Journal of Microbiology, Immunology and Infection (2017) 50, 718–722

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

BRIEF COMMUNICATION

Bacterial contamination of water samples in Gabon, 2013

Jonas Ehrhardt [a,b], Abraham S. Alabi [a,b], Peter G. Kremsner [a,b], Wolfgang Rabsch [c], Karsten Becker [d], Francis Tsombeng Foguim [b], Thorsten Kuczius [e], Meral Esen [a,b], Frieder Schaumburg [b,d,*]

[a] *Institut für Tropenmedizin, Eberhard Karls Universität Tübingen, Deutsches Zentrum für Infektionsforschung, Tübingen, Deutschland, Germany*
[b] *Centre de Recherches Médicales de Lambaréné (CERMEL), Lambaréné, Gabon*
[c] *National Reference Centre for Salmonella and Other Enteric Pathogens, Robert Koch Institute, Wernigerode Branch, Wernigerode, Germany*
[d] *Institute of Medical Microbiology, University Hospital Münster, Münster, Germany*
[e] *Institute of Hygiene, University Hospital Münster, Münster, Germany*

Received 22 June 2015; received in revised form 17 March 2016; accepted 24 March 2016
Available online 13 May 2016

KEYWORDS
Africa;
contamination;
extended spectrum beta-lactamase;
Salmonella;
water

Abstract Contamination of water is a major burden in the public health setting of developing countries. We therefore assessed the quality of water samples in Gabon in 2013. The main findings were a contamination rate with coliforms of 13.5% and the detection of a possible environmental reservoir for extended spectrum beta-lactamase-producing bacteria.

Introduction

In 2001, more than 600,000 deaths in sub-Saharan Africa were attributed to unsafe water, sanitation, and hygiene.[1] A recent meta-analysis showed that the burden of diarrheal diseases due to contaminated drinking water may be greatly underestimated and highlights the need for continual monitoring of water quality.[2] *Escherichia coli* is especially recommended as an indicator organism for fecal pollution, and total coliforms as indicator organisms for the cleanliness and integrity of distribution systems.[3]

Water, particularly drinking water, can also be a vector and reservoir for antimicrobial-resistant bacteria. For instance, 4% of drinking water bags and 40% of samples from sewer and river sites in Kinshasa, Democratic Republic

* Corresponding author. Institute of Medical Microbiology, University Hospital Münster, Domagkstrasse 10, 48149 Münster, Germany.
E-mail address: frieder.schaumburg@ukmuenster.de (F. Schaumburg).

http://dx.doi.org/10.1016/j.jmii.2016.03.009

of the Congo were contaminated with extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae.[4] Therefore, contaminated water bodies could give rise to colonization and infections with ESBL-producing Enterobacteriaceae in humans. The objective of this study was to assess microbial contamination with coliforms in Gabon and to test the antibiotic susceptibility.

Methods

During a cross-sectional study, 200 water samples were collected from different water sources in the provinces of Estuaire, Moyen-Ogooué, Ogooué-Ivindo, and Ngounié in Gabon in 2013 (Figure 1). The selection of samples was based on availability and accessibility, and therefore is not representative.

From each sampling site, 500 mL of water was collected in sterile bottles. Water taps and standpipes were allowed to run for 1 minute and were sanitized with an open flame before the water was aseptically collected. Samples were placed in a cool box before transport to the laboratory for analysis within a maximum of 4 hours after sampling. For each sample, the following items were recorded: date/time, type of water source (e.g., tap water, standpipe, open water body), temperature (e.g., water or environment), color (i.e., "colorless", "yellow", or "brown"), turbidity (i.e., "clear", "slight", "moderate", and "intensive"; a nephelometer was unavailable) and odor (i.e., "chlorous", "earthy", "sanious", or "inodorous") (Table 1).[5] Water sources were classified as "improved" (e.g., harvested rainwater, protected springs, boreholes, piped water sources) or "unimproved" (e.g., river, unprotected wells, and springs), based on the World Health Organization criteria.[2]

A pilot study of 10 samples (i.e., 8 improved and 2 unimproved water sources) using a standardized method (i.e., membrane filtration technique of 100 mL water) and culture on Columbia blood and MacConkey agar (Oxoid, Wesel, Germany; 24 hours, 37°C, ambient air) revealed high contamination by nonfastidious pathogens and did not allow for the quantification and subculture of single colonies.[5] We therefore decided to streak 500 μL of the water sample directly onto Columbia blood and MacConkey agar plates, which yielded a detection level of ≥200 colony-forming units (CFU)/100 mL. Samples containing >100 indicator organisms/100 mL were categorized as "high to very high" risk of fecal contamination, based on the World Health Organization criteria.[2,3]

All colonies growing on both agars (24 hours, 37°C, ambient air) were quantified and one colony of each

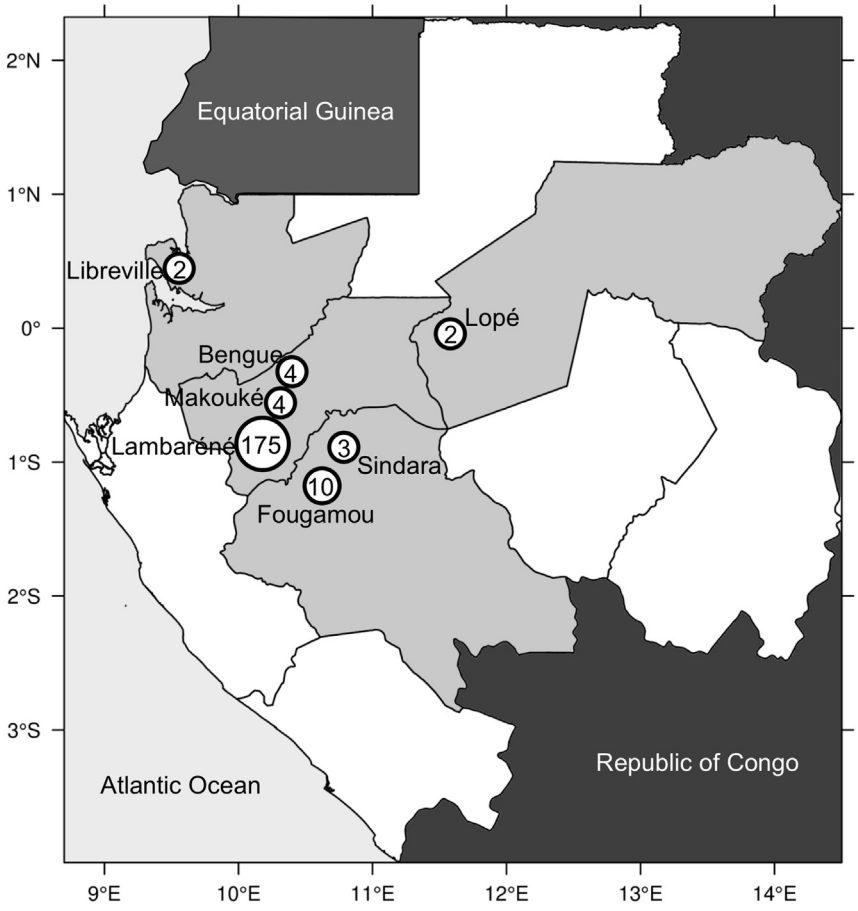

Figure 1. Map of Gabon. The provinces from where water samples were obtained are shaded in gray. The sites from where samples were obtained are indicated by circles and the number within indicates the number of samples taken. Vertical and horizontal scales indicate the degrees of longitude and latitude, respectively.

Table 1 Characteristics of water samples from Gabon, 2013.

Characteristics		Water source		
		Total (n = 200)	Improved (n = 184)	Unimproved (n = 16)
Mean temperature in Water °C (±SD)		32.1 (±11.5)	32.5 (±11.5)	27.8 (±3.5)
	Environment	27.4 (±1.9)	27.3 (±1.9)	28.3 (±1.9)
Color	Colorless	124 (29)	123 (66.8)	1 (6.3)
	Yellow	65 (32.5)	60 (32.6)	5 (31.3)
	Brown	11 (5.5)	1 (0.5)	10 (62.5)
Turbidity	Clear	174 (87)	173 (94.0)	1 (6.3)
	Slight	12 (6)	9 (4.9)	3 (18.8)
	Moderate	7 (3.5)	2 (1.1)	5 (31.3)
	Intensive	7 (3.5)	0 (0)	7 (43.8)
Odor	Chlorous	17 (8.5)	17 (9.2)	0 (0)
	Earthy	10 (5)	1 (0.5)	9 (56.3)
	Sanious	3 (1.5)	0 (0)	3 (18.8)
	Inodorous	170 (85)	166 (90.2)	4 (25.0)
Coliforms	Contamination rate	27 (13.5)	20 (10.9)	7 (43.8)
	Median cell count in contaminated samples, CFU/100mL (range)	3000 (200–80,000)	1800 (200–28,000)	4400 (2000–80,000)
E. coli	Contamination rate	1 (0.5)	0 (0)	1 (6.3)
	Cell count, CFU/100mL (range)	13,600	NA (NA)	13,600 (NA) [a]

[a] Only one isolate was detected.
Data are presented as n (%), unless indicated otherwise.
CFU = colony-forming unit; NA = not applicable; SD = standard deviation.

phenotype was identified by matrix-assisted laser desorption/ionization/time-of-flight (MALDI-TOF) in Münster, Germany (microflex LT; Bruker Daltonik, Bremen, Germany) using the MALDI Biotyper library (Version 3.3.2.0). Species of *Escherichia coli* were confirmed by Vitek 2 automated systems (bioMérieux, Marcy l'Étoile, France). *Salmonella* isolates were differentiated to the subspecies level by biochemical reactions listed in the White-Kauffmann–Le Minor scheme.[6] The serovars were detected by slide agglutination using antisera (SIFIN, Berlin, Germany). Susceptibility was tested by Vitek2 automated systems (bioMérieux) or agar diffusion test, based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Version 4.0). Only non-duplicate isolates per water sample were used when reporting the proportions of contamination and antimicrobial resistance.

Coliform bacteria were defined as *o*-nitrophenyl-beta-galactopyranoside-positive Enterobacteriaceae.[6] Extended spectrum beta-lactamase production was confirmed using the double disk diffusion test (Mast discs; Mast Diagnostics, Bootle, UK). The beta-lactamases *bla*TEM, *bla*SHV, and *bla*CTX-M were subtyped in all ESBL-producers.[7] Permits were not required to collect water samples from public water sources in Gabon.

Results

Most samples were drawn from improved sources (92%, n = 184), followed by unimproved (8%, n = 16) sources (Table 1). Improved sources included piped household connections (n = 123), standpipes (n = 27), piped yard connections (n = 25), boreholes (n = 8), and rainwater roof harvesting (n = 1).[2] Unimproved sources were rivers (n = 11), unprotected wells (n = 3), an unprotected spring (n = 1), and a multiple-use bottle (n = 1).[2] Only a small proportion of improved water sources were equipped with high-temperature water pipes (20%, n = 38).

Thirty-five coliforms were isolated in 27 samples (*Citrobacter freundii*, 7; *Enterobacter* sp., 7; *Escherichia coli*, 1; *Klebsiella pneumoniae*, 6; *Kluyvera ascorbata*, 1; *Leclercia adecarboxylata*, 1; *Pantoea dispersa*, 1; *Serratia marcescens*, 10; and *Yokenella regensburgei*, 1), which corresponded to an overall contamination rate of 13.5% with coliforms (Table 1).

Among coliforms, antimicrobial resistance was detected for ampicillin (91%, n = 31), cefotaxime (3%, n = 1), and cotrimoxazole (9%, n = 3). No resistance was detected against ciprofloxacin, imipenem, meropenem, and ertapenem.

One ESBL-producing *K. pneumoniae* isolate was found in an unimproved water source (i.e., the river "Petit Odavo", a rural sampling site with 1000 CFU/100 mL). This isolate carried *bla*TEM-1 and *bla*CTX-M-15, and was susceptible to ciprofloxacin and carbapenems; however, it was resistant to cotrimoxazole.

Three tap water samples from a hospital environment (i.e., ward, outpatient clinic, staff apartment) were contaminated with *Salmonella enterica* subsp. *salamae* (subspecies II 42:r:-). The contamination rates in these three tap water samples were 4000 CFU/100 mL (ward),

1200 CFU/100 mL (outpatient clinic), and 1000 CFU/100 mL (staff apartment). The three *Salmonella* isolates were detected in samples that were collected between April 21, 2013 and May 29, 2013. After this period, 21 additional water samples collected from other water sources of the same hospital showed no growth of *Salmonella* sp.

We reanalyzed *Salmonella* sp. isolated from clinical specimens between 2013 and 2015 ($n = 8$) in our hospital and did not find *Salmonella enterica* subsp. *salamae*. All clinical isolates belonged to *S. enterica* subsp. *enterica* (subspecies I) serovars Enteritidis ($n = 2$), Typhimurium ($n = 2$), Enugu ($n = 1$), Durham ($n = 1$), and Uppsala ($n = 1$). One strain was rough.

Discussion

Improved water sources are protected from outside fecal contamination and are safe drinking water sources.[2] The pollution rate (10.9%) of improved water sources with coliforms in this study was lower than the contamination rates of improved (i.e., piped) sources investigated in other studies in urban settings (27%) and rural settings (58%) in Africa.[8] Our method is less sensitive than the International Organization for Standardization (ISO) standard procedure (ISO 9308-1:2014); therefore, we may have underestimated the contamination by coliforms.

One ESBL-producing *K. pneumoniae* isolate was found in an unimproved water source (i.e., the river "Petit Odavo", a rural sampling site with 1000 CFU/100 mL). This isolate carried *bla*TEM-1 and *bla*CTX-M-15, and was susceptible to ciprofloxacin and carbapenems, but resistant to cotrimoxazole. The detection of ESBL-producing *K. pneumoniae* may be the "tip of the iceberg" because the threshold of detection was $\geq$200 CFU/100 mL. However, this finding is in line with high carrier rates of ESBL-producing Enterobacteriaceae in the community setting in Gabon (the community-associated carriage is 33.6%).[9] Open water bodies in remote regions are frequently used for personal hygiene. This could facilitate the spread of antimicrobial-resistant pathogens, which may become critical because in Gabon only limited treatment options are available for infections by ESBL producers.

The hospital where *S. enterica* subsp. *salamae* (II 42:r:-) was detected uses fresh filtered river water (filtered by a sand filter). Chlorination officially is performed in the respective water treatment plant, but the odor of the respective samples was not chlorous. We assumed that all *Salmonella* isolates had a common source because they belonged to the same rare serovar. Infections with *Salmonella* serovars of the subspecies II are frequently associated with reptiles, which argues for the river water as a likely source because it is the habitat of crocodiles, snakes, and turtles in Gabon.[10] We did not find *Salmonella enterica* subsp. *salamae* in clinical specimens between 2013 and 2015, which argues against an epidemic due to *Salmonella* subspecies II from drinking water. However, infections due to *Salmonella* subspecies II cannot be ruled out because stool cultures are only performed on a minority of people. People may have experienced episodes of diarrhea without consulting a doctor.

Some limitations of the study need to be addressed. First, we did not apply the membrane filtration techniques and did not use 500-mL water samples, the suggested standardized amount of water for the assessment of the quality of drinking water. This decision was based on the high contamination rates and turbidity of most samples. The deviation from standard operation procedures may hamper the comparison with other investigations because our approach increases the detection level of indicator organisms. Second, the proportion of improved and unimproved water sources was not well balanced and sample sites were restricted to four provinces in Gabon. Therefore, the results may not be representative for the whole country.

In conclusion, the contamination rates with coliforms were high in Gabonese water samples. Insufficient water treatment of surface water could give rise to outbreaks with *Salmonella* sp. and other enteric pathogens, including those exhibiting multiresistant antibiotic patterns. This may be a challenge for the local healthcare system because therapeutic options for infections with ESBL-producers are limited in Gabon.

Conflicts of interest

None.

Acknowledgments

We thank Harry Kaba, Lambaréné and Damayanti Kaiser (Münster, Germany) for the excellent technical assistance. This work was supported by the Deutsche Forschungsgemeinschaft (DFG; Bonn, Germany; EI 247/8-1).

References

1. Lopez AD, Mathers CD, Ezzati M, Jamison DT, Murray CJ. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet* 2006;**367**:1747—57.
2. Bain R, Cronk R, Wright J, Yang H, Slaymaker T, Bartram J. Fecal contamination of drinking-water in low- and middle-income countries: a systematic review and meta-analysis. *PLoS Med* 2014;**11**:e1001644.
3. World Health Organization (WHO). *Guidelines for drinking water quality*. 4th ed. Geneva, Switzerland: WHO; 2011.
4. De Boeck H, Miwanda B, Lunguya-Metila O, Muyembe-Tamfum JJ, Stobberingh E, Glupczynski Y, et al. ESBL-positive Enterobacteria isolates in drinking water. *Emerg Infect Dis* 2012;**18**:1019—20.
5. World Health Organization (WHO). *Rapid assessment of drinking-water quality: a handbook for implementation*. Geneva, Switzerland: WHO; 2012.
6. Leclerc H, Mossel DA, Edberg SC, Struijk CB. Advances in the bacteriology of the coliform group: their suitability as markers of microbial water safety. *Annu Rev Microbiol* 2001;**55**:201—34.
7. Monstein HJ, Östholm-Balkkhed Å, Nilsson P, Nilsson MV, Dornbusch K, Nilsson LE. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. *APMIS* 2007;**115**:1400—8.

8. Bain R, Cronk R, Hossain R, Bonjour S, Onda K, Wright J, et al. Global assessment of exposure to faecal contamination through drinking water based on a systematic review. *Trop Med Int Health* 2014;**19**:917—27.
9. Schaumburg F, Alabi A, Kokou C, Grobusch MP, Köck R, Kaba H, et al. High burden of extended-spectrum β-lactamase-producing Enterobacteriaceae in Gabon. *J Antimicrob Chemother* 2013;**68**:2140—3.
10. Pees M, Rabsch W, Plenz B, Fruth A, Prager R, Simon S, et al. Evidence for the transmission of Salmonella from reptiles to children in Germany, July 2010 to October 2011. *Euro Surveill* 2013;**18**(4). pii=20634.