REAL-TIME PCR DETECTION OF PATHOGENIC MICROORGANISMS IN ROOF-HARVESTED RAINWATER IN SOUTHEAST QUEENSLAND, AUSTRALIA

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Summary

The microbiological quality of roof-harvested rainwater was assessed by monitoring the concentrations of *E. coli* and enterococci in a range of urban rainwater tanks in Brisbane, Australia. Samples were also tested for the presence of potential pathogens using real-time PCR SYBR Green dye. Of the 72 samples, 8 (11%), 1 (1%), 4 (6%), 15 (21%) and 15 (21%) were PCR positive for *Aeromonas hydrophila lip* gene, *Campylobacter jejuni mapA* gene, *Legionella pneumophila mip* gene, *Salmonella invA* gene and *Giardia lamblia* β-giardin gene. None of the samples were positive for *Escherichia coli* O157, VT1 and VT2 genes. The presence or absence of the potential pathogens did not correlate with traditional faecal indicators suggesting that faecal indicators may not be adequate to assess the microbiological quality of rainwater and the consequent health risk.

Objective

To assess the microbiological quality of roof harvested rainwater in Brisbane, and to investigate the prevalence of pathogenic microorganisms, and their correlation with traditional faecal indicator bacteria.

Methodology

1. In all, 72 samples were collected from 52 rainwater tanks after a rain event.

2. The membrane filtration method was used to process the water samples for *E. coli* and enterococci enumeration.

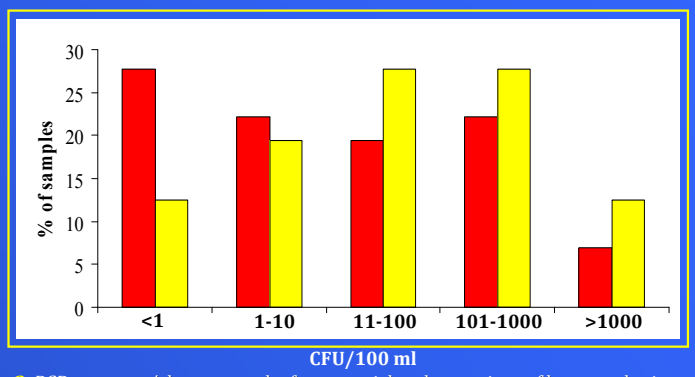
3. PCR primers were selected from published research literature.

4. From each water sample, DNA was extracted using DNeasy blood and tissue kit (Qiagen). Each water sample was tested for the potential PCR inhibitors, and the limit of detection of the real-time PCR assay was determined for each target.

5. Real-time PCR assay was performed using Rotor-gene 6000 (Corbett, Australia)

Results

1. Concentrations of *E. coli* □ and enterococci □ in samples (n=72) collected from roof-harvested rainwater



2. PCR presence/absence results for potential pathogens in roof harvested rain water samples

Target	PCR positive results/number of samples tested
A. hydrophila lip gene	8/72
C. jejuni mapA gene	1/72
E. coli O157 gene	0/72
E. coli verocytotoxin gene 1 (VT1)	0/72
E. coli verocytotoxin gene 2 (VT2)	0/72
L. pneumophila mip gene	4/72
Salmonella invA gene	15/72
Cryptosporidium oocyst wall protein gene	0/72
G. lamblia β -giardin gene	15/72

3. Binary logistic regressions were performed to identify the correlations between the concentrations of faecal indicator bacteria, and the presence/absence of potential pathogens. The presence/absence of the pathogens did not correlate with any of the indicator bacterial concentrations.

Indicators vs. pathogenic microorganisms	Nagelkerke's *R* square [a]	*P*-value [b]	Odd ratio
E. coli vs. *A. hydrophila*	.055	0.460	1.00
E. coli vs. *C. jejuni*	.008	0.775	1.00
E. coli vs. *L. pneumophila*	.006	0.640	1.00
E. coli vs. *Salmonella* spp.	.048	0.198	1.00
E. coli vs. *G. lamblia*	.019	0.484	1.00
Enterococci vs. *A. hydrophila*	.006	0.700	1.00
Enterococci vs. *C. jejuni*	.001	0.943	1.00
Enterococci vs. *L. pneumophila*	.007	0.555	1.00
Enterococci vs. *Salmonella spp.*	.016	0.388	1.00
Enterococci vs. *G. lamblia*	.001	0.928	1.00

Nagelkerke's *R* square, which can range from 0.0 to 1.0, denotes the effect size. Stronger associations have values closer to 1.0.

Conclusions

1. The presence of one or more pathogenic microorganisms along with high levels of faecal indicators could represent a significant health risks to users.

2. The results obtained also indicated a poor correlation between faecal indicators and potential pathogens tested. Therefore, testing faecal indicators may not be adequate to assess the microbiological quality of rainwater and consequent health risk.

3. As a part of the on-going research, we are currently using real-time PCR to quantify *C. jejuni*, *L. pneumophila*, Salmonella spp., and Giardia spp. in rainwater samples. Our future research will focus on Quantitative Microbial Risk Assessment (QMRA) for roof harvested rainwater.