Project Title: Naturalized *E. coli* and fecal indicators in drinking water wells: implications for testing, risk assessment and as potential environmental reservoirs for antimicrobial resistance.

Background:

As part of the growing need for the use, and thus protection of groundwater sources, which provide water for irrigation, and human and livestock consumption, and upon which 30% of Ontarians rely as their primary drinking water source, water is now recognized as a critical link to health; for example through pathogen dissemination, and has become a dynamic and ever evolving foundation for research. Water potability has traditionally been based upon surrogate markers of fecal contamination, with *Escherichia coli (E. coli*) being the most frequently assessed indicator (1) both in Canada and globally.

Indicator bacteria, such as *E. coli*, were originally thought to be present only in the intestinal tracts of warm-blooded animals, including humans (1), and as such the occurrence of *E. coli* in water and food has been used as an indicator of fecal contamination, signaling the possible presence of other fecal pathogens such as *Campylobacter* and *E. coli O157.* This is due, in part, to the observed correlation between elevated *E. coli* counts in water and the rate of occurrence of gastrointestinal symptoms or disease.(2).

Recent studies suggest that there is a significant portion of the *E. coli* population that are "naturalized" in various environments, flourishing outside of the human or animal intestinal tract (3,4,5). The existence of "naturalized" *E. coli* has challenged the notion that finding *E. coli* in water indicates recent fecal contamination and raises questions around the interpretation of *E. coli*-based water monitoring data and subsequent public health risk assessment, as "naturalized" *E. coli* may contribute to the *E. coli* numbers detected in water, resulting in assumptions regarding recent fecal pollution. Thus, there is an important need to differentiate enteric/fecal *E. coli* from the environmental bacteria, including naturalized *E. coli* populations, for use in the current practice of *E. coli*-based water quality monitoring. This study proposes the first ever investigation of naturalized *E. coli* in private drinking water wells in Canada.

Hypothesis/Rationale:

We hypothesize that *E. coli* adapts to the environment and persists outside of the animal intestinal tract, including within drinking water wells. As such, *E. coli* may not be the ideal indicator of fecal contamination for water quality determinations. Understanding the relationship and prevalence of naturalized *E. coli* in drinking water environments will inform both the interpretation of current water testing guidelines as well as future methodological development and policy. Further, we anticipate that these naturalized *E. coli* populations may serve as a previously unidentified reservoir of antimicrobial resistance (AMR) and antimicrobial resistance genes.

Aim: To evaluate and utilize comparative accessory gene fingerprinting to discriminate between naturalized and enteric/fecal *E. coli* in private drinking water samples in order to inform future testing methodologies, test interpretation and risk assessment, including potential reservoirs of AMR.

Methods:

Drinking well water samples (200 mL) will be collected from 200 drinking water wells in a select

geospatial region in rural Ontario. Waters will be filtered using the standard membrane filtration methodology with a 0.45 um filter. Filters will be transferred to Differential Coliform (DC) agar and incubated overnight at 37^0C. Isolates phenotypically identified as *E. coli* (purple on DC agar) will be selected and frozen/archived until further characterized. Fecal samples from the same geospatial context will be collected, including from dog, cat, cow, pig, sheep, bear, beaver, deer, horse, humans, geese, waste water treatment facilities, and others, as available, (approximately 100 isolates expected), plated to DC media and presumptive *E. coli* isolates selected and archived. In addition to looking at *E. coli* isolates from various well waters across a specific rural region, multiple *E. coli* isolates from single wells will also be studied, in order to assess diversity not only between wells but also within wells.

All water derived *E. coli* samples will be subsequently resurrected and plated to Blood Agar to achieve clonal isolates. 200 *E. coli* from varied drinking water samples will be selected for *E. coli* confirmation, using PCR (*uidA/tuf*) Similarly, *E. coli* isolated from the fecal samples will be cultured and confirmed as *E. coli*.

Naturalized versus enteric/fecal *E. coli* determinations will be made based on the method of Tymensen et al.; namely, assessing for the following genes, *iutA, ccdB, clpXET1, hra 1, phd, traT* previously shown to be differentially expressed in these two populations (4), and for the stress marker, *uspC-IS30-flhDC,* based on work by Zhi et al., 2016 (5).

Antimicrobial profiling will be performed according to standard methods, namely agar dilution and Etest. 25 *E. coli* isolates from water samples identified as naturalized, 25 *E. coli* isolates from water samples identified as fecal/enteric, and 25 *E. coli* from the various animal fecal samples will be further characterized for antimicrobial resistance. (eg. Ampicillin, Cefoxitin, Ceftazidime, Cefotaxime, Ertapenem, Amikacin, Gentamicin, Ciprofloxacin, Tetracycline, Colistin)

Microbial source tracking will be performed based on the *Bacteroidales* assays developed by Lee et al., 2010 (6)

Significance:

Currently, little is known regarding the contribution of private well waters to waterborne illness in Ontario, and current microbial indicators and the methods (culture) utilized to detect and report upon, are poorly understood, such that risk cannot be appropriately determined. Moreover, 1/3 of Canadians are known to rely on groundwater/well water as a primary drinking water source, including an estimated 98% of rural Ontarians. And, it is well documented that private water sources serve as a major vector for infectious diseases (2). Newer technologies, including qPCR, allow additional scientific insight into the varied bacterial populations within drinking well water, including the possible presence of naturalized *E. coli*. Determining the extent and prevalence of this environmentally adapted *E. coli* will shed light on the value and utilization of this globally recommended fecal indicator. As such, this work will serve as a pilot to future studies and inform testing, policy, risk assessment and the dynamic and ever changing environmental niches of antimicrobial resistance, as well as provide insight regarding environmental influences on the

establishment of environmental *E. coli* and AMR reservoirs. These findings could also be incorporated into assessment tools adopted by health authorities; both in Ontario, Canada, and elsewhere, in order to better inform public health and environmental interventions. Further, the information collected and generated by this study will allow for the development of geostatistical models which can be used to elucidate survival and transport mechanisms both above and below ground using open source geological and climate data as well as generate well susceptibility indices modelled on earlier work by Hynds et al, 2012 (7).

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