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Adhesion of Heterotrophic Bacteria on the Surface of Containers Used for Well Water Storage in Garoua (North Cameroon)

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Authors' contributions

This work was carried out in collaboration between all authors. Author MD designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors DEM and BG managed the analyses of the study. Authors MN and TN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The abundance of heterotrophic bacteria adsorbed on the walls of four types (polypropylene, glass, aluminium, clay) of containers used for well water storage in Garoua was compared.
Methodology: Water samples were obtained from two wells. In each well water sample, 3 sterilized slides of each type of container were introduced, at mid-depth from the surface. Their immersion times were respectively 24, 48 and 72 h. After an immersion time, slides were aseptically removed from the water samples and transferred into sterile test tubes containing 10 ml of saline. The resulting bacterial suspension was used to determine abundance of heterotrophic bacteria adsorbed on the slides.
Results: The maximum abundance of adsorbed bacteria (8.06×10^4 CFU/cm^2 to 2.47×10^6 CFU/cm^2)

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was obtained on the clay slide while the minimum (45 CFU/cm^2 to 5.90x10^3 CFU/cm^2) was recorded on the aluminium slide from first to third day of water storage. The bacterial abundance differs significantly depending on the slide type and the duration of water storage (P <0.001).

Conclusion: Rather than using massively clay pots for drinking water storage, people in North Cameroon are recommended to use metallic or glass containers to reduce bacterial adhesion.

Keywords: Adhesion; heterotrophic bacteria; household containers; well water.

1. INTRODUCTION

Stored water is one of the most important types of water supplies for domestic purposes in Cameroon. Well Water is stored in different types of containers at home for various purposes in Garoua (North Cameroon). Our investigations showed that approximately 60.7% of households store water at home for 24 hours, 23.3% for 48 hours, 9.3% for 72 hours and only 6.7% from 72 to 120 hours. Among the containers used, 75.7% are clay pots, 13.7% are plastic buckets, and 10.6% are plastic cans, metallic containers and others. The bacteriological quality of the water in these containers varies during the storage. The increase in bacterial abundance in stored water may be related to bacterial growth and biofilm formation [1]. According to Burkowska-But et al [2], the storage may affect water quality to such an extent that the water, which initially met the microbiological criteria for water intended for human consumption, may pose a health risk. Nola et al [3] found that storage of untreated groundwater for a long period would increase the health risk to the consumers in the short term if it contains potentially pathogenic bacteria due to their potential growth and activity. Microorganisms normally grow in water and on surfaces in contact with water as biofilms [4,2]. The biofilms interact with waterborne pathogens and affect their persistence. The persistence of these pathogens is considerably increased if they form a new biofilm or colonize an existing one [4]. The repeated use of the same containers for water storage results in biofilm formation containing live and metabolically active bacterial cells [2]. The material used for storage containers can differently support the development of biofilms [5]. The presence of biofilms leads to continuous contamination of the water phase resulting from the releasing of the attached growing biomass [6].

The majority of studies undertaken on water in Cameroon focused on surface and groundwater quality with little work being devoted to point-of-use water quality assessment.

Little is known about the detection of bacteria attached to household containers used for water storage. Our study aimed at comparing the development of biofilms on the walls of containers commonly used in Garoua, using water samples from two wells. The abundance of heterotrophic bacteria adsorbed on four types of slides (polypropylene, glass, aluminium, clay) was assessed during three days of water storage.

2. MATERIALS AND METHODS

2.1. Study Site

The Garoua metropolis (North Cameroon) is located at latitude 9°18’ N, longitude 13°24’ E at the bottom of the Benue River basin and the main tributary waterway of the left bank of the Niger River. It is characterized by a typical Sudano-Sahelian climate with two seasons: the dry season from November to April and the rainy season from May to October [7,8]. Generally, rainfalls do not exceed 985 mm in the year. The monthly average temperature varies from 18°C to 42°C; the highest value is often noted during March and the lowest in January.

Two wells (W1, W2) were chosen as sampling sites based on:

- geographic location in order to follow spatial variability of groundwater quality;
- uses, the wells used as drinking water source were privileged;
- number of users, more used wells were preferred.

2.2 Water Sample Collection

At each sampling point (W1, W2), two water samples were collected, one in a 500 ml sterile glass bottle and the other in a 1000 ml polyethylene bottle. Four water samples (n=4) were collected bimonthly at each sampling point during dry season (February-March) in order to determine the well water characteristics. The

sample in the polyethylene bottle was used for physico-chemical analysis [9]. Samples collected in glass bottles were destined for bacteriological analyses and experiment in laboratory.

2.3 Physicochemical Analysis of Well Water

The main physicochemical parameters considered were temperature, total dissolved solids (TDS), pH, electrical conductivity (EC), salinity, dissolved oxygen, dissolved carbon dioxide, and alkalinity. These parameters were chosen in accordance with their general importance on bacterial metabolism and the availability of our laboratory equipments. Temperature, pH, electrical conductivity, TDSs and salinity were measured using a portable pH/conductivity/TDS/salinity meter, Extech EC500. The dissolved oxygen was measured using an oximeter, Extech DO 400. Measurement of the redox potential was made by potentiometric determination of electron activity with an ORP meter, Extech RE300. Calibration and standardization of apparatus were performed according to the manufacturer's instructions before in situ use. Alkalinity was determined using colorimetric titration with hydrochloric acid (0.02 N) and mixed bromcresol green–methyl red indicator, as outlined by American Public Health Association [9]. The estimation of dissolved carbon dioxide (CO_2) was done by titrimetric method using a slight excess of sodium hydroxide solution (0.025 N), checked by means of phenolphthalein indicator, to neutralize the carbon dioxide immediately after water sampling, and titration with hydrochloric acid solution (0.02 N) was carried out later in the laboratory [10].

2.4 Bacteriological Analysis of Well Water

Heterotrophic bacteria were enumerated using the spread plate method with plate count agar (Bio-Rad, France). 0.1 ml raw/diluted water sample was pipetted onto the surface of a plate count agar in sterile 90×15 mm Petri dishes (Gosselin a Corning Brand, France). Inoculum was spread using a bent glass rod over the surface of the plate. Inoculum was let being absorbed completely into the medium before incubating at 37℃ for 72 h. Two repetitions were done using dilutions in order to select the plate that contains 30–300 CFU. The counting of colonies was performed using a colony counter (Jouan CC120, France) according to recommendations from American Public Health Association [9].

Membrane filtration was used to enumerate qualitative microbial indicators [total coliforms (TCs), faecal coliforms, *Escherichia coli*, and faecal streptococci (FS)] according to the standard methods [9]. For each well, raw/diluted water sample was filtered through a sterile 47 mm, 0.45 μm-pore-diameter, gridded membrane filter, under partial vacuum. Funnel was rinsed with three 30 ml portion of sterile dilution water. Filter was removed with a sterile forceps and placed on agar in 55×9 mm Petri dish (Gosselin a Corning Brand, France). The m-Endo LES (Difco Laboratories, Detroit, MI, USA) agar was used for the enumeration of TCs and faecal coliforms and *E. coli*, after 24 h incubations at 37 and 44℃, respectively. The typical coliform colony on m-Endo LES has a pink to dark-red colour with a metallic surface sheen. The resulting metallic green sheen colony forming unit (CFU) were subsequently identified according to Holt et al. [11]. The identification of each *E. coli* colony was confirmed using API 10S system after a subculture on a standard agar medium.

Slanetz–Bartley and bile esculin azide (BEA) agars (Biokar Diagnostics, Beauvais, France) were used for FS counts after 24 h incubation at 37℃ [12]. Brownish-black colonies with brown halos confirm the presence of FS on BEA. Each sample was analysed in duplicate 100 and 10 ml (after appropriate dilutions using 0.85 g/l NaCl solution). All analyses were carried out in triplicate.

2.5 Adhesion of Heterotrophic Bacteria on the Surface of Slides in Well Water

In each well water sample, 3 sterilized slides of each type of container held by clamps attached to a wire of 0.1 mm diameter were introduced into the water, in a flask, at mid-depth from the surface. Their immersion times were respectively 24, 48 and 72 h. After an immersion time, slides were aseptically removed from the flasks and transferred into sterile test tubes containing saline (10 ml). To release attached microorganisms, the slides were washed four times in the saline, by shaking for 20 seconds using a Vortex Genie 2 (Scientific Industries) at 1000-1200 rpm. The resulting bacterial suspension (10 ml) was used to determine abundance of heterotrophic bacteria adsorbed on the slides. Heterotrophic bacteria were

enumerated using the spread plate method with Plate Count Agar (Bio-Rad, France), incubated at 37℃ for 72 hours [9]. Appropriate dilutions were made using sterile saline contained in test tubes. All analyses were performed in triplicate.

2.6 Data Analysis

Bacterial count (x) expressed in CFU/cm^2 was calculated using the formula:

$$X = \frac{\text{Bacterial abundance } (\text{CFU}.mL^{-1})}{\textit{Slide surface } (cm^2)} .10mL$$

Changes in bacterial densities across time for different slides were plotted using the Sigma Plot 10.0 software. The values of bacterial counts (x) are transformed using the equation $y = \log_{10}(x + 1)$. The arithmetic mean of the log-transformed (base 10) values was utilized to summarize bacterial abundance at a given time. Two ways Analysis of variances (ANOVA) was performed using the software XLSTAT 2007 to determine potential effects of storage time and type of slide on bacterial adhesion.

3. RESULTS AND DISCUSSION

3.1 Physicochemical and Bacteriological Characteristics of Well Water

The results of the physicochemical analysis show that water samples range from acidic to basic with low to high mineralization. The TDS and salinity of water samples also varied with water source. The well W2 had the highest pH value, a relatively higher salinity, TDS and also the highest electrical conductivity (Table 1). According to Djaouda et al. [13], these fluctuations are likely linked to anthropogenic influences to which they are subject, location and/or geological substrate of watershed.

Abundance of each bacterial group isolated from sampled wells (Table 1) indicates clear evidence of faecal water contamination. The concentrations of Total coliforms, Faecal Coliforms, Heterotrophic bacteria and Faecal streptococci varied from one well to another, with the highest values recorded at well W2 (Table 1). However, *E. coli* were more present in well W1 water samples.

Several contemporary studies have shown that the presence of FCs and streptococci in groundwater is a frequent phenomenon. The presence of coliforms in drinking water has been regarded as an important marker of water safety [14]. Well W2 is more exposed to contamination than well W1. The bacterial pollution of well water is mostly due to poorly constructed and located latrines, open rubbish dumps and open drains [15]. Due to space limitations and a lack of a proper drainage network, the traditional latrines system is extensively used in this area, and faecal matter from these latrines into the nearby wells might have contaminated the well water sources. There is a need to educate the public about the quality of their water sources and the importance of clean and healthy surroundings near water sources and to implement measures to prevent the contamination of water sources in the community [13]. Boiling and/or filtering water is advised until disinfection and retesting to confirm that the contamination has been eliminated.

3.2 Adsorbed Heterotrophic Bacteria

The results revealed that the maximum bacterial abundance for the well W1 (8.06×10^4 to 1.76×10^6 CFU/cm^2) was obtained on the clay slide from first to three days of water storage. The minimum bacterial abundance (45 to 5.90×10^3 CFU/cm^2) was obtained on the aluminium slide during water storage. In well W2 water sample, the highest bacterial abundance (1.11×10^5 to 2.50×10^6 CFU/cm^2) was also obtained on the clay slide from first to three days of water storage. The minimum bacterial abundance (3.30×10^2 to 5.25×10^3 CFU/cm^2) was observed on the aluminium slide during the water storage. The ANOVA test performed on all data obtained showed that the abundance of heterotrophic bacteria differs very significantly depending on the slide and the duration of water storage ($P < 0.001$). Variations in abundance of bacteria attached to slides are represented in Fig. 1. The abundance of adsorbed heterotrophic bacteria increases throughout the water storage period.

These results are similar to those obtained by Wingender et al [6]. It was demonstrated that adhered bacteria are much less affected by changes in the physico-chemical characteristics (temperature, pH, nutrient levels, and metabolic, toxic substances) of water than planktonic bacteria [16]. Biofilm-forming bacteria are far less sensitive to environmental factors, an attribute which guarantees their survival even under extremely unfavorable conditions [17].

Table 1. Characteristics of sampled wells and mean (and standard deviation) values of physico-chemical and bacteriological parameters of water samples

Parameter	Well	
	W1	**W2**
GPS location (altitude)	N9.342 E13.389 (246 m)	N9.32437 E13.396 (221 m)
Quarter	Base Aérienne	Roumdé Adjia
Depth (m)	11	8.6
Water temperature (℃)	30.3 (0.3)	30.2 (0.6)
Water pH (CU)	6.85 (0.26)	7.52 (0.15)
Water electrical conductivity (µS/cm	626 (49.94)	1154 (96.77)
Water dissolved oxygen (mg/l)	2.2 (0.9)	2.7 (0.5)
Water salinity (ppm)	315 (24.85)	586 (45.40)
Water alkalinity (mg/l)	28.7 (11.4)	34.0 (17.1)
Water TDS (mg/l)	435 (36.39)	819 (65.25)
Water dissolved CO_2 (mg/l)	7.6 (2.0)	5.3 (0.1)
HPC (CFU/100 ml)	3.45×10^6 (5.0×10^4)	7.03×10^7 (1.15×10^4)
Total coliforms (CFU/100 ml)	1.83×10^4 (8.19×10^2)	3.06×10^4 (1.24×10^3)
Faecal coliforms (CFU/100 ml)	9.33×10^3 (3.21×10^2)	2.56×10^4 (3.83×10^3)
Escherichia coli (CFU/100 ml)	3.53×10^3 (5.80×10^1)	2.93×10^2 (1.10×10^1)
Faecal streptococci (CFU/100 ml)	3.57×10^2 (3.8×10^1)	3.67×10^3 (2.08×10^2)

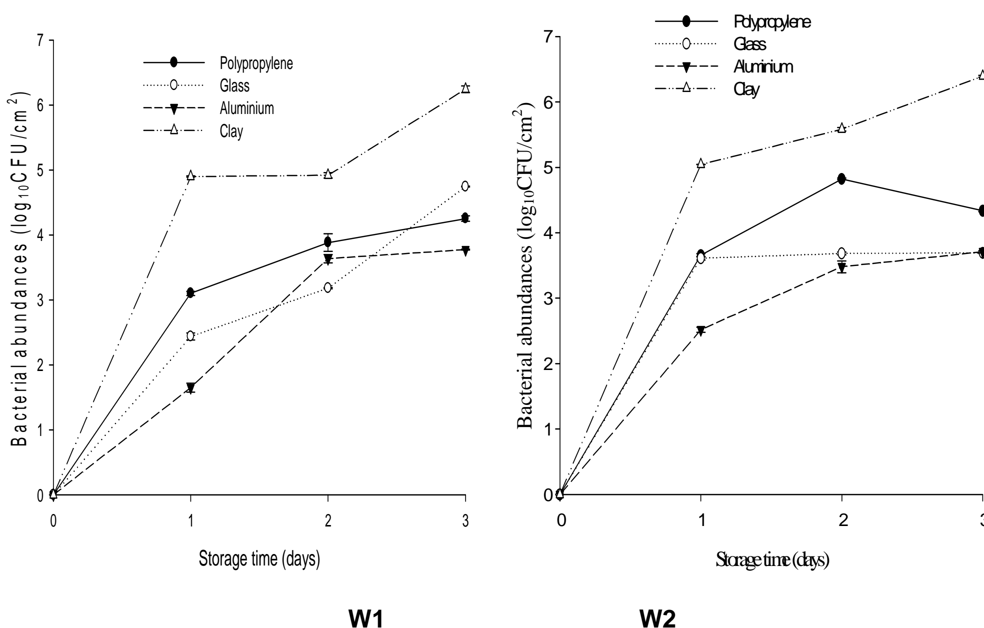

Fig. 1. Variation in average densities ($log_{10}CFU/cm^2$) of heterotrophic bacteria that were incorporated into biofilms on the surfaces of slides (aluminium, clay, polypropylene, glass) during storage of wells (W1, W2) water

Mean ± S.E.M = Mean values ± Standard error of means of three experiments

Biodegradable organic compounds in water favour the formation of biofilms [18]. The walls of the storage containers of water have very different behaviours with respect to the adhesion of bacteria present in the water. The adhesion of bacteria also varies depending on the slide, the nature and concentration of dissolved ions in water [19-21].

The high number of adsorbed heterotrophic bacteria on the clay slide could be explained by various factors determining the formation of biofilms. The clay pot used to make the slide has a good porosity which allows its water to maintain its freshness. According to Farkas et al [22], a hydrophobic slide having crevices and deposits is more easily colonized by bacteria. While the organic matters in the clay are removed by oxidation during the manufacturing process of pot, some chemical compounds may later on attach to the pores on its walls and stimulate the growth of microorganisms in the biofilm [22]. As highlighted in this work, other studies have reported relatively high abundance of bacteria attached to the plastic surface. Plastic materials can release biodegradable compounds increasing bacterial growth [23,24]. The low abundance of bacteria attached to the aluminium and glass are related to the low amount of nutrients released from these materials. Furthermore, the chemical properties of the slide surface can influence biofilm formation [25-27]. The pH of the slide due to its chemical composition influences the formation of biofilms. The optimum of biofilm formation is obtained for a neutral to slightly alkaline pH [28]. In addition, the topography of each slide has an influence on bacterial adhesion. Bacterial colonization seems faster on the polypropylene than the glass and aluminium due to higher roughness of the first [29].

Biofilms can degrade water quality through microbial activities, and in some cases represent a serious health risk to consumers, due to the potential presence and growth of pathogenic bacteria within them [30,31,5,6]. The slides used in this study allow the adhesion of bacteria at varying levels according to their physico-chemical nature. Abundance of adsorbed heterotrophic bacteria is higher in well W1 than well W2 samples. These observations could be linked to the physico-chemical properties of water and the number of planktonic bacteria initially present in water [6].

4. CONCLUSION

The results of this study indicated that well waters in Garoua are subject to faecal contamination and while stored at home for further uses, bacteria significantly grow on the container's wall. The bacterial adhesion on the containers' walls depends on the chemical nature of the substrate. Physicochemical and bacteriological water properties could also influence the biofilms formation and development.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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