Improving quantitative microbial risk assessments (QMRAs) with next generation sequencing

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Highlights

- The human infectious potential of *Campylobacter* can be estimated using multilocus sequence typing and incorporated into QMRAs to provide more accurate recreational risk assessments.
- Recreational risk was reduced by ~50% when considering *Campylobacter* infectivity, suggesting the default QMRA assumption that all pathogens are human infectious may overestimate risk.

Introduction

Recreational water quality guidelines determine if the public health risk to recreators is acceptable. These guidelines use faecal indicator organism (FIO) density to gauge the risk of illness from aquatic recreation on the basis that studies have previously shown a positive correlation between FIO density and the rate of contracting gastroenteritis (ANZECC, 2000; European Union, 2006; USEPA, 2012). However, most of these guidelines are based on epidemiological studies conducted in marine waters with known point sources of human faecal contamination, and may not be representative of the risk of illness in freshwater catchments affected by non-point, non-human sources (Fleisher et al., 1996; Kay et al., 1994).

Quantitative microbial risk assessments (QMRAs) look beyond FIOs and consider specific and reference pathogens to assess the risk to human health. QMRAs have been applied to marine, freshwater, and riverine systems to evaluate the risk associated with aquatic recreation. However, there are uncertainties within the QMRA process, such as how to represent the proportion of organisms that may be human infectious. Traditionally, it is assumed that all pathogenic organisms present in aquatic environments are human infectious until proven otherwise due to quantification methods not differentiating between pathogenic and saprophytic species. Although, this may be an overly conservative postulation that can result in premature closure of recreational hotspots and unnecessary investment in remediation if the actual risk of illness is lower. The aim of this study was to explore the effect of conservatism in QMRAs by comparing risk results using an evidence-based approach for pathogen infectivity against traditional assumptions. Preliminary research of a predominantly forested and agricultural freshwater catchment indicated the faecal pathogen *Campylobacter* spp. was driving the recreational risk. As such, *Campylobacter* spp. was used as a case study to incorporate its human infectivity as derived from multilocus sequence typing into the QMRA process to provide a more accurate risk assessment.

Methodology

Environmental monitoring

Water samples from a river in Victoria, Australia were collected at 5 site groupings along the length of the river under dry and wet weather conditions during November 2012-August 2014 and June 2017-June 2020. Five litre grab and flow-weighted composite water samples were analysed for FIOs *Escherichia coli* (*E. coli*), *Enterococcus* spp. and pathogens *Campylobacter* spp., *Salmonella* spp., *Giardia* spp., *Cryptosporidium* spp., adenoviruses, and enteroviruses using Australian standard methods. Noroviruses were assumed present at maximum concentrations consistent with virus detection. Concentration results

that were below/above the detection limit were assumed present at the lower/upper detection limit for subsequent analyses. *Campylobacter* spp. isolates were stored at -80°C for genetic sequencing.

***Campylobacter* spp. multilocus sequence typing (MLST) and infectivity**

Campylobacter spp. isolates were sequenced using the Illumina NextSeq500 with TruSeq Sequencing-by-Synthesis V2 chemistry in 150 bp paired-read format. The obtained reads for each isolate underwent *de novo* assembly using the Nullarbor (v2.0) software package with comparison to *C. jejuni* NTCT81116 (NC_009839.1) and *C. coli* RM4661 (NZ_CP007181) reference genomes.

The following criteria were applied to estimate the human infectious potential of *Campylobacter* spp.: 1) speciation, 2) matching with international databases, and 3) association with human disease. *C. jejuni* and *C. coli* isolates were compared to the PubMLST database (https://pubmlst.org/; Jolley et al. 2018) to match with sequence types (STs) existing prior to this project and identify potential human disease risk. STs that did not match were assumed not human infectious. *Campylobacter* spp. infectivity was estimated as:

$$\left(\frac{\text{Total } C.jejuni}{\text{Total } Campylobacter \text{ spp.}}\right) * \left(\frac{C.jejuni \text{ infectious}}{\text{Matched } C.jejuni}\right) + \left(\frac{\text{Total } C.coli}{\text{Total } Campylobacter \text{ spp.}}\right) * \left(\frac{C.coli \text{ infectious}}{\text{Matched } C.coli}\right) \quad \text{(Eq. 1)}$$

Quantitative microbial risk assessment

The risk of illness per single recreational exposure was determined for primary and secondary contact activities using Monte Carlo simulations (N=100,000). Primary contact was defined as swimming, or submerged from the chest and below (Dufour et al. 2006). Secondary contact was defined as wading, or submerged from the waist and below, fishing, kayaking, canoeing, rafting, or tubing activities (Dorevitch et al. 2011). Two infectivity scenarios were assessed: (1) all pathogens were assumed to be 100% human infectious, and (2) *Campylobacter* spp. infectivity was estimated from MLST data of riverine isolates, but all other pathogens were assumed 100% human infectious. Dose-response models and parameters will be fully detailed in the presentation.

Results and discussion

Under the baseline Scenario 1, *Campylobacter* spp. was responsible for 60%-80% of the total pathogen risk and the recreational risk increased with downstream distance. However, MLST data indicated that only 4%-49% of *Campylobacter* spp. were human infective, and infectivity decreased with downstream distance. When *Campylobacter* spp. infectivity was applied to the QMRA in Scenario 2, there was a 40%-94% reduction in *Campylobacter* spp. risk and 29%-74% reduction in total pathogen risk (Table 1).

Table 1. Total pathogen risk of illness from aquatic recreation in a Victorian river. Samples were collected under dry and wet weather conditions. Site groups are ordered from upstream (#1) to downstream (#5). Site groups 1-4 are freshwater and site group 5 is estuarine. Risks were evaluated for two scenarios: (1) all pathogens were 100% human infectious, (2) *Campylobacter* spp. infectivity from MLST data, and all other pathogens were 100% human infectious.

	Scenario 1				Scenario 2			
Site	Primary Contact		Secondary Contact		Primary Contact		Secondary Contact	
Group	Mean	95th perc.	Mean	95th perc.	Mean	95th perc.	Mean	95th perc.
1	1.4%	5.9%	0.3%	1.3%	1.0%	4.3%	0.2%	0.9%
2	2.4%	9.1%	0.6%	2.4%	1.4%	5.4%	0.3%	1.1%
3	2.3%	7.8%	0.5%	2.3%	0.6%	2.1%	0.1%	0.4%
4	4.3%	14.2%	1.1%	4.4%	2.0%	7.2%	0.4%	1.4%
5	3.4%	13.4%	0.9%	4.3%	1.9%	8.4%	0.4%	1.7%
All Sites	2.9%	10.6%	0.7%	3.3%	1.5%	5.9%	0.3%	1.2%

To date, only one QMRA study was found to attempt to incorporate the human infectivity of *Campylobacter* spp. using species-focused methods, where the authors assumed only *C. jejuni* and *C. coli* were infectious to humans (Soller et al. 2017). However, this methodology may not be sufficient due to *Campylobacters'* genetic diversity. In particular, not all strains of *C. jejuni* or *C. coli* have been previously associated with human disease (Carter et al. 2009, Sheppard et al. 2009, Shrestha et al. 2019). As shown, MLST allows further insight into the human infectivity of *Campylobacter* spp., which can be integrated into the QMRA process to provide more accurate risk assessments.

Conclusions and future work

This study has proven that not all organisms detected were human infectious, and assuming otherwise may result in an overestimate of recreational risk. Multilocus sequence typing can be used to estimate *Campylobacter* spp. infectivity in order to provide more accurate and meaningful risk assessments. This study represents a conceptual framework that can be extended to other pathogenic organisms. Organism infectivity can then be applied to future QMRA studies, and be used to develop site-specific recreational water quality guidelines, especially for waters impacted by non-human sources. Further research is needed to refine the human infectivity calculation for *Campylobacter* spp.. In particular, how to utilize new sequence types, which may be regionally specific. Future work should explore virulence markers and if these genes can be examined to indicate *Campylobacter* spp. human infectivity of new sequence types.

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