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

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INTRODUCTION

Minas Gerais

Belo Horizonte

0   75   150   300 Km

Doce River

1:2.200.000

0   22   44   66   88 Km

Projeção: Latitude/Longitude

● **Doce River Valley**

INTRODUCTION

✓ The Drinking Water Treatment System surveyed in this study captures water from one stream and one River, inside the basin of the River Doce, where environmental degradation has been intensified during the last years due to unregulated urban development.

✓ It is the largest plant in the municipality and has a Conventional treatment Technology. Its production reaches 180 L/s and serves 120.000 people.

✓ The Water Treatment Works supplies about 55% of the water demand of the city

✓ During the recent drought, it had to pump approximately 25% of its flowrate from a river deteriorated by unregulated urban development.

STUDY AREA

Health Care

-19.654160, -43.233430
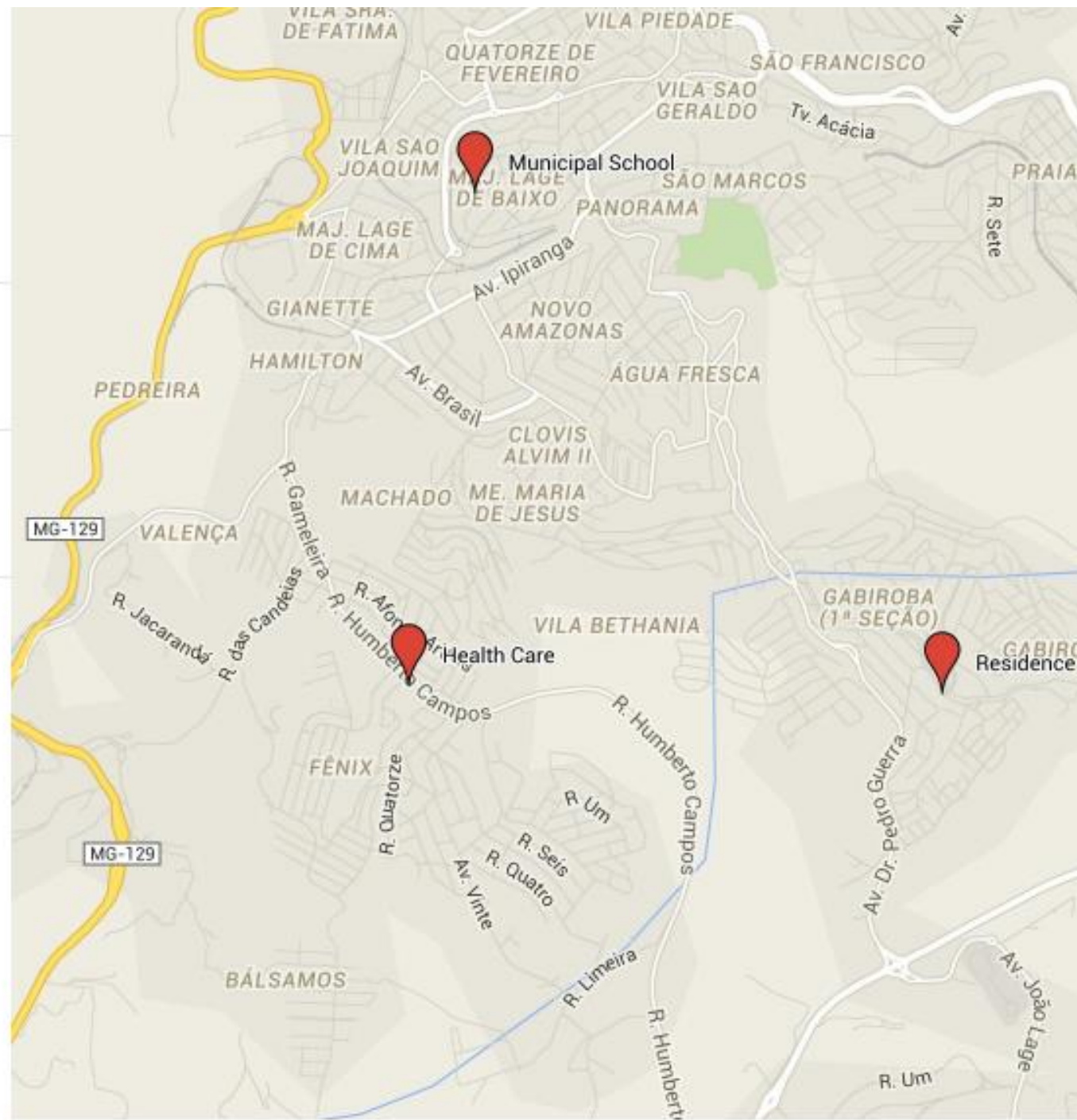
Residence

-19.654500, -43.210060

In total 18 samples

WTP-Pureza

-19.663790, -43.200698

Municipal School

-19.633870, -43.230540

Samples were collected in 3 points of the distribution system from July to December 2015 in

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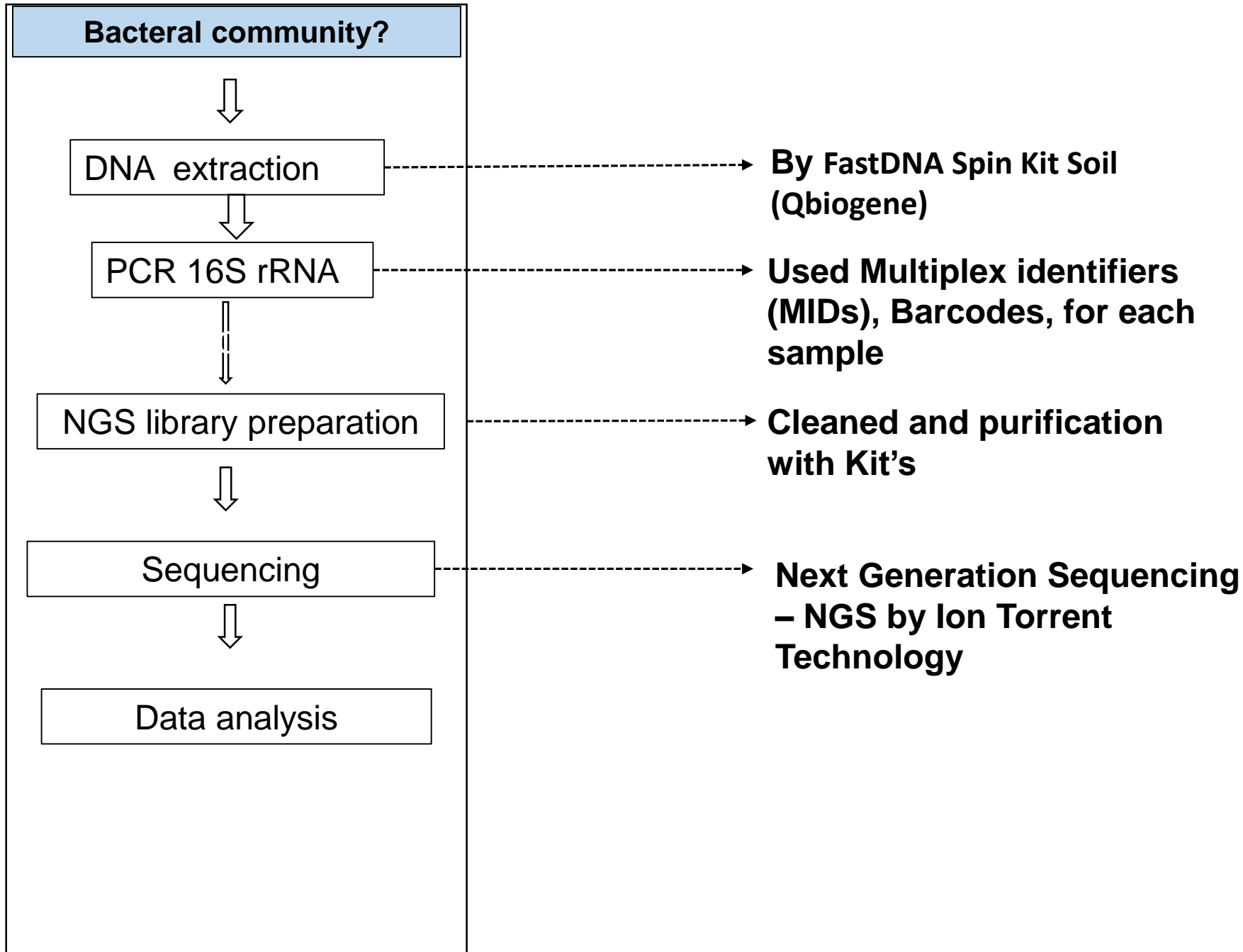
Point	Type of water	Sampling points	Filtered volume (July to Octorber)	Filtered volume (November and December)
1		Municipal School (point with the lowest altitude in the DWS)	2 L	4 L
2	Treated water (DWS)	Health Center (intermediate point)	2 L	4 L
3		Residence (farthest point from the water treatment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

A sample of 2 L was collected at point each month and filtered with a filter 0.22 um porosity (Acetate cellulose) for DNA extraction. Flasks of 100 mL was used to *E. coli* analysis. The water temperature, Free chlorine and turbidity were obtained *in-situ*.
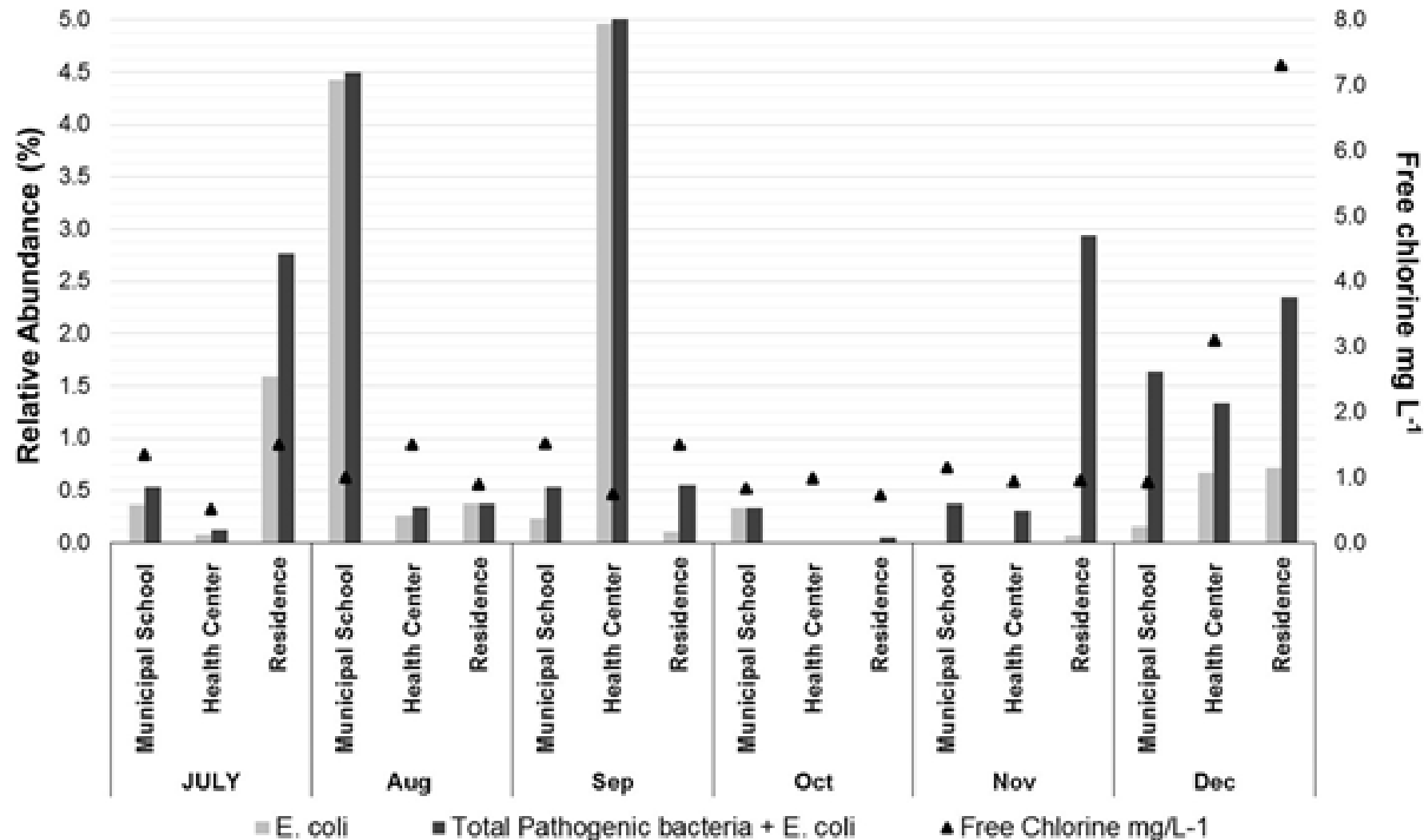
Bacteral community?

DNA extraction → **By FastDNA Spin Kit Soil (Qbiogene)**

PCR 16S rRNA → **Used Multiplex identifiers (MIDs), Barcodes, for each sample**

NGS library preparation → **Cleaned and purification with Kit's**

Sequencing → **Next Generation Sequencing – NGS by Ion Torrent Technology**

Data analysis

MATERIAL AND METHODS
E. coli and Coliforms analysis

Using ONPG-MUG - Colilert® -
APHA 9223B

A   **B**   **C**

Read and Interpretation results

- In total, NGS generated 131.941 OTUS's, based on Silva's ribosomal RNA gene database.

- The enzymatic method did not detect *E. coli* or coliforms in any of the samples throughout the study.

- Spearman correlation between *E. coli* and total pathogenic bacteria was not significant (0.05%).

RESULTS

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

NGS detected *E. coli* and several pathogenic bacteria in all but two samples (Fig), at relative abundance as high as 5% of the total community for one point.

Samples that had the highest free chlorine concentrations also showed high relative abundance of pathogenic bacteria

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

A total of 19 species of bacterial pathogens were detected in this study. Some of these bacteria are opportunistic and can cause disease in patients with debilitated immune systems (Pagani *et al.* 2003; Murphy 2012).

RESULTS

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

Pathogenic Species	Points/ month			Disease	Reference
	MS	**HC**	**R**		
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

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.

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 presence of a diverse community of pathogenic bacteria in the water distribution network investigated suggests that the system is in dire need of operational changes to improve water quality.

- 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.

ACKNOWLEDGMENTS

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Obrigada
Thank you