Microbiological safety of a small water distribution system: is *E. coli* a suitable indicator during a severe drought?

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Abstract

This study evaluated the microbiological safety of the water distribution system of a city in the state of Minas Gerais (Brazil), population 120000 inhabitants. During the study, the city suffered from a severe drought that had a significant impact on water availability and quality of the river that supplies water to the city. Samples (2 liters) were collected from the distribution system through a period of six months, which included wet and dry months, from three points: the point with the lowest altitude in the distribution network, the farthest point from the Water Treatment Works (WTW), and an intermediate point. Free chlorine was measured *in situ* using a Hach kit. DNA was extracted using a FastDNA Spin Kit Soil (Qbiogene). Advanced sequencing techniques (Ion Torrent) were used to identify and quantify pathogenic bacteria present in the samples. Coliforms and *E. coli*, indicators currently used worldwide to assess microbiological safety of drinking water, were measured on all samples using an enzyme substrate method (ONPG-MUG Colilert®). Results showed the presence of *E. coli* and pathogenic bacteria, even in the presence of free chlorine. The OTUs related to pathogen bacterial was present in all months on DWS and, in general, with high relative abundance.

Keywords
Pathogenic bacteria; coliforms; *E. coli*; drinking water distribution system (DWS); chlorine.

INTRODUCTION

Brazil has approximately 18% of the earth´s total freshwater (UNESCO, 2012), including rivers, lakes and reservoirs that have multiple uses, such as drinking water supply and power generation (ANA, 2013). Approximately 80% of all freshwater in Brazil is located in the Pantanal and Amazon regions, which present low population. The remaining freshwater is distributed in the Southeast, South and Northeast regions, which house the vast majority of the country's population (78%, IBGE, 2010). Since late 2013, the southeast region of Brazil is facing a serious water crisis as a result of both drought and bad water management, which has forced 27% of the country's municipalities to declare state of emergency in 2015 (ANA, 2015).

The significant reduction in water availability in rivers and reservoirs has forced several water companies to catch water from multiple sources in order to meet demand. Some of these sources receive untreated sewage from municipalities and industries, increasing the risk of drinking water contamination by pathogenic organisms (Revetta *et al.* 2016). This was the case of the city in Minas Gerais (southeast Brazil), subject of the current study. During the recent drought, it had to pump approximately 25% of its flowrate from a river that suffers from unregulated urban development.

The monitoring of the microbiological safety of water distribution systems (DWS) is extremely important in order to minimize risks to public health, especially during severe drought events as they cause further deterioration of freshwater quality. In Brazil, the current drinking water legislation (MS 2914/11) requires a minimum free chlorine concentration of 0.2 mg L^{-1} in the entire distribution network and only requires the monitoring of coliforms and *E. coli* to evaluate the microbiological safety of water supply systems. These indicators can be inadequate, as some pathogenic bacteria are more resistant to chorine than coliforms/*E. coli* (USEPA 2000). Research is still needed to assess the suitability of coliforms/*E. coli* as indicators for pathogenic organisms (Field 2007). The purpose of this study was to evaluate, using advanced molecular methods, if the well-known indicator *E. coli* is suitable to indicate the presence of pathogenic bacteria in a water distribution system severely stressed by drought.

MATERIAL AND METHODS

The water supply system

The Drinking Water Treatment Plant (DWTP) has an average flowrate of 180 L/s, reaching 200 L/s on peak days, serving approximately 120000 people. The DWTP uses conventional treatment, including coagulation, flocculation, sedimentation, single media filtration and addition of free chlorine before distribution. The DWTP has four single layer (1.8 m thickness), rapid, up flow filters, that are are backwashed every 12 hours with treated water. Free chlorine is added to filtered water at a concentration of 1.5 mg Cl_2/L (contact time of approximately 30 min), and fluoride is added at a concentration of 1 mg L^{-1}.

Sampling: Samples

Were collected from tree points in the drinking water distribution system (DWS) (Table 1), from July to December-2015, including the end of the dry season (July to September) and the beginning of the wet season (October to December).

Table 1. Sampling points in the water distribution system.

Point	Type of water	Sampling points	Filtered volume (July to Octorber)	Filtered volume (November and December)
1	Treated water (DWS)	Municipal School (point with the lowest altitude in the DWS)	2 L	4 L
2		Health Center (intermediate point)	2 L	4 L
3		Residence (farthest point from the water treatment works)	2 L	4 L

Samples were collected on taps that received water directly from the distribution network and were kept in sterile glass bottles containing 1 tablet of 10% sodium thiosulfate to inactivate free chlorine. One hundred mL of water were used for the quantification of coliforms and *E. coli* using ONPG-MUG Colilert®. The samples were processed immediately after sampling in accordance to APHA 9223B. Samples were filtered (total volumes stated on Table 1) using cellulose acetate membrane filters (GF1 filter, 0.2 μm, Macherey and Nagel). The filters were preserved at –20°C for genomic DNA extraction. Water temperature and free chlorine concentrations were measured in *in situ* using a calibrated thermometer and a 21055 colorimeter pocket Hach kit (Hach Lange, UK). Turbity was measured using a 2100Q portable turbidimeter.

DNA Extraction

DNA extraction from filters was performed using a FastDNA Spin Kit Soil (Qbiogene), according the manufacturer's instructions. Concentration and purity of DNA extracts were determined using a Nanodrop 1000 spectrophotometer (Thermo Scientific).

PCR amplification and purification

The PCR reaction was performed with Multiplex identifiers (MIDs), Barcodes forward, for each sample. The primer set used (9515F/926R) allowed the amplification of the V4 and most of the V5 region of the 16S rRNA gene. The PCR reaction was carried out using FastStart High Fidelity PCR System and the PCR Nucleotide Mix (Roche Diagnostics GmbH, Mannheim, Germany). This kit contains: NH^{4+} buffer, $MgCl_2$, dNTP's, and DNA polymerase. For the PCR, 5 μL of the 1:10 dilution for each of the DNA extracts was added to 45 μL of amplification mixture, resulting in a mix of 5 μL FastStart High Fedelity Reaction Buffer, 1μL dNTP, 1 μL of each primer, 2.5 μL of Enzyme blend and 40.5 μL of nuclease free water in a final volume of 50 μL. The following PCR program was used: initial denaturation 95 ° C for 4 min, followed by 25 cycles of 95 ° C for 1 min, 55 ° C (annealing) 45 sec, 72 ° C (extension) for 1 min and final elongation of 72 ° C for 7 min. PCR products were checked by electrophoresis, run at 100 V for 45 min, using 5μl of product/extract plus loading buffer on 2% agarose gels, containing Nancy-520 DNA Gel Stain (Sigma-Aldrich), and in 1 x Tris-acetate-EDTA buffer. Gels were visualized by a UV illumination using a Bio-Rad Fluor-S Multi Imager (Bio-Rad, UK). PCR purification was made using theAgencourt AMPure XP PCR Purification system, which utilizes Agencourt's solid-phase paramagnetic bead technology for high-throughput purification of PCR amplicons. The resulting purified PCR product was used directly in next-generation sequencing workflow.

Amplicon library quantification

The quantification of the individual amplicon libraries was carried out using a Qubit 2.0 Fluorometer, Qubit ds DNA HS reagent Assay kit and Qubit™ assay tubes. A Qubit working solution was prepared for all samples by diluting the Qubit dsDNA HS reagent 1:200 in Qubit dsDNA HS buffer. Each sample tube required 199 μl of Qubit working solution and 1 μl sample. The amplicon libraries were pooled in equimolar quantities into a unique solution for downstream template preparation procedure for clonal amplification on Ion Spheres. The diluted library was freshly prepared before being used on the Ion OneTouch2 System.

Next Generation Sequencing (NGS) by Ion Torrent

The samples was sequenced using the Ion Torrent PGM (200 bp) with the 316™ sequencing chip using the manufacturer's instructions (Life Technologies, USA). Data

were analyzed with SILVA ribosomal RNA gene database project. The final results were given in relative abundance (%).

Identification and relative abundance of pathogenic bacteria

Pathogenic bacteria were identified based on the NGS data, species level (Level 7 on Silva's database), by comparing the identified species with those reported on the medical literature (de Baets *et al.*. 2007; Retailliau *et al.* 1979; Patterson *et al.* 1991; Touchon *et al.* 2014; Loubinoux *et al.* 2003; Vaz-Moreira *et al.* 2011; Saeb *et al.* 2014; Okada *et al.* 2013; Lo *et al.* 2015; Auzias *et al.* 2003; Lindquist *et al.* 2003; Bathia *et al.* 2004; Gini *et al.* 1990, Lloyd-Puryear e t al 1990; Ryan *et al.* 2006; Park *et al.* 2011; Brooke 2012; Sharma & Kalawat 2010; Hironaga *et al.* 2008; Podschun *et al.* 2001; Siciliano *et al.* 2008; http://www.bacteRiver.cict.br; last full update, 29 August2016). The abundances of all pathogenic OTUs (operational taxonomic units) were summed, giving a total abundance of pathogenic bacteria.

RESULTS AND DISCUSSION

In total, NGS generated 131941 OTUS's, based on Silva's ribosomal RNA gene database. This value refers to the total of OTUs in the three sampling points throughout the monitoring period. The enzymatic method did not detect *E. coli* or coliforms in any of the samples throughout the study. However, NGS detected *E. coli* and several pathogenic bacteria in all but two samples (Figure 1), at relative abundance as high as 5% of the total community for one point. NGS has a very low detection limit (Roh *et al.* 2010). It is important to note that NGS does not indicate viability of the cells detected, as the presence of DNA fragments from dead cells would also be detected by NGS. Nonetheless, these results are worrisome, because pathogenic bacteria were only present at very low relative abundance on samples collected from the treated water reservoir that feeds the distribution network (data not shown). *E. coli* are frequently used as indicators to infer faecal pollution in water (USEPA, 2000). All strains of this indicator organism should experience the same persistence (maintenance of culturable cells) in water sources, although, some strains may have comparatively extended persistence outside the host, while others may persist very poorly in environmental waters (Anderson *et al.* 2005). *E. coli* is viable for at least three months under environmental conditions and could be viable for four months under laboratory conditions (Kudryavtseva 1972). The possible reason of the difference in viability between both conditions is that there are more microflora under natural than laboratory conditions, and an indicator organism that survives for long periods in the environment is not desired (Edberg *et al.* 2000). The absence of *E. coli* and the presence of pathogenic bacteria in the DWS studied could be attributed to differences in strains persistence. Furthermore, cells that are not active within the DWS could become active inside the human host (Pinto *et al.* 2012).

For most samples, *E. coli* were present when pathogenic bacteria were detected, except for the month of October at the farthest point from the water treatment works (Residence) and for the month of November at the point with the lowest altitude in the distribution network (Municipal School), giving two false negative results as indicator organisms. However, in both samples, only very low total abundances of pathogenic bacteria were detected (<0.4%). Conversely, *E. coli* were also present when pathogenic bacteria were not detected (Residence-August and Municipal School-October) or were detected at very low abundance (<0.05%, Health Centre-July, Municipal School-

August, Health Centre-September), giving five false positive results as indicator organisms. The samples with the highest abundance of *E. coli* did not coincide with those with the highest relative abundance of pathogenic bacteria. Spearman correlation between *E. coli* and total pathogenic bacteria was not significant (0.05%).

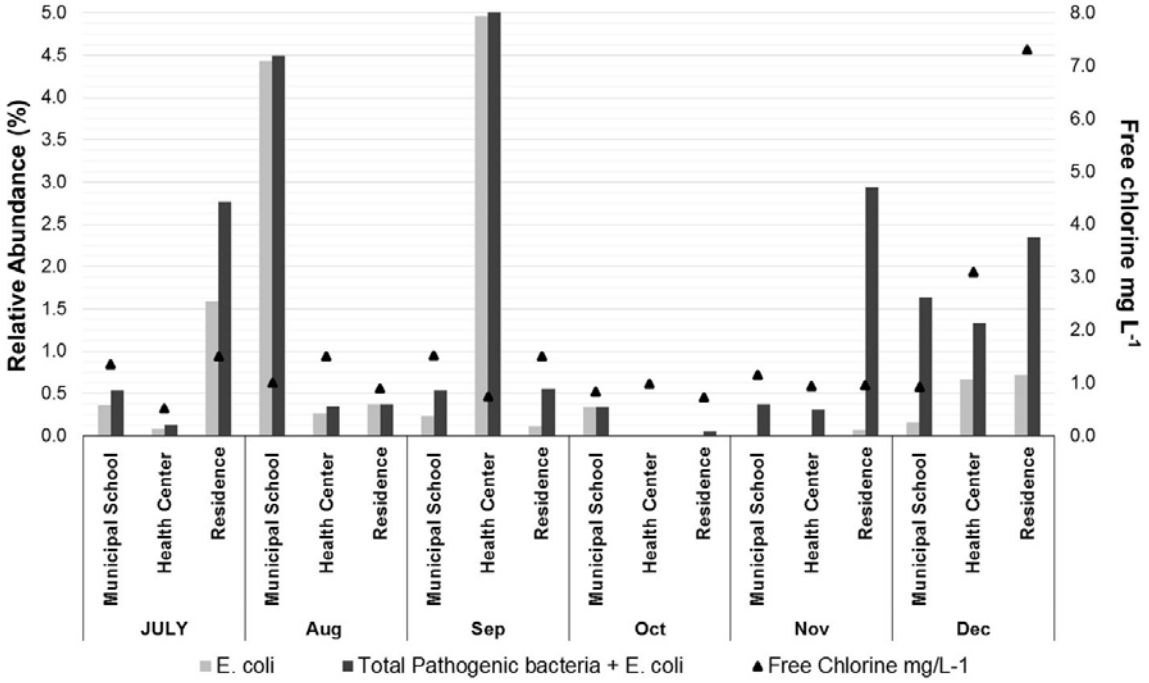

Figure 1. Relative abundance (%) of *E. coli* and pathogenic bacteria determined by NGS and free residual chlorine concentration (mg L^{-1})**.**

Free residual chlorine concentrations in virtually all samples met the minimum and maximum values stipulated by the Brazilian Regulation (MS 2914/2011, minimum of 0.2 mg L^{-1}, maximum of 5 mg L^{-1} combined chlorine residual), except for one sample collected at the farthest point in the distribution system in the wet month of December (7.3 mg L^{-1}). Operators reported that extra chlorine was added directly to the distribution system as a precautionary measure due to the detection of high turbidity in the distribution system. It has yet to be determined if the increase in turbidity was due to failure in the water treatment works or due to possible intrusion of untreated water in the distribution network. This will only be determined once the operators release all turbidity data on the water leaving the treatment works during the study. Both these possible causes are of concern due to the close association between high turbidity values and the presence of pathogenic organisms in water. Additionally, the practice of adding free chlorine directly into the distribution network when higher turbidity is detected is of concern due to its higher risk of forming trihalomethanes (THMs) in the drinking water distribution system (Ediberg *et al.* 2000) and because the network might not provide the necessary contact time required for disinfection, increasing risks to public health due to waterborne diseases (Wright *et al.* 2004). Samples that had the highest free chlorine concentrations also showed high relative abundance of pathogenic bacteria (Figure 1). However, the method used to determine the abundance of pathogenic bacteria does not indicate whether the pathogenic bacteria detected were alive or had

been inactivated by free chlorine. Pathogens are able to colonize water distribution systems even after disinfection by chlorination with chlorine (Pinto et al. 2012, 2014). Chloramine and chlorine could be a selective influence on the microbial community of drinking water distribution systems (Holinger et al. 2014). The occurrence of some pathogenic bacteria, such as *Legionella*, *Mycobacterium* and *Pseudomonas*, is influenced by the presence or absence of the disinfectant residual and the type of disinfectant residual used (Bautista-de los Santos et al. 2016).

A total of 19 species of bacterial pathogens were detected in this study (Table 2). Some of these bacteria are opportunistic and can cause disease in patients with debilitated immune systems (Pagani *et al.*. 2003; Murphy 2012). *P. acnes*, detected in two sampling points in two occasions, is generally considered non-pathogenic. However, evidence suggests that it can be a low-virulence pathogen in a variety of postoperative infections and other chronic conditions (Bathia *et al.* 2004).

Table 2. Pathogenic bacteria detected by NGS on samples from the water distribution network.

Pathogenic Species	Points/ month			Disease	Reference
	MS	**HC**	**R**		
1. Achromobacter xylosoxidans	JD	JAN	JD	Infection or colonisation in Cystic Fibrosis patients.	De Baets *et al.* 2007
2. Acinetobacter calcoaceticus	S		JD	Nosocomial infection	Patterson, *et al.* 1991; Touchon *et al.* 2014
3. Acinetobacter ursingii		N	JD	Bacteremia	Loubinoux *et al.*. 2003
4. Brucella spp	JSD	ON	JOND	Brucellosis, Osteoarthritis, Endocarditis and several neurological disorders.	Saeb *et al.* 2014
5. Chromobacterium haemolyticum		JND	SN	Bacteremia	Okada *et al.* 2013
6. Corynebacterium aurimucosum	S			Urinary Tract Infection	Lo *et al.* 2015
7. Corynebacterium durum			N	Respiratory Tract Infection	Riegel *et al.* 1997
8. Corynebacterium freneyi			JN	Bacteremia	Auzias *et al.* 2003
9. Coxiella	SN	AND	JSD	Q fever	Siciliano *et al.* 2008
10. *Dygonomonas* sp	ND			Infection gall bladder	Hironaga *et al.* 2008
11. Klebsiella	A			Nosocomial Infections	Podschun *et al.* 2001
12. Legionella nagasakiensis	J			Pneumonia	Yang *et al.* 2012
13. Massilia timonae	S	N		General Infections in Low Immunity patients	Lindquist *et al.* 2003
14. Propionibacterium acnes	JA		JD	Androgen stimulated seborrhoea, hyperkeratinisation and obstruction of the follicular epithelium and inflammation.	Bathia *et al.* 2004
15. Psychrobacter immobilis			D	Ocular infection, Meningitis	Gini *et al.* 1990, Lloyd-Puryear *et al.* 1990

16.	*Ralstonia pickettii*	A		J	Nosocomial Infections	Ryan *et al.* 2006
17.	*Rhodococcus erythropolis*			D	Septicaemia	Park *et al.* 2011
18.	*Shewanella putrefaciens*	S	S		Hepatobiliary disease, peripheral vascular disease, with chronic leg ulcer	Sharma & Kalawat 2010
19.	*Stenotrophomonas maltophilia*			JN	Nosocomial Infections	Brooke 2012

Legend: J=July, A=August, S=September, O=October, N=November, D=December. MS=Municipal School; HC=Health Center; R=Residence

Some OTUs were only detected in the dry months of July-September (*Ralstonia pickettii, Shewanella putrefaciens, Legionella nagasakiensis, Klebsiella, Corynebacterium aurimucosum*), whereas other species were detected only in the wet months of October-Decmeber (*Corynebacterium durum, Chromobacterium subtsugae, Dygonomonas* sp, *Psychrobacter immobilis).* This could suggest the effect of the water source on the pathogenic community of the distribution network, as water from a new intake built on a different river started to be pumped to the treatment works from October, during the peak of the water scarcity crisis. Revetta et al. 2016 observed that source water influences the resistance, survival and community composition of pathogens and indicators in water distribution systems.

CONCLUSIONS

The current work shows that *E. coli* was a fairly good indicator organism for pathogenic bacteria, based on next generation sequencing. However, it gave false positive or negative results as an indicator organism in 8 occasions, out of 18 samples. The current study also showed the presence of a diverse community of pathogenic bacteria in the water distribution network investigated, suggesting that the system is in dire need of operational changes to improve water quality. Furthermore, the current study highlights the importance of protecting catchment areas that are used for water supply in order to minimise risks to public health due to waterborne diseases. The current study also highlights the importance of effective maintenance of water distribution systems to prevent biofilm formation and accumulation of other particles that allow bacterial adhesion.

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REFERENCES

Agência nacional de águas ANA (Brasil). 2013 Sistema Nacional de Informações sobre Recursos Hídricos. Atlas Geográfico de Recursos Hídricos do Brasil - Seção 6: Infraestrutura. Rede de Abastecimento Urbano. 69 p. http:/www.snirh.gov.br/atlasrh2013livro/%3e (accessed 10 July 2016). In Portuguese Language.

Agência nacional de águas ANA (Brasil). 2015 Conjuntura dos recursos hídricos. Brasília, 107p. In Portuguese Language.

Auzias, A, Bollet, C., Ayari, R., Drancourt, M., Raoult, D. 2003 *Corynebacterium freneyi* Bacteremia. *Journal of clinical microbiology* **41** (6), 2777–2778.

Bautista-de los Santos, Q. M., Schroeder, J. L., Sevillano-Rivera, M. C., Sungthong, R., Ijaz, U. Z., Sloan, W. T.,Pinto, A. J. 2016 Emerging investigators series: microbial communities in full-scale drinking water distribution systems–a meta-analysis. *Environmental Science: Water Research & Technology* **2**, 1-14.

Bhatia, A., Maisonneuve, J.F., Persing, D.H. 2004 *Propionibacterium acnes* and chronic diseases. http://www.ncbi.nlm.nih.gov/books/NBK83685/ (accessed 10 July 2016)

Bouwer, E. J., Crowe, P. B. 1988 Biological processes in drinking water treatment. *Journal American of Water Works Association* **80**(9), 82−93.

BRASIL. 2011. Portaria n. 2914 de 12 de dezembro de 2011. Estabelece os procedimentos e responsabilidades relativos ao controle e vigilância da qualidade da água para consumo humano e seu padrão de potabilidade, e dá outras providências. DiáRiver Oficial, Poder Executivo, Brasília, DF. In Portuguese Language.

Brooke, J. S. 2012 *Stenotrophomonas maltophilia*: an Emerging Global Opportunistic Pathogen. *Clinical Microbiology Reviews* **25**(1), 2–41.

De Baets, F, Schelstraete, P., Van Daele, S., Haerynck, F., Vaneechoutte, M. 2007 *Achromobacter xylosoxidans* in cystic fibrosis: Prevalence and clinical relevance. *Journal of Cystic Fibrosis* **6**(1), 75–78.

Edberg, S.C., Rice, E.W., Karlin, R.J., Allen, M.J. 2000 *Escherichia coli*: the best biological drinking water indicator for public health protection. *Journal of Applied Microbiology* **88**, 106–116.

Engle, N.L., Lemos, M.C. 2010 Unpacking governance: Building adaptive capacity to climate change of river basins in Brazil. *Global Environmental Change* **20**, 4-13.

Euzéby J. P. 1997 List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. *International Journal of* Systematic and Evolutionary Microbiology **47**, 590–592.

Field, K., Samadpour, M., 2007 Fecal source tracking, the indicator paradigm, and managing water quality. Water Research, **41** (16), 3517-3538.

Gini, G. A. 1990 Ocular infection caused by *Psychrobacter immobilis* acquired in the hospital. *Journal of Clinical Microbiology* **28**(2), 400–401.

Hironaga, M., Yamane, K., Inaba, M., Haga, Y., Arakawa, Y. 2008 Characterization and antimicrobial susceptibility of *Dysgonomonas capnocytophagoides* isolated from human blood sample. *Japanese journal of infectious diseases* **61**, 212–213.

Holinger, E. P., Ross, K. A., Robertson, C. E., Stevens, M. J., Harris, J. K., Pace N. R. 2014 Molecular analysis of point-of-use municipal drinking water microbiology. *Water Research* **49**, 225-235.

Instituto Brasileiro De Geografia E Estatística IBGE. Censo demográfico 2010: características da população e dos domicílios. River de Janeiro, 2011. Available in: Access in: March 22, 2015

Kimberly, L. A., Whitlock, J.E., Harwood, V. J. 2005 Persistence and Differential Survival of Fecal Indicator Bacteria in Subtropical Waters and Sediments. *Applied and Environmental Microbiology* **71**(6), 3041–3048

Kudryavtseva, B.M. 1972 An experimental approach to the establishment of zones of hygienic protection of underground water sources on the basis sanitary-bacteriological indices. *Journal of Hygience, Epidemiology, Microbiology, and Immunology* **18**, 503–511.

Lindquist, D., Murrill, D., Burran, P., Winans, G., Janda, M., Probert, W. 2003 Characteristics of *Massilia timonae* and *Massilia timonae*-Like Isolates from Human Patients, with an Emended Description of the Species. *Journal of Clinical Microbiology* **41**(1), 192–196.

Lloyd-puryear, M., Wallace, D., Baldwin, T., Hollis, D. G. 1991Meningitis caused by *Psychrobacter immobilis* in an Infant. *Journal of Clinical Microbiology* **29** (9), 2041–2042.

Lo, S., Thiam, I., Fall, B., Ba-Diallo, A., Foly, D. O., Diagne, R., Lamine Dia, M., Ka, R., Marceline, S. A., Sow, A. I. 2015 Urinary tract infection with *Corynebacterium aurimucosum* after urethroplasty stricture of the urethra: a case report. *Journal of Medical Case Reports* **9** (156), 2-3.

Loubinoux, J., Mihaila-Amrouche, L., Le Fleche, A., Pigne, E., Huchon, G., Grimont, P.A. D., Bouvet, A. 2003 Bacteremia caused by *Acinetobacter ursingii. Journal of Clinical Microbiology* **41**(3), 1337–1338.

Marengo, J. A. 2006 Mudanças climáticas globais e seus efeitos sobre a biodiversidade: caracterização do clima atual e definição das alterações climáticas para o território brasileiro ao longo do Século XXI. Brasília, DF: MMA, 163 p. In Portuguese Language

Ministério do Meio Ambiente. Secretaria de Recursos Hídricos. 2006 Plano Nacional de Recursos Hídricos: panorama e estado dos recursos hídricos do Brasil. Volume I. Brasília, DF, BRASIL. In Portuguese Language

Okada, M., Inokuchi, R., Shinohara, K., Matsumoto, A., Ono, Y., Narita, M., Ishida, Y., Kazuki, C., Nakajima, S., Yahagi, N. 2013 *Chromobacterium haemolyticuminduced* bacteremia in a healthy young man. *Bio Med Central Infectious Diseases* **13**(406), 2-4.

Park, S.D., Uh, Y., Jang, I.H., Yoon, K.J., Kim, H.M., Bae, Y.J. 2011*Rhodococcus erythropolis* septicaemia in a patient with acute lymphocytic leukaemia. *Journal of Medical Microbiology* **60,** 252–255.

Patterson, J.E., Vecchio, J., Pantelick, E.L., Farrel, P., Mazon, D., Zervos, M.J. Hierholzer, J.W. 1991 Association of Contaminated Gloves with Transmission of *Acinetobacter caicoaceticus* var. anitratus in an Intensive Care Unit. *The American Journal of Medicine* **91**(5), 479-483.

Philip, C.B. 1943 Nomenclature of the pathogenic *rickettsiae*. *American Journal of Hygiene* **37**, 301–309.

Pinto A. J., Schroeder J., Lunn M., Sloan W., Raskin L. 2014 Spatial-temporal survey and occupancy-abundance modeling to predict bacterial community dynamics in the drinking water microbiome. *mBio* **5**(3), e01135-14.

Pinto, A. J., Xi, C., Raskin. L. 2012 Bacterial Community Structure in the Drinking Water Microbiome Is Governed by Filtration Processes. *Environmental Science and Technology* **46**, 8851−8859.

Podschun, R., Pietsch, S., Holler, C., Ullmann, U. 2001 Incidence of *Klebsiella* Species in Surface Waters and Their Expression of Virulence Factors. *Applied and Environmental Microbiology* **67**(7), 3325-3327.

Retailliau, H., Hightower, A., Dixon, R., Allen, J. 1979 *Acinetobacter calcoaceticus*: A Nosocomial Pathogen with an Unusual Seasonal Pattern. *The Journal of Infectious Diseases* **139**(3), 371-375.

Revetta R.P., Gomez-Alvarez V., Gerke T.L., Santo Domingo J.W., Ashbolt N.J. 2016 Changes in bacterial composition of biofilm in a metropolitan drinking water distribution system. *Journal of Applied Microbiology* **121**(1), 294-305.

Riegel, P., Heller, R., Prevost, G., Jehl, F., Monteil, H. 1997 *Corynebacterium durumsp*. nov., from human clinical specimens. *International Journal of* Systematic and Evolutionary Microbiology **47**, 1107–1111.

Roh, S. W., Abell, G. C., Kim, K. H., Nam, Y. D., & Bae, J. W. 2010 Comparing microarrays and next-generation sequencing technologies for microbial ecology research. *Trends in Biotechnology*, **28**(6), 291-299.

Ryana, M.P., Pembrokeb, J.T., Adley, C.C. 2006 *Ralstonia pickettii*: a persistent Gram-negative nosocomial infectious organism. *Journal of Hospital Infection* **62**, 278–284.

Saeb, A.T.M., David, S.K., Al-Brahim, H. 2014 In Silico Detection of Virulence Gene Homologues in the Human Pathogen *Sphingomonas* Spp. *Evolutionary Bioinformatics* **10,** 229–238.

Sharma, K.K., Kalawat, U. Emerging Infections: *Shewanella* – 2010 A Series of Five Cases. *Journal of Laboratory Physicians* **2**(2), 61–65.

Siciliano, R. F., Ribeiro, H. B., Furtado, R. H. M., Castelli, J. B., Sampaio, R. O., Santos, F. C. P., Colombo, S., Grinberg, M., Strabelli, T.M.V. 2008 Endocardite por *Coxiella burnetii* (febre Q): doença rara ou pouco diagnosticada? Relato de caso. *Revista da Sociedade Brasileira de Medicina Tropical* **41**(4), 409-412. In Portuguese language

Standard Methods for the Examination of Water and Wastewater 2012 22th edn, American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

Touchon, M., Cury, J., Yoon, E. J., Krizova, L., Cerqueira, G. C., Murphy, C., Grillot-Courvalin, C. 2014 The genomic diversification of the whole *Acinetobacter* genus: origins, mechanisms, and consequences. *Genome biology and evolution* **6**(10), 2866–2882.

UNESCO. United Nations Education, Scientific and Cultural Organization Managing Water under Uncertainty and Risk. UNESCO, Second Part. http:/www. wwdr4, 2012. p. 230 – 370 (accessed 21 July 2016).

United States Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: enterococci and Escherichia coli EPA-821/R-97/004. U.S. Environmental Protection Agency, Washington, DC, USA.

Wilkes, G., Edgeb, T., Gannonc , V., Jokinenc, V. C., Lyauteyd, E., Medeirose, D., Neumannf, N., Rueckerg, N., Toppd, E., Lapena, D.R. 2009 Seasonal relationships among indicator bacteria, pathogenic bacteria, *Cryptosporidium* oocysts, *Giardia* cysts, and hydrological indices for surface waters within an agricultural landscape. *Water Research* **43**(8), 2209–2223.

Wingender, J. Flemming, H.C. 2011 Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health* **214**, 417-423.

Wright, J., Gundry, S., Conroy, R. 2004 Household drinking water in developing countries: a systematic review of microbiological contamination between source and point-of-use. *Tropical Medicine and International Health* **9**(1), 106-117.

Yang, G., Benson, R.F., Ratcliff, R.M., Brown, E.W., Steigerwalt, A.G., Thacker, W.L., Daneshvar, M.I., Morey, R.E., Saito, A., Fields, B.S. 2012 *Legionella nagasakiensis* sp. nov., isolated from water samples and from a patient with pneumonia. *Journal of clinical microbiology* **41**(6), 2777–2778.