

Neighborhood diversity of potentially pathogenic bacteria in drinking water from the city of Maroua, Cameroon

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ABSTRACT

This study examined the spatial variation of potential gastrointestinal pathogens within drinking water sources and home storage containers in four neighborhoods in Maroua, Cameroon. Samples were collected from source ($n = 28$) and home containers ($n = 60$) in each study neighborhood. Pathogen contamination was assessed using quantitative polymerase chain reaction, targeting *Campylobacter* spp., Shiga toxin producing *Escherichia coli* (virulence genes, *stx1* and *stx2*), and *Salmonella* spp. Microbial source tracking (MST) targeted three different host-specific markers: HF183 (human), Rum2Bac (ruminant) and GFD (poultry) to identify contamination sources. *Staphylococcus aureus* and the tetracycline-resistance gene (*tetQ*) were assessed to measure human hand contact and presence of antibiotic-resistant bacteria. Pathogen/MST levels were compared statistically and spatially, and neighborhood variation was compared with previously collected demographic information. All the test fecal markers and pathogens (except *Arcobacter*) were detected in home and source samples. Two neighborhoods tested positive for most pathogens/MST while the others only tested positive for one or two. Spatial variation of pathogens/MST existed between sources, storage containers, and neighborhoods. Differing population density and ethno-economic characteristics could potentially explain variation. Future research should explore the influence of demographic and ethno-economic factors on water quality during microbial risk assessments in urban Africa.

Key words | antibiotic resistance, home drinking water storage, informal water distribution system, microbial source tracking, Shiga toxin producing *E. coli*

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INTRODUCTION

One of the greatest advances in public health in the West during the early twentieth century was the reduction of the number of enteric pathogens in drinking water. In the USA, [Cutler & Miller \(2004\)](#) estimated that mortality rates dropped 30% between 1900 and 1936 after the implementation of public drinking water and sanitation systems, and that 43% of this decrease in mortality was due to the

increased access to clean water. Replicating the success of Western public drinking water and sanitation system improvement in developing countries has proved challenging. A great disparity in mortality due to waterborne pathogens between developed and developing countries persists today ([Bern et al. 1992](#); [Rahman et al. 2014](#)). This study, combining demographic, ethnographic and biological data,

sought to examine factors usually not involved in traditional water quality studies that influence the spatial variation of potential pathogens in drinking water, and which may contribute to the persistence of poor drinking water quality even after the implementation of drinking water chlorination systems.

It is known that resource constraints in many developing countries are one reason for persistent poor water quality. Improvements that have been made to increase drinking water quality and access frequently can only partially fill the need gap (Bain *et al.* 2014). While there has been an increase in the number of individuals who have access to improved drinking water sources (Bartram *et al.* 2014), the number of households in which water is piped directly into the household remains low (Clasen & Bastable 2003; Fewtrell *et al.* 2005; Batterman *et al.* 2009). Lack of a complete delivery system between the original water source and point of use has created a context in which informal delivery systems have evolved and the number of informal water distributors have increased in number to fill the gap (Kjellén & McGranahan 2006). Further complicating the matter, frequent water rationing and water shortages force households to store water in containers within their households (Levy *et al.* 2008; Firth *et al.* 2010; Harris *et al.* 2013; Kayser *et al.* 2013).

Especially in growing urban areas, such as our study area, drinking water contamination can stem from not only increased human contact (e.g. the addition of informal drinking water vendors), but also emerging microbial pathogens (e.g. antimicrobial-resistant bacteria, zoonotic pathogens) in the neighborhood environment and increased livestock interaction in transitioning rural-to-urban zones. As we discuss below, drinking water quality at the point of use can vary markedly between neighborhoods in the same urban setting, even when it is drawn from the same primary source.

Maroua, the administrative capital of the Far North Region of Cameroon, is home to nearly 300,000 people. Since the end of World War II, the city has experienced rapid population growth and diversification of ethnic groups (Seignobos & Iyebi-Mandjek 2000). A majority of residents have direct or indirect access to piped tap water, which is obtained locally from a deep aquifer and then chlorinated by the city's water supplier (Hervé

Doualla, personal communication). Frequent water rationing requires over 90% of inhabitants to store their drinking water in some sort of home drinking water storage containers, and 60% of inhabitants use an informal drinking water supplier to deliver water to their home (Healy Profítos *et al.* 2014). Conducting the study across neighborhoods in the city of Maroua provided the opportunity to assess the spatial variation of potential waterborne pathogens in a context that is becoming progressively more common throughout the developing world: a rapidly developing and diversifying city whose inadequate formal drinking water systems necessitate a patchwork of informal distribution systems to deliver water to most homes, which then must be kept in storage containers.

The study presented in this article stemmed from results from a project conducted in Maroua, Cameroon in 2013, which examined the quality of drinking water as it moved through formal and informal distribution systems and how drinking water quality at the point of use affected gastrointestinal health (Healy Profítos *et al.* 2014). Fecal indicator bacteria assessment found drinking water quality was poorest within home storage containers, which contained both human and livestock fecal contamination and evidence of antibacterial-resistant pathogens. Interestingly, home containers with low levels of *Escherichia coli* and the presence of tetracycline-resistant pathogens had the greatest risk of diarrheal illness. These results prompted further examination, as presented in this paper, of how pathogens varied spatially and what potential demographic and ethnographic factors contribute to any spatial patterns found. Furthermore, the presence of Shiga toxin producing *E. coli* (STEC) has become more apparent in the region (Kagambèga *et al.* 2012; Martikainen *et al.* 2012). This moved us to determine all samples for STEC in order to confirm its presence within our study area as well as other pathogens.

Demographic and environmental information collected during health surveys during the summer of 2013 was analyzed in conjunction with previous demographic and ethnographic data (Seignobos & Iyebi-Mandjek 2000) to elucidate potential explanations of any potential spatial variation. The results describe the spatial variation of drinking water pathogens in the region, and contribute valuable

knowledge on the variation of potential contamination that can occur in drinking water in urban settings in Africa.

MATERIALS AND METHODS

Drinking water samples were collected from water storage containers and drinking water sources of households located in four different Maroua neighborhoods between June 1 and August 1 2013. The neighborhoods were selected by choosing neighborhoods that exhibited high (Doualaré), medium (Lopéré and Dougoy), and low (Domayo) cholera incidence rates during the cholera outbreak that lasted from 2009 to 2011 (Mouhaman 2015). Selection of these neighborhoods also allowed us to study neighborhoods that exhibited a variation in socio-economic levels and public infrastructure.

Water sample collections occurred in conjunction with health surveys, which focused on collecting demographic, household economic and diarrheal data (Healy Profítos et al. 2014). The total household sample size ($n = 120$) was calculated using the relative proportion of each neighborhood's contribution to the city's overall population: Doualaré, $n = 50$ households; Lopéré, $n = 20$ households; Domayo, $n = 30$ households; and Dougoy, $n = 20$ households. A map of each neighborhood was printed and divided into numbered squares using a 2 cm × 2 cm grid. Squares were then randomly selected by drawing numbered pieces of paper, and one household within each square was surveyed.

To ensure participants' anonymity, each household was assigned a unique identification code and none of the participants' names were entered into the database. All participants were notified that they could stop the survey at any time, for any reason. The project was reviewed and approved by The Ohio State University Institutional Review Board (IRB 2010B004 and Amendment 032013), and permission to conduct research was given by the local Cameroon Ministry of Health, local government officials, and traditional community leaders.

Drinking water sample collection

One water company is responsible for the distribution system that supplies treated tap water throughout the city.

Frequent electricity rationing cuts power to the pumps that push water through the piped distribution system, prompting households to store their water in order to ensure a continuous supply for drinking and other household needs, such as cooking, cleaning, and hygiene. Drinking water samples could only be collected from households ($n = 60$) which had enough water to supply the field team with a sample. Volumes of 300–750 ml of stored drinking water were taken from the household's storage container, which was typically a traditional wide-mouth clay jar (called *canari* in French). *Canari* are commonly used across West Africa because the clay pots 'sweat', which allows the container to keep water cool in a climate that can experience temperatures up to 45 °C during the hot season (Figure 1).

As much as possible, source drinking water samples were also collected from the source where the household normally obtained their drinking water ($n = 28$). Most sources were public stand taps, but additionally included water distributors' jerry cans, open wells, and borehole wells. From the total number of household stored water samples and total number of source samples, 25 paired household–source samples were matched. All samples were collected using single, sterile 800-ml Whirl-Pak bags (Nasco, Fort Atkinson, WI, USA). Sampling bags were filled with water using the same method that individuals in

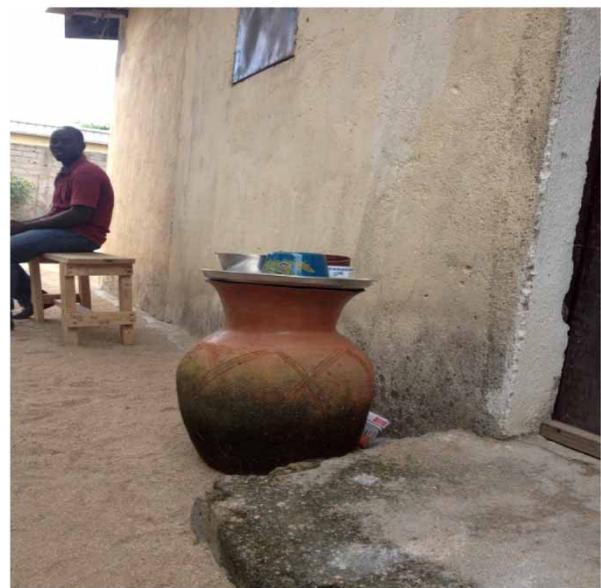


Figure 1 | *Canari* in a household's compound being used for drinking water storage.

the household used to remove water from the storage container to drink. Immediately after sampling, samples were placed in a cooler and taken to the field laboratory for initial chemical and microbial analyses.

Water quality measurements

Sixty samples from storage containers and 28 samples from drinking water sources were successfully collected in the field. Aliquots of 50–100 ml of each sample were tested for chemical and microbial quality. Total chlorine (mg/L), free chlorine/bromine (mg/L), pH, total alkalinity (mg/L), total hardness (mg/L of CaCO₃), and cyanuric acid (mg/L) levels were assessed to determine the chemical quality of the water by using a six-way paper-testing strip (Kokido Development Ltd, Hong Kong). Initial evaluation of microbial quality of the drinking water was done in the field using Coliplates (Bluewater Biosciences, Inc., Ontario, Canada). The Coliplates measured the most probable number of colony forming units (CFU/100 ml) of both *E. coli* and total coliforms (Gronewold & Wolpert 2008). Two samples (one home sample, one source sample) were lost during DNA extraction for a total of 59 home samples and 27 source samples were analyzed for their microbial quality.

Molecular detection

After initial chemical and biological in-field analysis, the remaining sample volume was then filtered through a sterile EMD Millipore Isopore™ Polycarbonate Membrane Filter (pore size: 0.45 µm, diameter: 47 mm) (Merck KGaA, Darmstadt, Germany), placed in a sterile 2 ml test tube and frozen immediately at –20 °C. The frozen filters were then transferred on ice to the laboratory at The Ohio State University's College of Public Health, where further analyses were performed.

The contamination of drinking water samples was assessed for three enteric pathogens: (1) *Campylobacter* spp. (targeting the 16S rRNA gene of *C. jejuni*, *C. coli*, and *C. lari*); (2) STEC (targeting virulence genes *stx1* and *stx2*); and (3) *Salmonella* spp. To determine the major fecal contamination source, three different host markers were tested with all the samples: (1) HF183 (human); (2) Rum2Bac

(ruminants); and (3) GFD (avian). In addition, three more bacteria were determined; (1) tetracycline-resistant bacteria (*tetQ*); (2) *Staphylococcus aureus* (*nuc* gene) (Postollec *et al.* 2011); and (3) *Arcobacter*, an emerging pathogen.

Total coliforms and *E. coli* levels were assessed in order to rapidly identify the relative levels of potential fecal contamination among samples in the field. These assays revealed high *E. coli* levels within the majority of home containers tested, and greater levels in home compared to source samples (Healy Profitós *et al.* 2014). *Campylobacter* and *Salmonella* spp. were chosen as both are prevalent waterborne pathogens (also foodborne) that are present in developing countries, and are responsible for a large percentage of bacterial infections (Coker *et al.* 2002; Van Dyke *et al.* 2010; Bain *et al.* 2014; Crump & Heyderman 2014; Geta-mesay *et al.* 2014). *Arcobacter* spp. were chosen in order to determine if this emerging food and waterborne pathogen was present in the study area (Lastovica 2006; Hsu & Lee 2015). *S. aureus* was assessed to measure potential contact of drinking water with human skin (i.e. hand contact) (Mintz *et al.* 2001). STEC was assessed based on the high levels of *E. coli* detected in home drinking water samples and to verify the presence of this emerging pathogen in this part of West Africa.

Microbial source tracking (MST) fecal markers were used in an attempt to identify the potential source of fecal contamination: human sources identified with marker HF183 (Bernhard & Field 2000), ruminant sources with Rum2Bac (Mieszkin *et al.* 2010), and avian sources with GFD (Green *et al.* 2012). The presence of tetracycline-resistant bacteria was targeted with the *tetQ* gene, which also indicated potential human-and livestock-associated fecal contamination (Lee *et al.* 2012) as tetracycline is commonly used in both human and veterinary medicine in the area.

Sample processing and nucleic acid extraction

Concentration for bacterial DNA extraction was conducted using the procedure described in Lee *et al.* (2012). The filter membrane was then transferred to a sterile 2 ml tube with 0.1 mm and 0.5 mm diameter sterilized glass beads (0.3 mg each; Biospec Products, Bartlesville, OK, USA). After bead beating using a Mini-Beadbeater 96 apparatus (BioSpec Products, Bartlesville, OK, USA) at 13,200 oscillations/min for

3½ min, the supernatant was transferred to a new 2 ml microcentrifuge tube, followed by DNA extraction using a QIAamp DNA stool kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions, and then suspended in 200 µl of elution buffer. The final eluates were used immediately or stored at –80 °C until further processing.

Quantitative polymerase chain reaction assays

The sequences of the primers that were used in quantitative polymerase chain reaction (qPCR) assays to detect *Campylobacter* spp., *Salmonella* spp., Shiga toxin genes (*stx1* and *stx2*), human-specific faecal contamination (HF183), ruminant-specific *Bacteroidales* (Rum2Bac), avian-specific faecal contamination, e.g. gull, goose, chicken and duck (GFD), antibacterial resistance (*tetQ*), *Staphylococcus* spp., and *Arcobacter* spp. are listed in Supplementary Table 1 (available with the online version of this paper). All experiments were conducted in duplicate using the ABI 48-well StepOne™ real Time System (Applied Biosystems, Foster City, CA, USA). When some of the samples showed the presence of PCR inhibition (tested with Sketa22 assay; Haugland et al. 2010), the DNA extracts were diluted.

For the quantification of HF183 (Haugland et al. 2010), *Salmonella* (Hein et al. 2006), Rum2Bac (Gourmelon et al. 2010), *stx1* and *stx2* (Ibekwe et al. 2002), the TaqMan-based real-time qPCR analysis was performed. The total volume of qPCR mixture was 20 µL containing 2 µL DNA template,

10 µL TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 500 nM primers, and 250 nM Probe. SYBR Green qPCR analysis was used for *tetQ* (Nikolich et al. 1994), *Campylobacter* (Josefsen et al. 2004), *Arcobacter* (Lee et al. 2012), *Staphylococcus* (Hagi et al. 2010), and GFD (Green et al. 2012) detections. The total volume of qPCR mixture was 20 µL including 2 µL DNA template, 10 µL SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), and 500 nM primers. A mixture of all PCR reagents with nuclease-free water (Fisher Scientific, Fair Lawn, New Jersey, USA) were used as a negative control for each PCR reaction. The PCR cycling conditions were composed of an initial cycle at 50 °C for 2 min and 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing and extension under proper conditions followed by reference conditions (Table 1). After amplification, melting curve analysis (SYBR analysis only) was performed by heating samples to 95 °C for 30 s, cooling them to 62 °C for 1 min, and then heating them to 95 °C at a rate of 0.2 °C/s.

Statistical and spatial analyses

Statistical analyses were performed using Microsoft Excel 2008 (Release ver. 12.3.6 Microsoft Excel) and Stata 13.1 (StataCorp Lp, College Station, TX, USA) software. Because the distribution of water contamination was non-normal, Mann–Whitney U tests were used to compare mean

Table 1 | Water quality descriptive statistics for home and source samples (home, $n = 59$; source, $n = 27$)

	Source samples (log number of gene copies/100 ml)			Home samples (log number of gene copies/100 ml)		
	Mean ^a	Median	Range	Mean ^a	Median	Range
<i>Campylobacter</i> spp.	0.98	0.01	0–4.4	0.99	1.02	0–1.02
<i>Salmonella</i> spp.	1.26	1.26	0–1.3	2.92	2.50	0–3.46
<i>S. aureus</i>	3.47	3.47	0–3.7	4.78	3.96	0–5.49
<i>stx1</i>	n.d. ^b	n.d.	n.d.	4.12	4.06	0–4.67
<i>stx2</i>	n.d.	n.d.	n.d.	3.55	1.30	0–4.15
HF183	8.0	8.00	0–8.3	6.26	5.97	0–6.73
Rum2Bac	4.66	4.62	0–5.1	5.46	4.87	0–6.36
GFD	4.37	4.37	0–4.4	0.70	0.70	0–0.70
<i>tetQ</i>	3.80	3.32	0–4.5	3.59	3.31	0–4.30

^aAll means reported are arithmetic.

^bn.d. = none detected.

contamination levels between source and home samples and Kruskal–Wallis tests were used to measure the difference of mean contamination between home storage container samples taken from the four study neighborhoods. Median values have also been reported and Mood's median tests were used to compare median contamination values between source, home storage container, and neighborhood samples. Map generation and visual analysis were performed with Quantum Geographic Information System (QGIS) 2.6.1 Brighton (Quantum GIS Development Team 2014).

RESULTS

With the exception of *Arcobacter* spp., which was not found in any sample, molecular measurements detected all the listed bacterial markers in Table 1 in various amounts in source and home storage container samples. The concentrations of all pathogens in the home and source samples did not differ significantly (Figure 2), although home samples did have higher median concentrations than source samples of *Campylobacter* spp., *Salmonella* spp., *stx1*, *stx2*, Rum2Bac, and *S. aureus* (Table 2).

When each of the home storage container and source sample groups was examined at levels of finer classification

(e.g. by source type or by neighborhood), there were variations in the pattern of drinking water contamination. In the case of source samples (all source types included), water contamination exhibited variation in what types of pathogenic bacteria and/or MST markers were present depending on the type of source that the water came from: open well, borehole, jerry can or tap (Figure 3).

Although the sample size of most of the source type subgroups was very small, it is important to note that samples taken from stand taps or from jerry cans that had been filled with chlorinated tap water exhibited a surprising amount of microbial contamination in terms of the variety of pathogens and/or MST markers that were detected in them compared to open well and the borehole well samples. Stand taps tested positive for *Salmonella* spp. (median concentration 1.26 log gene copies/100 ml), *Campylobacter* spp. (0.95 log gene copies/100 ml), *S. aureus* (3.04 log gene copies/100 ml), GFD (4.37 log gene copies/100 ml), HF183 (8.3 log gene copies/100 ml), Rum2Bac (4.65 log gene copies/100 ml) and *tetQ* (3.07 log gene copies/100 ml). Jerry cans tested positive for *S. aureus* (3.68 log gene copies/100 ml), HF183 (3.54 log gene copies/100 ml), Rum2Bac (4.67 log gene copies/100 ml) and *tetQ* (3.32 log gene copies/100 ml). Whereas borehole samples only tested positive for *Campylobacter* spp (1.02 log gene copies/100 ml), Rum2Bac (0.7 log gene copies/100 ml),

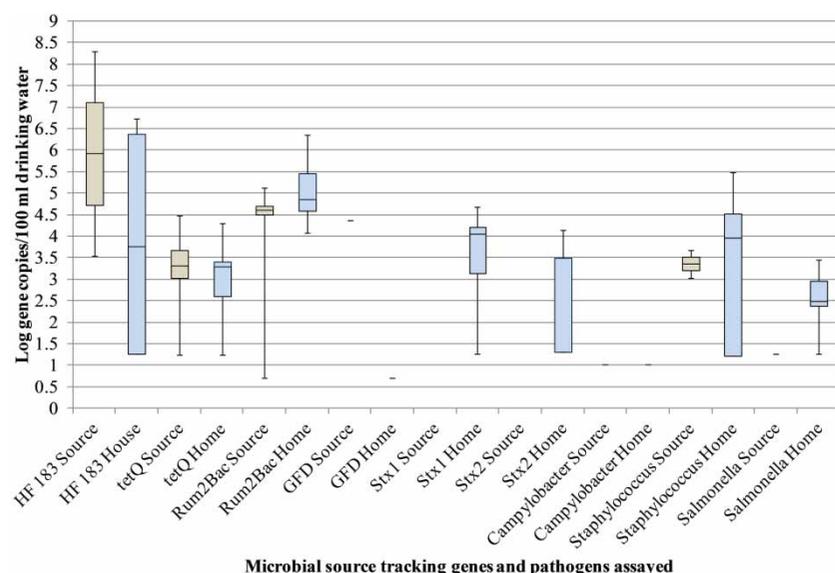
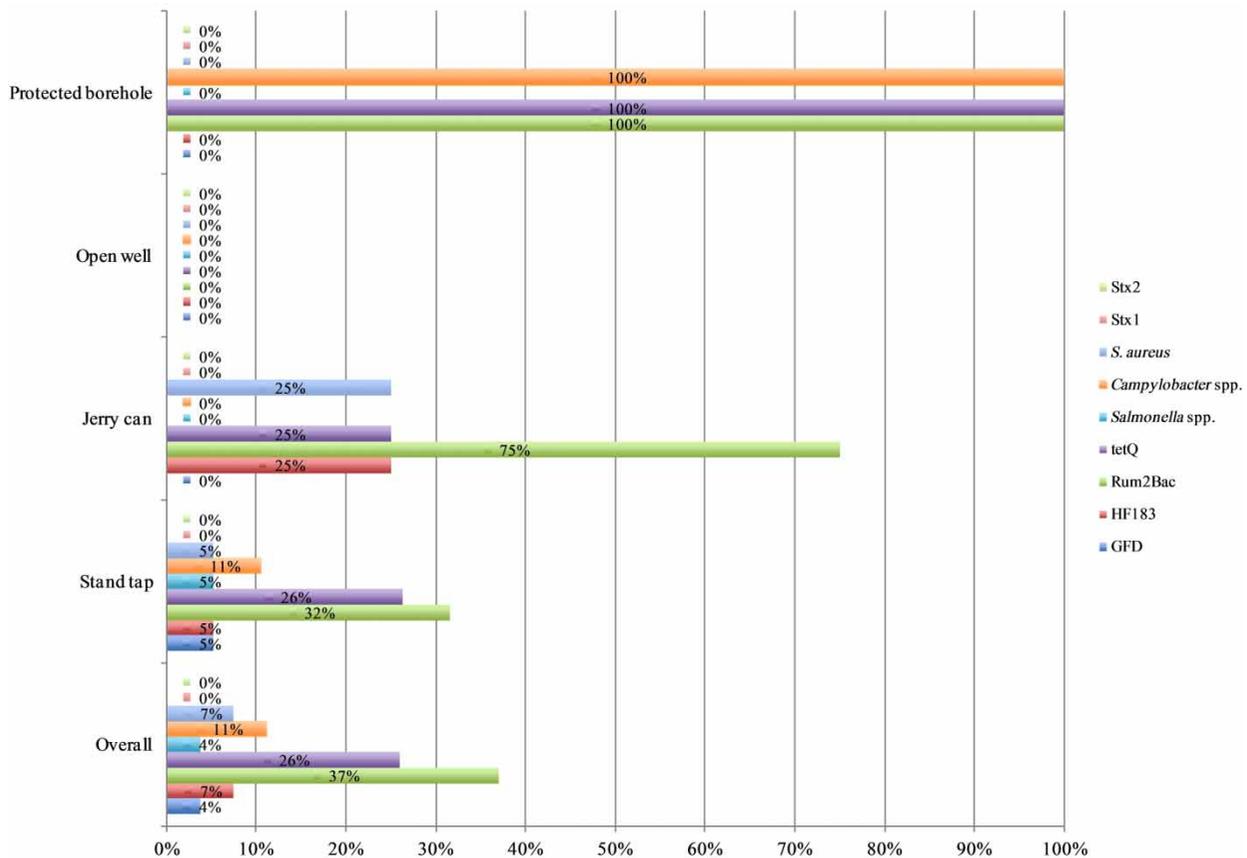


Figure 2 | Box plot of MST and pathogen results for all positive home and source samples.

Table 2 | Descriptive statistics of the four study neighborhoods for each of the microbial quality parameters assayed (Kruskal–Wallis)

	Doualaré (n = 28)		Lopéré (n = 10)		Domayo (n = 13)		Dougoy (n = 8)	
	Average log gene copies/100 ml	% of (+) assays	Average log gene copies/100 ml	% of (+) assays	Average log gene copies/100 ml	% of (+) assays	Average log gene copies/100 ml	% of (+) assays
Total coliforms ^{a,*}	1932.8	89%	1851.8	80%	1125.5	69%	1701.6	100%
<i>E. coli</i> ^{a,*}	929.5	96%	187.0	80%	287.3	62%	167.1	100%
<i>Campylobacter</i> spp.	1.0	25%	1.02	10%	0.95	15%	n.d. ^b	n.d.
<i>Salmonella</i> spp.	2.95	25%	n.d.	n.d.	3.06	15%	n.d.	n.d.
<i>S. aureus</i>	4.90	32%	n.d.	n.d.	3.79	23%	n.d.	n.d.
<i>stx1</i> [*]	4.22	32%	n.d.	n.d.	3.36	23%	n.d.	n.d.
<i>stx2</i>	3.61	25%	n.d.	n.d.	1.30	8%	n.d.	n.d.
HF183	6.38	11%	n.d.	n.d.	1.26	8%	n.d.	n.d.
Rum2Bac	5.23	32%	4.91	20%	5.97	23%	4.65	13%
GFD	0.70	4%	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
<i>tetQ</i>	3.14	14%	4.07	20%	n.d.	n.d.	3.31	25%

^aConcentration measured in CFU/100 ml.^bn.d. = none detected.^{*}*p*-value < 0.05, Kruskal–Wallis equality of populations rank analysis.**Figure 3** | Comparison of frequency of pathogen and MST markers between the types of drinking water source.

and *tetQ* (4.48 log gene copies/100 ml), and open well samples did not test positive for any pathogen or MST marker. It should also be noted that STEC (both *stx1* and *stx2*) were not found in any type of source and that STEC was the only pathogen parameter found exclusively in home samples (Figure 2).

In the case of home samples, due to the small number of samples taken from homes in three out of the four neighborhoods (Doualaré = 28; Lopéré = 10; Domayo = 13; and Dougoï = 9), limited statistical analysis was possible. The descriptive statistics for each parameter are described in Table 2. Kruskal–Wallis equality-of-populations rank analysis revealed that there were only statistically significant differences between neighborhoods in terms of average total coliforms, *E. coli*, and *stx1* gene levels, although *tetQ* levels did approach statistical significance ($p = 0.07$). But when the median value of each assay was compared using Mood's median test, no neighborhood's median value for any parameter was statistically different from the others.

When the contamination in home storage containers in each neighborhood was analyzed visually, a potential pattern of contamination suggested by the statistical analyses was easier to see (Figure 4). Certain neighborhoods had positive detections of all or nearly all of the biological parameters assessed (Doualaré and Domayo), while some only had measurable positive frequency percentages of one or two parameters (Lopéré and Dougoï). Simple visual inspection revealed no clear clustering of any specific type of pathogen or fecal marker detected in home containers or in source samples taken in any neighborhood. It is also interesting to note that the neighborhood with the highest cholera incidence rate, Doualaré, had the highest concentration of microbial contamination in terms of the number of positive detects of the pathogens or MST markers of interest. The levels of contamination in the other three neighborhoods did not demonstrate a correspondence with their relative cholera incidence rates.

Further statistical analysis conducted on the chemical quality of all water samples demonstrated that there was

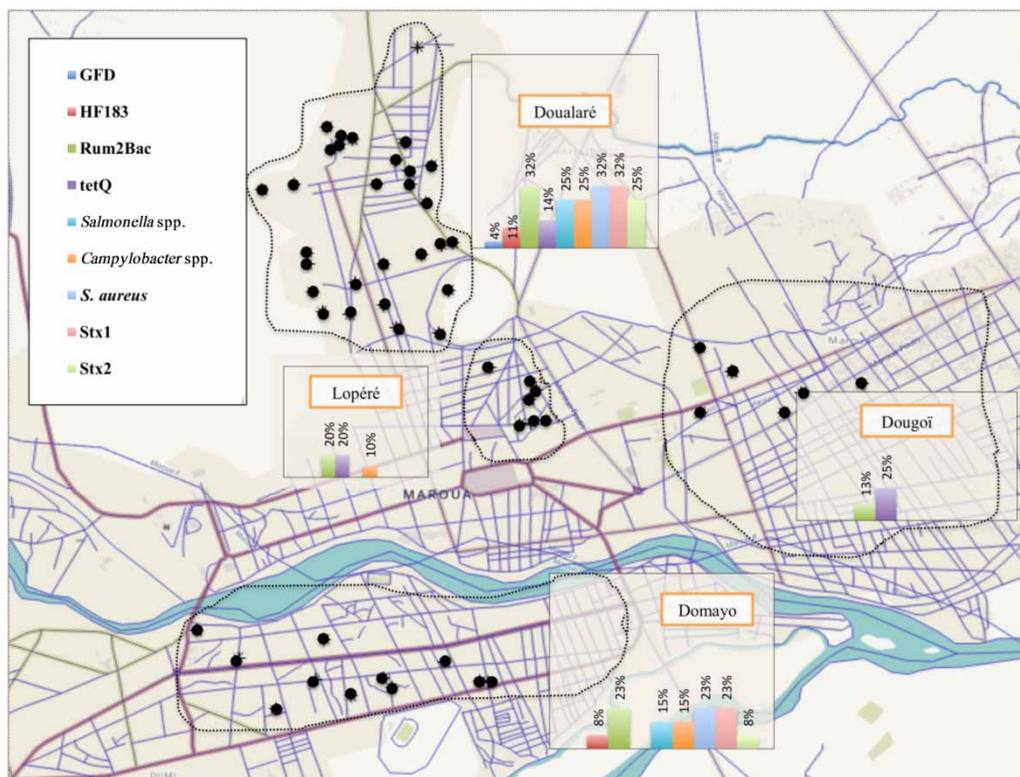


Figure 4 | Map of positive detections of pathogens or MST markers in stored drinking water taken from homes in each study neighborhood.

only a statistically significant difference between home containers and source samples in the level of total chlorine. There was no apparent relationship between any chemical parameter tested and any of the microbial quality results detailed above.

DISCUSSION

Results from the initial analyses conducted on collected water samples demonstrated that as water moved through the informal distribution system, it became more contaminated (Healy Profítós *et al.* 2014), a finding which is consistent with numerous other drinking water quality studies from around the world (Mintz *et al.* 2001; Momba 2002; Levy *et al.* 2008; Copeland *et al.* 2009; Firth *et al.* 2010; John *et al.* 2014). Further examination of the data presented in this article revealed that 70% (13/19) of the tap samples analyzed tested positive on at least one of the microbial parameters measured. While it is impossible to know if this contamination originated in the water itself, or in contamination of the tap opening, it is nevertheless of concern.

As noted above, while home samples were more generally contaminated based on bacterial parameters (i.e. total coliforms, *E. coli*, *Salmonella* spp., *Campylobacter* spp., and *S. aureus*), it was source samples that had higher median concentrations of fecal and antibiotic resistance markers HF183, GFD and *tetQ* genes. Currently we do not have a good understanding of the cause or causes behind the decrease in the median concentration of MST markers and the increase in the median concentration of pathogens as drinking water moves through the distribution system and enters home storage containers. Possibly, bacteria carrying the fecal marker gene copies (e.g. *Bacteroidales*) die off quickly or decay after they leave the source water, enter into the informal distribution system (e.g. more exposure to oxygen) and, subsequently, the home storage container.

Although resource constraints limited the number of drinking water samples that were taken in each neighborhood and the inherent limitation of using molecular methods (e.g. qPCR), such as its inability to discriminate between viable and non-viable organisms, our results suggest that a varying pattern of contamination between

neighborhoods may exist. Review of the demographic data collected through our health surveys and more historical demographic information compiled by Seignobos & Iyebi-Mandjek (2000) revealed some potential underlying causes behind the potential spatial variation of home contamination patterns suggested by study neighborhoods.

Since the end of World War II, the population of Maroua has expanded rapidly, with major population growth seen particularly between 1976 and the 1990s (Seignobos & Iyebi-Mandjek 2000). The primary driver of the population increase was immigration, and as new immigrants arrived in the city, new neighborhoods became established quickly and without much oversight from city planners. Doualaré, one of the youngest and fastest growing neighborhoods, is presently a sprawling neighborhood that is home to most of the more recent migrants who have come to Maroua from rural areas of the region. While population density increased in both old and new neighborhoods alike (Seignobos & Iyebi-Mandjek 2000), Doualaré's density continues to be one of the highest.

Yet, population density itself cannot fully explain the differences seen in pathogen spatial variation as Domayo, Lopéré and Dougoy have similar population densities (Seignobos & Iyebi-Mandjek 2000) yet dissimilar patterns of contamination. Ethno-cultural and economic reasons may play more of a role in how water is managed. Historically, neighborhoods in Maroua have been settled based along a mixture of ethnic and economic lines, as occupations are often tied to ethnicity (Seignobos & Iyebi-Mandjek 2000). This was supported by the results of our health surveys, which found variation in the ethnic compositions of our study neighborhoods, and clear differences between the main income-generating activities for families in each neighborhood. For example, while Dougoy is also rapidly growing, it also contains more wealthy merchants and vendors than Doualaré, where occupations are often low-paying jobs, such as mototaxi drivers. Land tenure is another contributing issue. Many residents of Doualaré, especially newly arrived migrants, do not own their own land. As they are constantly worried about being removed, they are also less likely to invest in the agricultural activities that comprise a large part of the city's overall economy.

The economic differences between neighborhoods, in addition to rapid expansion, have led to certain

neighborhoods (Dougoy, Domayo) having increased access to cleaner drinking water sources and more complete sanitation systems. Results from the health surveys also noted that Doualaré residents were more likely to be forced to find alternative ways to obtain drinking water (e.g. higher utilization of unsafe open wells) and deal with increasing human and animal waste without complete sanitation systems compared to the more established neighborhoods of Domayo and Lopéré or wealthier neighborhoods, such as Dougoy.

CONCLUSIONS

Efforts to increase the access to quality drinking water have grown in the recent past, driven in part by the motivation of developing countries to achieve development targets set through the Millennium Development Goals (Demarest *et al.* 2013). Yet as many clean water access programs have discovered, increasing the access to clean drinking water for the over 700 million people in the world who still lack it is not a simple task (WHO & UNICEF 2014). As found in this study, even an urban population with relatively high access to an improved drinking water source still has issues with microbial contamination both at the point of access (i.e. stand taps) and at the point of use (i.e. home containers).

While our findings are exploratory and our sample sizes limited, the results of this study suggest that further research is required to better understand how ethno-cultural and economic differences could affect the spatial variation of potential waterborne pathogens within improved drinking water systems in developing countries.

Greater amounts of fecal markers in source samples and greater amounts of bacterial contamination in home samples reveal another layer of potential complexity in the challenge of providing microbially-safe drinking water for people in developing countries. The finding that source samples had relatively low levels of indicator organisms (total coliforms and *E. coli*) compared to home container samples, but had relatively high levels of fecal markers, demonstrates that the way in which drinking water is monitored and evaluated needs to be reconsidered. The variation in contamination within the drinking water

distribution systems (from stand taps to jerry cans) and between neighborhoods within the same city highlights that drinking water quality projects should consider adapting different strategies or developing more targeted quality baselines based on the specific water management systems within homes that are unique to each geographic location or even neighborhood. Further research is required to increase the knowledge of how drinking water quality varies within growing urban centers in developing countries.

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REFERENCES

- Bain, R., Cronk, R., Wright, J., Yang, H., Slaymaker, T. & Bartram, J. 2014 [Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis](#). *PLoS Med.* **11**, e1001644.
- Bartram, J., Brocklehurst, C., Fisher, M. B., Luyendijk, R., Hossain, R., Wardlaw, T. & Gordon, B. 2014 [Global monitoring of water supply and sanitation: history, methods and future challenges](#). *Int. J. Environ. Res. Public Health* **11**, 8137–8165.
- Batterman, S., Eisenberg, J., Hardin, R., Kruk, M. E., Lemos, M. C., Michalak, A., Mukherjee, B., Renne, E., Stein, H., Watkins, C.

- & Wilson, M. C. 2009 Sustainable control of water-related infectious diseases: a review and proposal for interdisciplinary health-based systems research. *Environ. Health Perspect.* **117**, 1023–1032.
- Bern, C., Martinez, J., de Zoysa, I. & Glass, R. I. 1992 The magnitude of the global problem of diarrhoeal disease: a ten-year update. *Bull. World Health Organ.* **70**, 705–714.
- Bernhard, A. E. & Field, K. G. 2000 A PCR assay to discriminate human and ruminant feces on the basis of host differences in *Bacteroides-Prevotella* genes encoding 16S rRNA. *Appl. Environ. Microbiol.* **66**, 4571–4574.
- Clasen, T. F. & Bastable, A. 2003 Faecal contamination of drinking water during collection and household storage: the need to extend protection to the point of use. *J. Water Health* **1**, 109–115.
- Coker, A. O., Isokpehi, R. D., Thomas, B. N., Amisu, K. O. & Obi, C. L. 2002 Human campylobacteriosis in developing countries. *Emerg. Infect. Dis.* **8**, 237–243.
- Copeland, C., Beers, B., Thompson, M., Pinkerton, R., Barrett, L., Sevilleja, J. E., Alencar, S., Lima, A. & Guerrant, R. 2009 Faecal contamination of drinking water in a Brazilian shanty town: importance of household storage and new human faecal marker testing. *J. Water Health* **7**, 324–331.
- Crump, J. A. & Heyderman, R. S. 2014 Invasive *Salmonella* infections in Africa. *Trans. R. Soc. Trop. Med. Hyg.* **108**, 673–675.
- Cutler, D. & Miller, G. 2004 *The Role of Public Health Improvements in Health Advances: The 20th Century United States*. National Bureau of Economic Research, Cambridge, MA.
- Demarest, J., Pagsuyoin, S., Learmonth, G., Mellor, J. & Dillingham, R. 2013 Development of a spatial and temporal agent-based model for studying water and health relationships: the case study of two villages in Limpopo, South Africa. *J. Artif. Soc. Soc. Simul.* **16**, 3. <http://jasss.soc.surrey.ac.uk/16/4/3.html> (accessed 2 December 2014).
- Fewtrell, L., Kaufmann, R. B., Kay, D., Enanoria, W., Haller, L. & Colford, J. M. 2005 Water, sanitation and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *Lancet Infect. Dis.* **5**, 42–52.
- Firth, J., Balraj, V., Muliylil, J., Roy, S., Rani, L. M., Chandrasekhar, R. & Kang, G. 2010 Point-of-use interventions to decrease contamination of drinking water: a randomized, controlled pilot study on efficacy, effectiveness and acceptability of closed containers, *Moringa oleifera* and in-home chlorination in rural South India. *Am. J. Trop. Med. Hyg.* **82**, 759–765.
- Getamesay, M., Getenet, B. & Ahmed, Z. 2014 Prevalence of *Shigella*, *Salmonella* and *Campylobacter* species and their susceptibility patterns among under five children with diarrhea in Hawassa Town, South Ethiopia. *Ethiop. J. Health Sci.* **24**, 101–108.
- Gourmelon, M., Caprais, M. P., Mieszkin, S., Marti, R., Wéry, N., Jardé, E., Derrien, M., Jadas-Hécart, A., Communal, P. Y., Jaffrezic, A. & Pourcher, A. M. 2010 Development of microbial and chemical MST tools to identify the origin of the faecal pollution in bathing and shellfish harvesting waters in France. *Water Res.* **44**, 4812–4824.
- Green, H. C., Dick, L. K., Gilpin, B., Samadpour, M. & Field, K. G. 2012 Genetic markers for rapid PCR-based identification of gull, Canada goose, duck and chicken fecal contamination in water. *Appl. Environ. Microbiol.* **78**, 503–510.
- Gronewold, A. D. & Wolpert, R. L. 2008 Modeling the relationship between most probable number (MPN) and colony-forming unit (CFU) estimates of fecal coliform concentration. *Water Res.* **42**, 3327–3334.
- Hagi, T., Kobayashi, M. & Nomura, M. 2010 Molecular-based analysis of changes in indigenous milk microflora during the grazing period. *Biosci. Biotechnol. Biochem.* **74**, 484–487.
- Harris, A. R., Davis, J. & Boehm, A. B. 2013 Mechanisms of post-supply contamination of drinking water in Bagamoyo, Tanzania. *J. Water Health* **11**, 543–554.
- Haugland, R. A., Varma, M., Sivaganesan, M., Kelty, C., Peed, L. & Shanks, O. C. 2010 Evaluation of genetic markers from the 16S rRNA gene V2 region for use in quantitative detection of selected *Bacteroidales* species and human fecal waste by qPCR. *Syst. Appl. Microbiol.* **33**, 348–357.
- Healy Profítos, J., Mouhaman, A., Lee, S., Garabed, R., Moritz, M., Piperata, B., Tien, J., Bisesi, M. & Lee, J. 2014 Muddying the waters: a new area of concern for drinking water contamination in Cameroon. *Int. J. Environ. Res. Public Health* **11**, 12454–12472.
- Hein, I., Flekna, G., Krassnig, M. & Wagner, M. 2006 Real-time PCR for the detection of *Salmonella* spp. in food: an alternative approach to a conventional PCR system suggested by the FOOD-PCR project. *J. Microbiol. Methods* **66**, 538–547.
- Hsu, T. T. D. & Lee, J. 2015 Global distribution and prevalence of *Arcobacter* in food and water. *Zoonoses Public Health* **62**, 579–589.
- Ibekwe, A. M., Watt, P. M., Grieve, C. M., Sharma, V. K. & Lyons, S. R. 2002 Multiplex fluorogenic real-time PCR for detection and quantification of *Escherichia coli* O157:H7 in dairy wastewater wetlands. *Appl. Environ. Microbiol.* **68**, 4853–4862.
- John, V., Jain, P., Rahate, M. & Labhasetwar, P. 2014 Assessment of deterioration in water quality from source to household storage in semi-urban settings of developing countries. *Environ. Monit. Assess.* **186**, 725–734.
- Josefsen, M. H., Jacobsen, N. R. & Hoorfar, J. 2004 Enrichment followed by quantitative PCR both for rapid detection and as a tool for quantitative risk assessment of food-borne thermotolerant *Campylobacter*s. *Appl. Environ. Microbiol.* **70**, 3588–3592.
- Kagambèga, A., Martikainen, O., Lienemann, T., Siitonen, A., Traoré, A. S., Barro, N. & Haukka, K. 2012 Diarrheogenic *Escherichia coli* detected by 16-plex PCR in raw meat and beef intestines sold at local markets in Ouagadougou, Burkina Faso. *Int. J. Food Microbiol.* **153**, 154–158.
- Kaysar, G. L., Moriarty, P., Fonseca, C. & Bartram, J. 2013 Domestic water service delivery indicators and frameworks

- for monitoring, evaluation, policy and planning: a review. *Int. J. Environ. Res. Public Health* **10**, 4812–4835.
- Kjellén, M. & McGranahan, G. 2006 *Informal Water Vendors and the Urban Poor*, International Institute for Environment and Development.
- Lastovica, A. J. 2006 Emerging *Campylobacter* spp.: the tip of the iceberg. *Clin. Microbiol. Newsl.* **28**, 49–56.
- Lee, C., Agidi, S., Marion, J. W. & Lee, J. 2012 *Arcobacter* in Lake Erie Beach waters: an emerging gastrointestinal pathogen linked with human-associated fecal contamination. *Appl. Environ. Microbiol.* **78**, 5511–5519.
- Levy, K., Nelson, K. L., Hubbard, A. & Eisenberg, J. N. S. 2008 Following the water: a controlled study of drinking water storage in northern coastal Ecuador. *Environ. Health Perspect.* **116**, 1533–1540.
- Martikainen, O., Kagambèga, A., Bonkoungou, I. J., Barro, N., Siitonen, A. & Haukka, K. 2012 Characterization of Shigatoxigenic *Escherichia coli* strains from Burkina Faso. *Foodborne Pathog. Dis.* **9**, 1015–1021.
- Mieszkin, S., Yala, J.-F., Joubrel, R. & Gourmelon, M. 2010 Phylogenetic analysis of *Bacteroidales* 16S rRNA gene sequences from human and animal effluents and assessment of ruminant faecal pollution by real-time PCR. *J. Appl. Microbiol.* **108**, 974–984.
- Mintz, E., Bartram, J., Lochery, P. & Wegelin, M. 2001 Not just a drop in the bucket: expanding access to point-of-use water treatment systems. *Am. J. Public Health* **91**, 1565–1570.
- Momba, M. 2002 Regrowth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Res.* **36**, 3023–3028.
- Mouhaman, A. 2015 A multiscale analysis and modeling of cholera incidence in the far north of Cameroon. Unpublished Doctoral Dissertation. University of Maroua.
- Nikolich, M. P., Hong, G., Shoemaker, N. B. & Salyers, A. A. 1994 Evidence for natural horizontal transfer of tetQ between bacteria that normally colonize humans and bacteria that normally colonize livestock. *Appl. Environ. Microbiol.* **60**, 3255–3260.
- Postollec, F., Falentin, H., Pavan, S., Combrisson, J. & Sohler, D. 2011 Recent advances in quantitative PCR (qPCR) applications in food microbiology. *Food Microbiol.* **28**, 848–861.
- Rahman, A. E., Moinuddin, M., Molla, M., Worku, A., Hurt, L., Kirkwood, B., Mohan, S. B., Mazumder, S., Bhutta, Z. A., Raza, F., Mrema, S., Masanja, H., Kadobera, D., Waiswa, P., Bahl, R., Zangenberg, M., Muhe, L. & Persistent Diarrhoea Research Group 2014 Childhood diarrhoeal deaths in seven low- and middle-income countries. *Bull. World Health Organ.* **92**, 664–671.
- Seignobos, C. & Iyébi-Mandjek, O. 2000 Atlas de la province Extrême-Nord Cameroun (Paris: Yaoundé: Institut de recherche pour le développement; République de Cameroun, Ministère de la recherche scientifique et technique, Institut national de cartographie) (in French).
- Van Dyke, M. I., Morton, V. K., McLellan, N. L. & Huck, P. M. 2010 The occurrence of *Campylobacter* in river water and waterfowl within a watershed in southern Ontario, Canada: *Campylobacter* in river water. *J. Appl. Microbiol.* **109**, 1053–1066.
- WHO & UNICEF 2014 Progress on sanitation and drinking-water: 2014 update. World Health Organization and United Nations Children's Fund.

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