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ORIGINAL ARTICLE

Survival and growth of *Vibrio cholerae* and *Escherichia coli* in treated groundwater consumed in northern Cameroon

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Abstract

Treated groundwater is a major source of drinking water but subject to potential contamination of fecal–oral pathogens. To understand ecology of the pathogens in the treated water, this study evaluated survival and growth of *Vibrio cholerae* and *Escherichia coli* in the treated groundwater in northern Cameroon. *E. coli* and *V. cholerae* O1 were isolated from human feces. Water samples were collected from the following sources: a well, tap water from the Cameroon Water Utilities Company, and mineral and borehole waters sold in Maroua, respectively. These waters were treated by one or more processes, including autoclaving, filtration, chlorination and ozonation and were used for the constitution of microcosms. *E. coli* and *V. cholerae* were inoculated into each microcosm at respective concentrations of 50 CFU/10 mL (separately) and 40 CFU/10 mL each (together). All bacterial strains survived in all microcosms were used. The ability to survive and grow varied with the bacterial strain and microcosm ($P < 0.05$). When inoculated separately into the same type of microcosms, *V. cholerae* grew faster than *E. coli* with the latter even showing decrease in concentration in mineral water. When inoculated together, *V. cholerae* grew faster than *E. coli*, except in autoclaved well water and filtered and autoclaved well water. Autochthonous ultramicroflora inhibited the growth of *E. coli* in filtered well water ($P < 0.05$).

Keywords *Vibrio cholerae* · *Escherichia coli* · *Drinking water* · Northern Cameroon

Introduction

Drinking unsafe water is one of main causes of many waterborne diseases, especially in developing countries (Nanfack et al. 2014). According to the World Health Organization, unsafe drinking water is responsible for 70% of waterborne diseases in developing countries (2013).

In Cameroon, diarrheal disease is responsible for 15–20% of total human deaths per year (Ngwe and Banza-Nsungu 2007). On average, only, 13.5% of population living in rural areas and 17% of population in urban areas have access to improved drinking water (BAD 2010). The poor supply of potable water is due to many reasons, primarily uncontrolled urbanization, extreme poverty of the population, and insufficient funds from the public authorities (UN-Habitat 2001). The majority of the population must therefore find ways and means to cope with this challenge (GWP 2010). According to a survey by the INS (2013), 90.2% of the population living in the city of Yaounde (capital of Cameroon) have access to treated water (72.7% to tap water, 14.3% to public tap water, and 3.2% to mineral water). The rest 9.8% use protected (3.8%) and unprotected (2.2%) springs, boreholes (3.2%), and well waters (0.6%).

For more than a decade, cholera epidemics have been recorded every two years in the Lake Chad Basin spanning northern Cameroon, Chad, Nigeria and Niger (Kaas

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et al. 2016; UNICEF 2016). Between 2004 and 2013, 46,172 cholera cases were reported in Cameroon with 979 deaths in the Far North Region (Momba and El-Liethy 2017; UNICEF 2013). According to the WHO (2011), consuming treated water reduces the risk of waterborne diseases such as dysentery and cholera. During the cholera outbreaks, medical authorities usually recommend that people use tap water, mineral water, or disinfect well and borehole waters by chlorination, filtration and/or boiling. Emphasis is also placed on promoting hygienic practices to reduce contamination of water supplies and foods by the fecal–oral pathogens (UNICEF 2016).

Traditionally, drinking water, regardless of its sources, treated or not, is stored in individual households for daily use. Yet, the stored water is subject to contamination by the microorganisms at different points thereby their growth in the water (Djaouda et al. 2010, 2013; Burkowska-But et al. 2015). The quality of stored water is affected by different factors such as water source, container characteristics, duration and condition of storage, conditions of transportation from its source, and non-compliance with hygienic measures (Ngnikam et al. 2007; Djaouda et al. 2010). The survival of fecal bacteria (e.g., *E. coli*, *Salmonella* sp., and *V. cholerae*) in treated well water would depend on the bacterial strain and water treatment applied (Djaouda et al. 2013).

In general, among the microorganisms typically present in water of tropical environments, main pathogenic bacteria include *E. coli* O157-H7, *V. cholerae*, *Salmonella* spp. and *Shigella* spp. (WHO 2013). During a cholera outbreak, occurrence of fecal contamination of stored water could come with finding of *V. cholerae* and commensal enteric bacteria such as *E. coli* in the water. Although previously treated, such water could contribute to the spread of the epidemic. Treated water, stored in households, may be contaminated by fecal bacteria during cholera outbreaks. Many fecal bacteria are likely to remain in the water and be responsible for a gradual deterioration of water quality. Accidental contamination of water supply can occur following repairs on the distribution system and, if this is associated with pathogenic *V. cholerae*, a cholera outbreak can happen. Few studies focused on the ecology of pathogenic *V. cholerae* O1 in water sources and analysed its role in cholera transmission through its interaction with abiotic factors (Islam et al. 2020). However, very few data are available on the survival and growth of fecal bacteria in treated groundwater in northern Cameroon. In addition, in the event of treated water contamination, the dynamics of *V. cholerae* and commensal enteric bacteria such as *E. coli* remain largely unknown. This information is crucial because the transmission of cholera is plausibly mediated by the survival of *V. cholerae* in treated water contaminated during handling and storage.

The objective of this research was to evaluate the survival and growth of *E. coli* and *V. cholerae* in treated groundwater, including tap, well, mineral and borehole sources.

Materials and methods

Study site

Northern Cameroon is located between 9° and 13° north latitude and between 13° and 15° east longitude with fairly varied terrain consisting of plains and mountains. This region has a typical Sudano-Sahelian climate with two distinct seasons: the rainy season from May to September and the dry season from October to April (M'Biandoun et al. 2003; Ndongo et al. 2015). Rainfall is extremely variable with an annual average not exceeding 1000 mm. The temperature varies between 18 and 41 °C (Sighomnou 2004).

In this region, groundwater is the main source for drinking (Djaouda et al. 2014; Healy Profitós et al. 2016). The public water distribution system uses chlorinated groundwater. For those without access to chlorinated tap water, wells or boreholes serve as the main water source. Wells' depth is comprised between 2.3–10 m, and these water sources serve 34% of the population (Zoua et al. 2020). Before drinking, well water is usually treated by filtration and/or boiling by few poor households that are advised on the health risks related to unsafe water. The bottled mineral water is much more expensive and usually consumed by the richer households because of concerns over potential risk of the tap water and its organoleptic quality (particularly the taste).

Choice of water types for the study

Tap, well, borehole, and mineral waters were chosen because of their wide use for drinking and domestic purposes. The borehole and mineral waters were, respectively, treated by ozonation and ozonation followed by chlorination. Tap water was treated by chlorination (0.1 mg/L residual chlorine). Well water was treated by physical disinfection techniques (filtration and/or autoclaving).

Water sampling

Before taking sample from the taps, water was flushed for 5 min and then the mouth of the hand pipe was sterilized with a spirit of lamp flame and then cooled by running water. Samples were collected from wells, and flasks were filled in the same way as people normally use to fill their water containers. Bottled borehole and mineral waters in 1.5L bottles were purchased at the Central Market of Maroua and immediately transported to the laboratory at ambient temperature (24 °C).

Choice of bacterial strains

E. coli and *V. cholerae* were selected for their importance in the environment and public health. *E. coli* is an excellent indicator of fecal contamination of water (Edberg et al. 2000; Chippaux et al. 2002; Valenzuela et al. 2009). *V. cholerae* O1 has been the cause of recent cholera outbreaks in northern Cameroon. The *E. coli* strain was isolated in the laboratory from human feces. Its identification was made according to the techniques described by Holt et al. (2000). The strain of *V. cholerae* O1 was obtained from the Pasteur Center of Cameroon. The biochemical and serological tests were used for confirmation of the bacterial strain (CDC 2015).

Preparation of bacterial stocks

For the preparation of bacterial stocks, a colony forming unit of either *E. coli* and *V. cholerae* from standard agar medium was inoculated into 100 mL of nutrient broth for 24 h at 37 °C. The strain of *V. cholerae* was grown on alkaline nutrient agar and *E. coli* on standard non-selective plate count agar (Bio-Rad) for later use. Cells were then harvested by centrifugation at 3000 g for 10 min at ambient temperature and washed twice with sterile NaCl solution (8.5 g/L). The washed cells were suspended in sterile saline for inoculation of studied microcosms.

Microcosms

Well water samples were treated by filtration (0.22 µm) and/or autoclaving at 121 °C for 15 min. Borehole, mineral and tap water, disinfected at the industry level, did not undergo any additional treatment. Seven types of microcosms were performed: sterile saline (SS), filtered well water (FWW), autoclaved well water (AWW), filtered-autoclaved well water (FAWW), chlorinated tap water (CTW), ozonated borehole water (OBW) and mineral water (MW) treated by ozonation and chlorination. For each type of treated water, three microcosms of 100 mL each were made in flasks.

Bacteriological control of microcosms

A microbiological quality control of the different microcosms was carried out before inoculation of the bacterial strains. 10 mL of each type of treated water was collected and analyzed, using the membrane filtration technique and the EMB (Eosine Methylene Blue) agar (Abteck Biologicals Laboratries, Liverpool, England) for *E. coli* and TCBS (Thiosulfate Citrate Biles Salts Sucrose agar) (Merck KGaA, Darmstadt, Germany) for *V. cholerae* seeking (APHA 2012). All analyses were repeated three times. The water samples with no these microorganisms found were used for the constitution of the microcosms.

Inoculation of microcosms

To approach the natural conditions of bacterial contamination of water, the various microcosms were contaminated by bacterial suspensions in saline solution. One hundred microliters of water from each microcosm (flask of 100 mL water) was removed and replaced with 100 µL of bacterial suspension. A first series of different microcosms was contaminated at level of 50 CFU/10 mL of *E. coli*, a second series at 50 CFU/10 mL of *V. cholerae*. The third set was contaminated with both 40 CFU/10 mL *E. coli* and 40 CFU/10 mL V. *cholerae*. The bacterial concentrations used for the inoculation of microcosms were inspired by those usually observed under natural conditions in the study area (Djaouda et al. 2014). Flasks were then incubated without shaking, in the dark, at room temperature (30 ± 2 °C) in a similar way to Elmahdy et al. (2018).

Evaluation of the survival and growth of *V. cholerae* and *E. coli* in water microcosms

The local people use containers that allow water storage for up to three days, but normally households store the water for 24 h (Djaouda et al. 2013). For this reason, our study used one day as observation time unit. The initial bacterial concentration of *V. cholerae* and *E. coli* was immediately determined after contamination of the microcosms (J0). The water storage lasted for three days, and analyses were performed after 24 h, 48 h, and 72 h (J3) to determine bacterial concentrations. Experiments were done thrice. Bacterial cultures were incubated at 37 °C. The membrane filtration technique (APHA 2012) was used to enumerate *E. coli* and *V. cholerae* in ten millilitres of raw or diluted water sample from each microcosm. Plate count agar (PCA) and alkaline nutrient agar were used to enumerate *E. coli* and *V. cholerae*, respectively. In order to determine its potential influence on the survival and growth of *V. cholerae* and *E. coli*, concentration of autochthonous ultramicroflora was simultaneously determined on PCA in FWW microcosm. It was found difficult to distinguish colonies of the experimental bacteria from those of the autochthonous ultramicroflora on PCA. Therefore, 0.22 µm filtered FWW water was used for enumeration of autochthonous ultramicroflora on PCA. Expression of bacterial concentration was given in colony forming unit (CFU) per volume of water.

Data analysis

The variations in bacterial concentrations as a function of time in each microcosm were explored using the Sigmaplot 10.0 software. Due to their skewed distributions, the bacterial counts (x) were log-transformed (Daumas 1982). Two nonparametric tests (Kruskal–Wallis and Wilcoxon) were performed to investigate whether there was any difference in the median concentration of *E. coli* and *V. cholerae* among different microcosms and water storage periods using R (R Core Team, Version 3.6.3). The Spearman correlation test to determine the relation between the autochthonous ultramicroflora and the experimental fecal bacteria was performed using also R (R Core Team, Version 3.6.3).

Results

Dynamics of concentration of *V. cholerae* and *E. coli* inoculated separately in microcosms

Variations in the concentrations of *V. cholerae* and *E. coli*, inoculated separately into each microcosm type, for the three days period of water storage, are shown in Fig. 1.

Escherichia coli

Overall, the concentration of *E. coli* increased from J0 to J3 in all microcosms, except for mineral water (MW) (Fig. 1). The median concentration of *E. coli* increased from J0 (1.71 $\log_{10}$CFU/10 mL) to J3, considering saline (SS) (3.84 $\log_{10}$CFU/ 10 mL, interquartile range (IQR) = 1.46), tap water (CTW) (1.78 $\log_{10}$CFU/10 mL, IQR = 1.11), well water (AWW) (2.08 $\log_{10}$CFU/10 mL, IQR = 1.69) and filtered and autoclaved well water (FAWW) (2.80 $\log_{10}$CFU/10 mL, IQR = 2.18), borehole water (OBW) (1.87 $\log_{10}$CFU/10 mL, IQR = 2.00) and filtered well water (FWW) (1.72 $\log_{10}$CFU/10 mL, IQR = 2.18) microcosms. On the other hand, the median concentration of *E. coli* in the mineral water microcosm (MW) decreased from J0 to J3 (0.90 $\log_{10}$CFU/10 mL, IQR = 0.66).

Vibrio cholerae

The median concentration of *V. cholerae* increased from J0 (1.71 $\log_{10}$CFU/10 mL) to J3, resulting in a median concentration of 2.59 $\log_{10}$CFU/10 mL (IQR = 0.19), 3.42 $\log_{10}$CFU/10 mL (IQR = 0.59), 2.17 $\log_{10}$CFU/10 mL (IQR = 0.54), 2.29 $\log_{10}$CFU/10 mL (IQR = 0.58) for the microcosms FWW (Fig. 1f), MW (Fig. 1b), AWW (Fig. 1d) and FAWW (Fig. 1e), respectively. During the three days of water storage in the microcosm CTW, a small increase in *V. cholerae* concentration was noted (from 1.71 $\log_{10}$CFU/10 mL to median concentration 2.35 $\log_{10}$CFU/10 mL, IQR = 0.58) (Fig. 1c). In the microcosms OBW (Fig. 1g) and SS (Fig. 1a), *V. cholerae* concentration increased rapidly from J0 to J3 (from 1.71 $\log_{10}$CFU/10 mL to median concentration 3.15 $\log_{10}$CFU/10 mL, IQR = 0.95 and from 1.71 $\log_{10}$CFU/10 mL to median concentration 3.16 $\log_{10}$CFU/10 mL, IQR = 0.26, respectively). Overall, concentration of *V. cholerae* increased in all microcosm waters during the three days of water storage. However, this increase was lower in CTW microcosm.

Dynamics of concentration of *V. cholerae* and *E. coli* inoculated together in microcosms

Variations in the concentration of *V. cholerae* and *E. coli*, inoculated together into each microcosm type, for the three days period of water storage are presented in Fig. 2.

The median concentration of *E. coli* slightly increased from J0 (1.61 $\log_{10}$CFU/10 mL) to J3, resulting in a median concentration of 1.66 $\log_{10}$CFU/10 mL (IQR = 0.52) and 1.63 $\log_{10}$CFU/10 mL (IQR = 0.94) for the microcosms MW (Fig. 2b) and AWW (Fig. 2d), respectively. More increase in *E. coli* concentration was observed in the microcosms SS, OBW and FAWW, varying from 1.61 $\log_{10}$CFU/10 mL (J0) to 2.64 $\log_{10}$CFU/10 mL (IQR = 0.72), 2.06 $\log_{10}$CFU/10 mL (IQR = 0.88) and 2.41 $\log_{10}$CFU/10 mL (IQR = 0.51) (J3), respectively. On the other hand, the median concentration of *E. coli* in the CTW and FWW microcosms decreased from J0 to J3 (1.34 $\log_{10}$CFU/10 mL, IQR = 0.58) and 0.84 $\log_{10}$CFU/10 mL, IQR = 0.95, respectively).

Overall, the concentration of *V. cholerae* increased from J0 to J3 in all microcosms, except for AWW and FAWW microcosms (Fig. 2). The median concentration of *V. cholerae* increased from J0 (1.61 $\log_{10}$CFU/10 mL) to J3, considering saline (SS) (2.10 $\log_{10}$CFU/10 mL, IQR = 0.91), mineral water (MW) (2.06 $\log_{10}$CFU/10 mL, IQR = 0.92), tap water (CTW) (2.02 $\log_{10}$CFU/10 mL, IQR = 0.81), borehole water (OBW) (2.32 $\log_{10}$CFU/10 mL, IQR = 0.65) and filtered well water (FWW) (1.85 $\log_{10}$CFU/10 mL, IQR = 0.41) microcosms. In FAWW, *V. cholerae* median concentration slightly decreased on J3 to 1.56 $\log_{10}$CFU/10 mL (IQR = 0.14). For the microcosm AWW (Fig. 2d), during the three days of water storage, the concentration of *V. cholerae* remained almost unchanged (1.61 $\log_{10}$CFU/10 mL, IQR = 0). These results indicated a weak growth of *V. cholerae* in autoclaved water (AWW, FAWW) after three days of water storage.

Relation between autochthonous ultramicroflora, *E. coli* and *V. cholerae* inoculated in filtered well water

The relation between autochthonous ultramicroflora, *E. coli* and *V. cholerae* inoculated separately or together in filtered

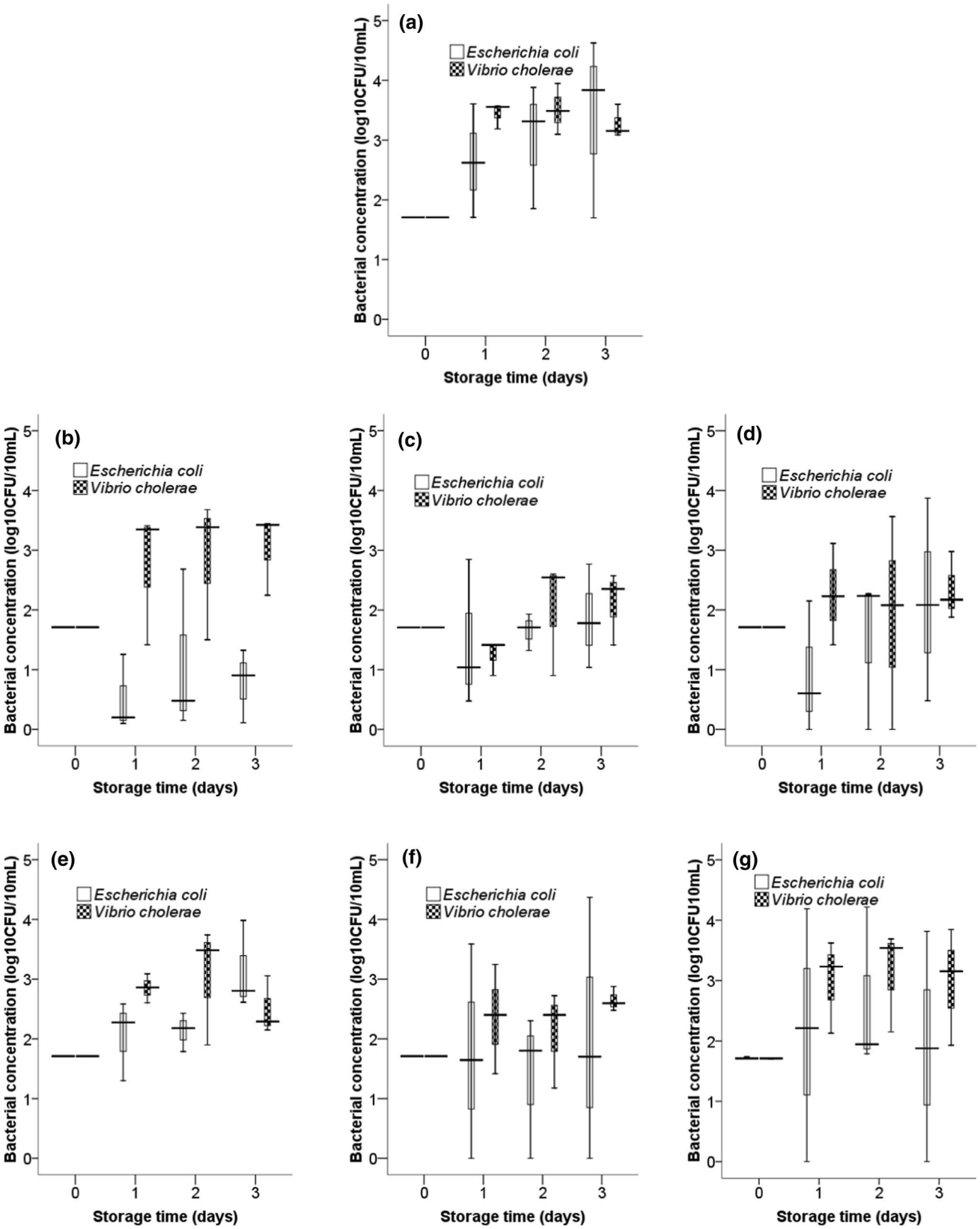

Fig. 1 Variations in concentrations of *E. coli* and *V. cholerae* inoculated separately in sterile saline **a**, mineral water **b**, chlorinated tap water **c**, autoclaved well water **d**, filtered-autoclaved well water **e**, filtered well water **f** and ozonated borehole water **g** during storage (Boxplot, $n = 3$)

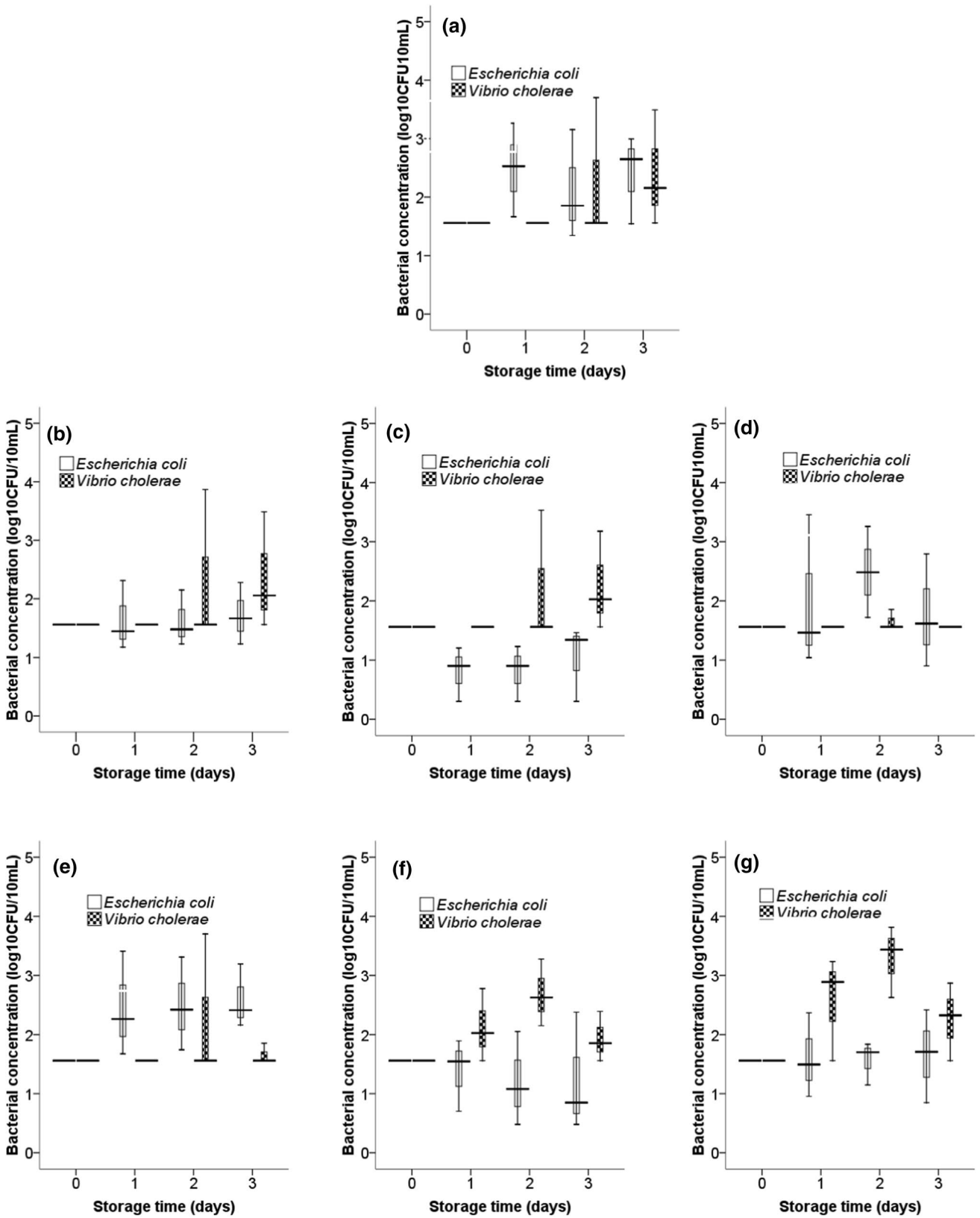

Fig. 2 Variations in concentrations of *E. coli* and *V. cholerae* inoculated together in sterile saline **a**, mineral water **b**, chlorinated tap water **c**, autoclaved well water **d**, filtered-autoclaved well water **e**, filtered well water **f** and ozonated borehole water **g** during storage (Boxplot, $n = 3$)

well waters was examined by the Spearman correlation test. Concentration of ultramicroflora in filtered well water was not significantly correlated with *E. coli* and *V. cholerae* when these bacteria were inoculated separately into the medium. However, increasing autochthonous ultramicroflora in filtered well water would not inhibit the growth of *V. cholerae* and *E. coli*. The population increase in autochthonous ultramicroflora was negatively correlated with *E. coli* and positively to *V. cholerae* when these bacteria were inoculated together into filtered well water ($P < 0.05$). The decay of *E. coli* influenced by the presence of autochthonous ultramicroflora would be favorable for the multiplication of *V. cholerae* in the medium.

Discussion

Variations of concentration of *V. cholerae* and *E. coli* inoculated separately in microcosms

E. coli

The concentration of *E. coli*, inoculated alone, increased from J0 to J3 in all microcosms, except for the MW microcosm, where this concentration decreased during storage of water. The Kruskal–Wallis test showed that at least one of the microcosms presented a median concentration of *E. coli* that was significantly different ($P < 0.05$). The concentration of *E. coli* underwent significant variations depending on the types of treated water. This variability was also noted by Djaouda et al. (2013) in a previous study. Wilcoxon test showed that *E. coli* concentration was significantly lower in mineral water than in saline ($P < 0.05$). The decrease in the concentration of *E. coli* in the mineral water was likely related to the water treatment techniques. Indeed, the chlorination following ozonation during the production of mineral water ensures long-term disinfection due to residual chlorine (Vital 2010; Goncharuk 2014).

Concentration of *E. coli* was significantly higher in saline than in tap water ($P < 0.05$). The low growth of *E. coli* in tap water was likely due to the combination of several factors. Directly after the water chlorination, *E. coli* cells are usually killed or inactivated (Al-Bahry et al. 2014). However, the growth of *E. coli* was weakly inhibited during the storage of tap water. According to Al-Bahry et al. (2014) and LeChevallier (2003), the effect of chlorine on bacteria decreases over time due to its interaction with other chemical elements in the water.

There was a statistically significant difference between borehole water (OBW) where there was a large increase in *E. coli* concentration and mineral water (MW) where there was a decrease in this concentration ($P < 0.05$). The treatment of water by ozonation as in the case of borehole water would not guarantee the protection of water from further bacterial contamination. Bouteleux (2005) showed that the treatment of water by ozonation does not affect the growth of *E. coli* in case of further contamination. The combination of chlorination and ozonation, however, provided effective protection of the mineral water. These results corroborate those of Bouteleux (2005) and Serrano et al. (2012) who showed that the concentration of *E. coli* decreases in mineral water after 24 h of storage.

Vibrio cholerae

The Kruskal–Wallis test showed that at least one of the microcosms presented a median concentration of *V. cholerae* that was significantly different ($P < 0.05$). The results of this study showed a weak growth of *V. cholerae* in filtered well water (FWW) compared to physiological saline (SS) and borehole water (OBW) ($P < 0.05$). Djaouda et al. (2013) also showed an inhibition of *V. cholerae* growth in filtered well water during its storage. Moreover, filtration also reduces a large quantity of the particles present in the water (Vital 2010), although, during filtration, a fraction of the autochthonous microflora (ultramicroflora) is able to pass through the pores of the filter apparatus (Hahn 2004). This ultramicroflora is likely to grow using natural organic carbon available in water. The competition between autochthonous ultramicroflora and *V. cholerae* cells for the same vital resource would thus be at the origin of this weak growth of *V. cholerae* in filtered well water.

However, the comparison of the median values did not show significant difference in concentrations of *V. cholerae* between FWW, FAWW and AWW. During autoclaving, a fraction of autochthonous bacteria, that possibly passes through the filter, are destroyed. This destruction would allow the formation of assimilable organic carbon in large quantities, a nutrient essential for the growth of *V. cholerae* (Wang et al. 2007; Vital 2010; Pandit and Kumar 2013; Goncharuk 2014). In FAWW, filtration reduces the amount of organic matter in the water (Soppe et al. 2015). Autoclaving hydrolyzes the remaining organic matter in the previously filtered water with release of assimilable organic carbon (LeChevallier 2003). The availability of assimilable organic carbon is believed to be responsible for the growth of *V. cholerae*. However, the median concentration of *V. cholerae* was significantly higher in saline compared to autoclaved well water and filtered and autoclaved well water ($P < 0.05$). Saline, although not containing organic matter, is able to stabilize *V. cholerae* cells and allow them to grow thanks to their reserves. The results of this study showed that the median concentration of *V. cholerae* was significantly lower in autoclaved well water compared to that of borehole water ($P < 0.05$). Both ozonation and autoclaving result in the hydrolysis of organic matter in the water

(Pandit and Kumar 2013). Moreover, these two techniques do not guarantee residual protection of the water in the event of further bacterial contamination. The survival and growth of *V. cholerae* in well water, filtered and autoclaved or not, would mostly depend on the physicochemical properties of the water.

Wilcoxon test showed a significant difference between the median concentration of *V. cholerae* between borehole and tap water ($P < 0.05$).That result was in alignment with some other similar studies (Mary et al. 2001; Bouteleux 2005; Serrano et al. 2012). Bouteleux (2005) showed that *V. cholerae* grows in water treated with ozonation in case of further contamination. As in the case of *E. coli*, *V. cholerae* concentration was significantly higher in saline than in tap water ($P < 0.01$). Residual chlorine in tap water was very likely the limiting factor that affects the growth of *V. cholerae*.

The Wilcoxon test revealed higher median concentration of *V. cholerae* in mineral water than in tap water ($P < 0.05$). This could be explained by possible conditions in mineral water reducing the impact of residual chlorine on *V. cholerae*. Indeed, some ions such as HCO_3^- and Na^+ in mineral water might decrease the efficacy of chlorine disinfection by increasing the pH of the water (McGuire, 2018).

When *E. coli* and *V. cholerae* were inoculated separately in microcosms, concentration of *E. coli* remained higher than those of *V. cholerae* in SS and FAWW. This corresponds to one of the criteria (greater ability to survive in water than pathogenic germs) that made it possible to choose *E. coli* as a bioindicator (Edberg et al. 2000). However, the growth of these bacteria is low in water treated by ozonation and/or chlorination. *E. coli* would not be a good indicator of contamination of chemically treated water.

Variations of concentration of *V. cholerae* and *E. coli* inoculated together in microcosms

Overall, concentration of *E. coli* increased slightly in all microcosms except in CTW and FWW where it decreased during water storage. Residual chlorine in tap water would have inhibited the growth of *E. coli* during water storage (LeChevallier 2003; Vital 2010; String et al. 2020). According to Mary et al. (2001), the survival of allochthonous bacteria such as *E. coli* in filtered water could be reduced by the presence of "ultramicrocells" of autochthonous bacterial microflora, due to nutrient competition exerted by bacteriolytic enzymes of membrane vesicles produced by native microflora.

In all microcosms, concentration of *V. cholerae* remained higher than that of *E. coli*, except in autoclaved well water and filtered and autoclaved well water where *E. coli* concentration was higher. *V. cholerae* was able to survive in groundwater under storage conditions and even in competition with other microorganisms (Djaouda et al. 2013). The growth of *V. cholerae* in well water autoclaved and filtered or not, as shown precedently when not mixed with *E. coli*, would mostly depend on physicochemical properties of water.

When inoculated together, the median concentration of *V. cholerae* was significantly higher than the median concentration of *E. coli* in microcosms CTW ($P < 0.01$), FWW ($P < 0.05$) and OBW ($P < 0.05$) following 72 h of storage (J3).

Variations in water physicochemical properties and microcosm type presented different influence on survival and growth of *V. cholerae* and *E. coli*.

Influence of autochthonous ultramicroflora on dynamics of concentration of *V. cholerae* and *E. coli* inoculated separately in filtered well water

The Spearman correlation test performed suggests that autochthonous ultramicroflora in filtered well water did not significantly affect *E. coli* and *V. cholerae* when these bacteria were inoculated separately into the medium. According to Messi et al. (2002), *E. coli* was less able to survive than the autochthonous ultramicroflora and is no longer detectable after several days of water storage. The availability of dissolved nutrients in FWW reduced the influence of the autochthonous ultramicroflora on *E. coli* growth.

The increase in the autochthonous ultramicroflora population had a significantly negative impact on *E. coli* but not *V. cholerae* when these bacteria were inoculated together into filtered well water. Autochthonous ultramicroflora feeds on organic matter and organic carbon. *E. coli* feeds mainly on the organic matter present in the water, while *V. cholerae* feeds mainly on assimilable organic carbon. When *E. coli* and *V. cholerae* are inoculated together, competition between *E. coli* and autochthonous ultramicroflora is unfavorable for *E. coli*. Dead *E. coli* cells would constitute organic matter for the autochthonous ultramicroflora. Thus, the proliferation of autochthonous ultramicroflora that feeds on organic matter would be beneficial to *V. cholerae* whose diet is based on organic carbon. Similar results were obtained by Vital (2010) and Bouteleux (2005) with Enterobacteriaceae. According to Djaouda et al. (2013), *V. cholerae* was able to survive in the presence of autochthonous ultramicroflora cells in stored filtered well water. Jubair et al. (2012, 2014) also demonstrated that *V. cholerae* persists even for 700 days in microcosms consisting of nutrient-poor lake waters containing other aquatic microorganisms.

Conclusion

The tap water, mineral water, and borehole water treated by chlorination, ozonation/chlorination and ozonation, respectively, were overall of good microbiological quality.

The treatment of well water by filtration, autoclaving, and filtration and autoclaving also contributed to water quality improvement. However, further contamination of any treated water, during home storage, compromised water quality. *E. coli* and *V. cholerae* survived in all treated waters that served as microcosms in this study. Inoculated separately in microcosms, *V. cholerae* presented higher abundance compared to *E. coli*, after three days, except in filtered-autoclaved well water. When these microorganisms were inoculated together in the microcosms, *V. cholerae* concentration remained higher than that of *E. coli* except in autoclaved well water and filtered and autoclaved well water where the reverse was noted. *E. coli* had a relatively lower survival ability than *V. cholerae* in the microcosms where they were inoculated together. In the presence of the autochthonous ultramicroflora, *V. cholerae* can develop in the treated water and is even able to multiply in competition with the ultramicrocells of the autochthonous microflora. The extended survival of *V. cholerae* in treated water may have serious public health implications in northern Cameroon where access to safe water and sanitary hygiene is limited. Chlorination was more effective than other water disinfection techniques. It is recommended that households without access to drinking water should disinfect all drinking water by chlorination and boiling, and keep treated water 'safely' stored to avoid further contamination.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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