Quantitative detection of pathogens in roof harvested rainwater

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Roof-harvested rainwater is an alternative water source. Though generally considered acceptable for potable use, the presence of pathogens has been reported in research literature [1]. Various zoonotic pathogens are present in faeces of animals that have access to the roof, and following rain events, pathogens may be transported to rainwater tanks via roof runoff. The microbiological quality of water is traditionally assessed by enumerating faecal indicators such as *Escherichia coli* and enterococci [2]. Significant limitations in using faecal indicators include their poor correlation with pathogens and faecal indicator concentrations cannot be used to assess public health risk when compared to the direct monitoring of pathogens [3]. Polymerase chain reaction (PCR)

based techniques enable rapid and direct detection/quantification of pathogens in water that are otherwise laborious to culture using traditional microbiological methods.

In this study, the microbiological quality of roof-harvested rainwater was assessed by enumerating faecal indicators and detecting zoonotic pathogens in samples from rainwater tanks. The significance of this study stems from the fact that, instead of measuring faecal indicators, pathogens that are capable of causing illness were directly measured using quantitative PCR (qPCR) methods. The pathogen concentration data will be used to perform quantitative microbial risk assessment (QMRA). This work forms part of the development of a 'Toolbox' of methodologies using qPCR based methods which can be used to detect and quantify more than 35 microorganisms commonly found in water (more information on the qPCR 'Toolbox' can be obtained from the corresponding author Dr. Warish Ahmed).

Eighty-four rainwater **s**amples were collected from 66 residential houses in Brisbane and Gold Coast regions. Membrane filtration method was used for *E. coli*, and enterococci enumeration. For PCR/qPCR analysis, *Aeromonas hydrophila lip* gene, *Campylobacter jejuni mapA* gene*, Campylobacter. Coli ceuE gene, E. coli* O157 LPS, VT1, VT2 genes, *L. pneumophila mip* gene, *Salmonella invA and spvC* genes, *G. lamblia* β-giradin gene and *Cryptosporidium parvum* Cryptosporidium oocyst wall protein (COWP) gene were selected. Most of these genes were selected based on their virulent properties. In addition, priority was given to those genes which are single copy genes (where possible) so that gene copy numbers could be directly converted to cell counts. DNA extraction from rainwater samples, PCR amplification, the standards for qPCR and the primers used for this study are described elsewhere [4]. For each target pathogen, PCR reproducibility, limit of detection, detection efficiency and PCR inhibitory effects were evaluated.

For the samples tested, 57 (65%) were positive for *E. coli.* The concentrations were: 18 (20%) between 1 and 10 CFU/100 ml, 16 (18%) between 11 and 100 CFU/100 ml, 17 (19%) between 101 to 1000 CFU/100 ml, and 6 (7%) had >1001 CFU/100 ml. For the 84 samples, 72 (82%) were positive for enterococci. The concentrations were: 16 (18%) between 1 to 10 CFU/100 ml, 27 (31%) between 11 to 100 CFU/100 ml, 20 (23%) between 101 to 1000 CFU/100 ml, and 9 (10%) had >1001 CFU/100 ml. The PCR positive results for potential pathogens are shown in Table 1.

TABLE 1. PCR positive results for potential pathogens

Gene of target pathogen	PCR positive results/Number of samples tested (% of sample positive)	Range of gene copies/100 ml
A. hydrophila lip gene	7/84 (8.3)	Not tested
Campylobacter coli ceuE gene	10/27 (37)	Not tested
C. jejuni mapA gene	1/84 (1.1)	Below qPCR detection limit
E. coli O157 LPS gene	0/84 (0)	Not tested
E. coli VT1 gene	0/84 (0)	Not tested
E. coli VT2 gene	0/84 (0)	Not tested
L. pneumophila mip gene	8/84 (9.5)	6 – 17
Salmonella invA gene	17/84 (20)	6.6 – 38
Salmonella spvC gene	0/27 (0)	Not tested
G. lamblia β-giradin gene	15/84 (18)	9 – 51
Cryptosporidium parvum COWP gene	0/84 (0)	Not tested

Quantitative PCR assays were performed on selected pathogens considering their prevalence and infectious dose. Though *C. jejuni mapA* gene was detected in one sample, the concentration was below qPCR detection limit. *L. pneumophila*, *Salmonella*, and *Giardia*

lamblia were detected in several samples (Table 1). *L. pneumophila mip* and *Salmonella invA* are single copy genes and were converted to cell numbers (i.e. 1 gene copy = 1 cell). *G. lamblia* β-giradin gene copies numbers were converted to cysts (16 gene copies = 1 cyst). Binary logistic regressions were also performed to identify the correlations between the concentrations of fecal indicator bacteria and the presence/ absence of potential target pathogens (Table 2). The presence/absence of the potential pathogens did not correlate with any of the indicator bacteria concentrations.

TABLE 2. The relationships between faecal indicators and the presence and absence of selected pathogens in samples from rainwater tanks

Indicators vs. pathogenic microorganisms	Nagelkerke's *R* square [a]	*P*-value [b]	Odds ratio
E. coli vs. *A. hydrophila*	0.055	0.460	1.00
E. coli vs. *C. jejun*	0.008	0.775	1.00
E. coli vs. *L. pneumophila*	0.006	0.640	1.00
E. coli vs. *Salmonella*	0.048	0.198	1.00
E. coli vs. *G. lamblia*	0.019	0.484	1.00
Ent vs. *A. hydrophila*	0.006	0.700	1.00
Ent vs. *C. jejuni*	0.001	0.943	1.00
Ent vs. *L. pneumophila*	0.007	0.555	1.00
Ent vs. *Salmonella*	0.016	0.388	1.00
Ent vs. *G. lamblia*	0.001	0.928	1.00

[a] Nagelkerke's *R* square, which can range from 0.0 to 1.0, denotes the effect size (the strength of the relationship); stronger associations have values closer to 1.0.

[b] *P*-value for the model chi square was <0.05 and the confidence interval for the odds ratio did not include 1.0. Greater odds ratios indicate a higher probability of change in the dependent variable with a change in the independent variable.

Roof-harvested rainwater can be of poor microbiological quality. The presence of one or more pathogenic microorganisms along with fecal indicators represents a health risks to users. The pathogens had a poor correlation with fecal indicators. Currently we are performing QMRA using Monte Carlo analysis to determine the likely numbers of infections resulting from these exposures. These outcomes in terms of the impact of using roof-harvested rainwater on the disease burden of South East Queensland region of Australia will be interpreted.

Acknowledgements

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References

1. Simmons, G. *et al.* (2001) Contamination of potable roof-collected rainwater in Auckland, New Zealand. *Water Res.* 35:1518-1524.
2. US Environmental Protection Agency. 2000. Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. Office of Science and Technology, Washington, DC. EPA/821/R-97/004.
3. Hörman, A. *et al.* (2004) *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and indicator organisms in surface water in south-western Finland, 2000–2001. *Appl. Environ. Microbiol*. 70, 87–95.
4. Ahmed, W. *et al.* (2008) Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Appl. Environ. Microbiol*. 74, 5490-5496.

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