate. The following equations (1 and 2) were used to calculate the number of attached bacteria and coliphages, respectively:

$$\text{cfu cm}^{-2} = \text{ND/surface area of slides} \quad (1)$$

$$\text{pfu cm}^{-2} = \text{ND/surface area of slides} \quad (2)$$

where N is the number of microorganisms, D the dilution factor.

RESULTS

Results of investigations

For the collection of drinking water, the results showed that 66, 21 and 13% of households use EJ, PE and GS containers, respectively. It was also revealed that 58% of households stored their water for 24 to 48 h, 26% for 12 to 24 h, 14% for 6 to 12h and 2% for about three days. Therefore, this study selected an average storage period of 48 h as the largest percentage (58%) of households which store their water for that period of time.

Physico-chemical quality of raw waters from well and drilling

The physico-chemical quality of raw waters from well and drilling is presented in Table 1. The temperature in all containers ranged from 26.5 to 30.6°C, which was relatively close to the annual mean of ambient temperature in Burkina Faso. Neutral pH range of 7.17 to 7.59 was recorded in all the containers. The turbidity ranged from 9.19 to 33.24 NTU and therefore did not meet the turbidity limit (<5 NTU) acceptable in Burkina Faso/WHO (2005) guidelines. The DOC ranged from 12.64 to 21.54 mg l⁻¹ and showed a significant presence of organic nutrients.

Table 2. Microbiological quality of raw waters from well and drilling.

Parameters	Well water (n=30)		Drilling water (n=30)	
	Ranges	Average	Ranges	Average
Total coliforms (cfu/100 ml)	996-3.19x10 ³	2.98x10 ³	76-1.98x10 ³	1.41x10 ³
<i>Escherichia coli</i> (cfu/100 ml)	54-135	84	<1-62	23
<i>Clostridium perfringens</i> (cfu/100 ml)	<1-27	11	<1-6	<1
Somatic coliphage (pfu/100 ml)	<1-650	432	<1-206	74
F-specific coliphage (pfu/100 ml)	<1-145	107	<1-35	19

Table 3. Growth of indicator microorganisms on the surface of containers during drinking water storage.

Parameters	Well water (n=30)						Drilling water (n=30)					
	EJ		PE		GS		EJ		PE		GS	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Total coliforms (cfu.cm ⁻²)	266	355	105	174	37	51	75	124	54	88	7	11
<i>Escherichia coli</i> (cfu.cm ⁻²)	9	5	5	2	4	3	7	11	4	5	2	3
<i>Clostridium perfringens</i> (cfu.cm ⁻²)	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Somatic coliphage (pfu.cm ⁻²)	7	9	4	6	1	2	11	13	7	9	5	8
F-specific coliphage (pfu.cm ⁻²)	2	5	1	3	2	3	8	12	5	7	2	5

Microbiological quality of raw waters from well and drilling

The microbiological quality of raw waters from well and drilling is presented in Table 2. Raw waters from well and drilling were contaminated by total coliforms (an average of 2.98×10^3 and 1.41×10^3 cfu/100ml, respectively), *E. coli* (an average of 84 and 23 cfu/100ml, respectively), *C. perfringens* (an average of 11 and <1 cfu/100ml, respectively), somatic coliphage (an average of 432 and 74 pfu/100 ml, respectively) and F-specific coliphage (average of 107 and 19 pfu/100 ml, respectively). The Burkina Faso/WHO (2005) guidelines for water quality required the absence of coliform bacteria in water. Therefore, the raw waters collected for this experimental study were not safe for drinking.

Growth of indicator microorganisms on the container walls during storage of drinking water

The growth of indicator microorganisms on the container walls during the storage of drinking water is presented in Table 3. Attached indicator microorganisms were observed during the experiment period. The yield (average count) of total coliforms, *E. coli*, *C. perfringens*, somatic coliphage and F-specific coliphage in all containers during the study period (48 h) was 7 to 355, 2 to 11, < 1, 8-13 and 3-12 pfu cm⁻², respectively. The lowest yield of indicator microorganisms was noted for *C. perfringens*, while the highest yield was noted for total coliforms. Moreover, the occurrence and survival of total

coliforms was greater on EJ container walls than PE and GS container walls. The regrowth of indicator microorganisms in all the containers occurred 48 h after their exposure to stored waters.

DISCUSSION

The experimental protocol described in this study was designed to evaluate the attachment of indicator microorganisms to container walls used in household for drinking water by rural communities in Burkina Faso. As a result, the adhesion of indicator microorganisms on the surface of EJ, PE and GS containers, was detected and cause formation of biofilms. Studies of Strathmann et al. (2013) showed that in water storage systems, microorganisms are mainly present on internal surfaces as attached bacteria as a biofilm, with a minor part in the water phase. Biofilm formation during water storage in this study is related to temperature, pH, turbidity, concentration of organic nutrients and indigenous microorganism's community of the stored water. Indeed, biofilm formation in water distribution systems depends on a variety of factors including the physico-chemical properties of water, the composition of biofilms, factors governing their formation and the effect and significance of these biofilms (Wu et al., 2015).

The adhesion of indicator microorganisms on the surface of water storage containers is supported by the level of temperature detected in the drinking water during storage. The ability of bacteria to grow and form a biofilm over a wide range of temperature has been studied.

Silhan et al. (2006) have shown that biofilm formation in drinking water systems were denser at about 35°C than at about 15°C. In this study, temperatures ranging from 26.5 to 30.6°C were recorded in all containers, creating favorable microorganism growing conditions. Therefore, for Strathmann et al. (2013), temperature is considered to be an important regulator of biofilm growth, especially in non-disinfected water. At the same time, during water storage, neutral pH was obtained in the different containers investigated. It is well known that neutral pH condition contributes considerably to increase in the formation of biofilm (Jones et al., 2015).

The turbidity level in different containers showed the presence of a concentration of suspended solids. It has been revealed that, there is a link between high turbidity level and the growth of microorganisms in water because the turbidity can serve as a source of nutrients for waterborne bacteria, protozoa and viruses (Wingender and Flemming, 2011). At the same time, the concentration of organic nutrients (DOC content) could be responsible for the microbial growth potential enabling the buildup of microorganisms on storage containers walls. The link between organic content and bacterial growth resulting in biological aggregates that may attach to the surface of distribution systems has been reported (Liu et al., 2013). Biofilm formation is usually promoted on the surface of a material if that material is able to supply the required nutrients for microbial growth (Strathmann et al., 2013). Therefore, the occurrence and persistence of microorganisms on the surface of EJ, PE and GS could be related to the level of turbidity and DOC in stored waters.

One of the other important factors involved in the attachment of indicator microorganisms is their occurrence and their persistence in stored waters. This is due to the original presence of indicator microorganisms in the raw waters (from well and drilling). The survival and regrowth of indicator microorganisms during biofilm formation is due to the occurrence of indigenous microorganism's community in the raw waters (Wu et al., 2015). As total coliform and *E. coli* were constantly present in raw waters, they were also found in stored waters in the different containers. However, only *C. perfringens* was recorded in stored waters at a low concentration during the study period. Concerning the coliphages, a higher count was found in the well water than in the drilling water. With the exception of *C. perfringens*, the survival of indicator microorganisms on the surface of household containers consisted of total coliform, and *E. coli*, somatic and F-specific coliphages, although the yield for coliphages was lower than that of total coliforms and *E. coli*. Whereas the occurrence and persistence of total coliforms and *E. coli* was always found during storage on the surface of containers, other microorganisms not investigated in this study, could occur. So competition of microorganism's community for limited organic nutrients could be responsible for the DOC concentration in the stored waters.

This study has shown that the regrowth of indicator microorganisms occurred 48 h after the exposure of water in containers. This regrowth was observed more with total coliforms than with other indicator microorganisms studied. It is well known that total coliforms include a heterogeneous group constituting the genera *Escherichia*, *Citrobacter*, *Klebsiella*, *Enterobacter*, *Serratia* and *Rahnella* (Richards et al., 2015). Although, these genera were not identified in this study, they could be released into the stored water and deteriorate its quality. Indeed, biofilm formation in drinking water systems may deteriorate water quality by microbial activity, and in some cases, constitute a serious health hazard for consumers, either due to pathogenic bacteria growing in the biofilm or since biofilms may provide a safe haven for intruding pathogens (Fabris et al., 2016). Moreover, the persistence of coliphages on the surface of storage containers was observed and their potential of regrowth was even minimized during the storage. However, their release into drinking water could lead to a risk of viral infection for consumers.

During water storage, clay-based material (EJ) supported more attachment of indicator microorganisms than the plastic-based material (PE) and the metal-based material (GS). So, there is a direct relationship between the type of surface of storage materials and the density of biofilm formation. Several investigations have shown significant differences in biofilm formation on various materials over several months (Waines et al., 2011), showing that container material influences considerably biofilm formation, at least, in the short term. Thus, the quality of water during storage depends on the type of surface of storage materials. In the present study, water quality is more affected in EJ than PE and GS. The study revealed that the level of DOC was higher in EJ-stored water than in PE-stored and GS-stored ones. This could also explain an increase in total coliform numbers on the surface of EJ than on PE and GS containers.

The results of this study has revealed that indicator microorganisms have survived and regrown in EJ, PE and GS containers during water storage with biofilm formation. This formation of biofilm on the surface of containers could deteriorate the quality of the water stored. Therefore, a suitable education and information program related to water safety and hygiene practices should be provided for rural communities in order to minimize microorganism's regrowth in water stored in containers. In this regard, it is also important to previously perform a disinfection of the drinking water before storage.

Conclusion

This study investigated the attachment of indicator microorganisms to water storage containers used in household drinking water by rural communities in Burkina Faso. The

results showed that, indicator microorganisms (total coliform, *E. coli*, Enterococci, *C. perfringens*, somatic and F-specific coliphages) were attached on the inner surface of EJ, PE and GS containers and this was linked to different parameters such as temperature, pH, turbidity, concentration of organic nutrients and indigenous microorganism's community. The survival and regrowth of indicator microorganisms on the inner surface of containers is due to the bad quality of raw waters before storage. Clay-based material (EJ) supported more attachment of indicator microorganisms than that of plastic-based material (PE) and metal-based material (GS). The lowest yield of biofilm formation by the indicator microorganisms was noted for *C. perfringens* while the highest was noted for total coliform. The persistence of indicator microorganisms on the surface of water containers could deteriorate the quality of drinking water and therefore, it is important to adopt simple water treatment regime such as chlorination before and during water storage in containers.

Competing interests

The authors declare that there is no conflict of interest.

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