Microbiological Quality of Drinking Water

from First Nation Communities in Manitoba, Canada

by

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# ABSTRACT

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This research examined the microbiological quality of drinking water distribution systems typically encountered in First Nation in Canada. Fecal bacteria and a wide range of antibiotic resistance genes (ARGs) were frequently present in tap water of a community that relies on lake water as the source water to its water treatment plant, whereas this was not the case in communities that rely on groundwater. Fecal bacteria and ARGs were predominantly detected in tap water of homes that had water distributed by a truck for storage in underground cisterns, and not in treated water of homes with piped water. Cistern samples that tested positive for fecal bacteria had free residual chlorine concentrations less than 0.2 mg/L, which is required to suppress microbial growth. We are urging all governments in Canada to strategize how to alternatively deliver clean, running drinking water to families living in these homes.

**FOREWORD**

This dissertation is written in manuscript style and is composed of two manuscripts. These two manuscripts have not yet been submitted to a journal.

The manuscripts include:

Manuscript I: Detection of Fecal Bacteria and Antibiotic Resistance Genes in Drinking Water Collected from Three First Nation Communities in Manitoba.

Manuscript II: Presence of Antibiotic Resistance Genes and Indicator Bacteria in Drinking Water from Cisterns in a First Nation Community in Manitoba

# ACKNOWLEDGEMENTS

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# 1. INTRODUCTION

## 1.1 Drinking water issues in First Nation communities in Canada

Access to clean drinking water is one of the most important determinants of human health (WHO 2011). However, many residents in First Nation communities across Canada have only limited access to safe drinking water. The latest report (Neegan Burnside Ltd. 2011) indicates that 72% on-reserve homes in Canada have water piped directly to their households from drinking water treatment plants (WTPs); 13.5% of the homes obtain their drinking water delivered by trucks where it is stored in underground or aboveground cisterns; and the remaining homes (14.5%) either rely on well water or do not have running water. In addition, Neegan Burnside Ltd. (2011) also reported that 39% of water systems on reserves are at a high overall risk, 34 % are at a medium overall risk, and 27 % are at a low overall risk. The risk level was determined based on the Indigenous and Northern Affairs Canada Risk Level Evaluation Guidelines (INAC 2011) in which scores were given to each of five parameters including 1) water source, 2) design, 3) Operation (and Maintenance), 4) reporting and 5) operators. The low, medium, and high overall risk were defined as:

**Low Risk (1.0 to 4.0):** Systems have minor deficiencies where water quality parameters usually do not exceed the maximum acceptable concentrations of substances as defined by the Guidelines for Canadian Drinking Water Quality (GCDWQ).

**Medium Risk (4.1 to 7.0):** Systems have deficiencies where water quality may pose risks that can affect human health. Immediate action is not required, but the deficiencies should be addressed.

**High Risk (7.1 to 10.0):** Systems have major deficiencies leading water quality parameters exceed the maximum acceptable concentrations of substances as defined by the GCDWQ. These

1

deficiencies may lead to human health and safety risks, or environmental risks. Once a system is classified under this category, immediate corrective action must be taken to minimize or eliminate deficiencies.

When water quality parameters exceed the maximum acceptable concentrations of substances as defined by the GCDWQ, Drinking Water Advisories (DWAs) are issued for the protection of public health. Drinking Water Advisories (DWAs) can be found online: https://www.canada.ca/en/indigenous-services-canada/services/short-term-drinking-water-advisories-first-nations-south-60.html. In brief:

**Boil Water Advisories:** Families in homes under a Boil Water Advisory are advised to boil their tap water for at least one minute before consuming the water to kill fecal bacteria present in water.

**Do Not Consume Advisories:** Families in homes under a Do Not Consume Advisory should not consume the water because the water system is contaminated by chemicals such as lead, which cannot be removed from the water by boiling.

**Do Not Use Advisories:** Families in homes under a Do Not Use Advisory should not use the water for consumption or other household activities because the water is contaminated by substances that may irritate skin, eye, or nose.

Ongoing DWAs in First Nation communities across Canada are reported on a website maintained by Health Canada (https://www.canada.ca/en/indigenous-services-canada/services/short-term-drinking-water-advisories-first-nations-south-60.html), with the exception of DWAs in First Nation communities issued in the Province of British Columbia and Province of Saskatchewan. For British Colombia, ongoing DWAs are reported on a website maintained by the First Nation Health Authority (http://www.fnha.ca/what-we-do/environmental-health/

drinking-water-advisories). Based on these websites, as of May 2018, there were 48 short-term and 76 long-term DWAs in First Nation across Canada, excluding the Province in Saskatchewan. For Saskatchewan, the drinking water quality in First Nation communities associated with the Saskatoon Tribal Council is reported on an annual basis (Saskatoon Tribal Council 2017). In 2016/2017, the Saskatoon Tribal Council deemed that 77% of wells and 29% of cisterns in these First Nation contained unsafe drinking water sources. The Government of Canada, under the leadership of Prime Minister Justin Trudeau, is working with First Nation communities to end long-term drinking water advisories by 2021 with the progress being reported on-line: https://www.aadnc-aandc.gc.ca/eng/1506514143353/1506514230742.

## 1.2 Microbial contamination in drinking water distribution systems

The majority (> 90%) of the DWAs in First Nation communities are Boil Water Advisories, indicating that drinking water is frequently contaminated with fecal bacteria, including the risk of the presence of pathogenic bacteria in the water. Chlorine is an effective disinfection method because it kills pathogenic bacteria, thereby providing safe drinking water for the end-user (Davies and Mazumder 2003). Although bacteria in source water can be successfully removed by water several studies have observed that the drinking water from taps in homes is of poorer quality than the treated water leaving the water treatment plants (Verberk et al. 2007; Vreeburg and Boxall 2007; Liu et al. 2013). This suggests that there is a possibility of the regrowth of bacteria during water distribution, and/or a risk of bacterial intrusion as treated water is distributed from the water treatment plant to homes.

Pipeline, truck delivery to cisterns, and wells are the three major drinking water distribution methods used by First Nation communities. A common cause for bacteria to enter pipelines is presence of leaks in the pipeline (a low pressure in the piped distribution system is an indication

of leaks) (Kleiner 1998; Sadiq et al. 2004). Bacteria can also enter at points in the distribution system where pipes connect (Kleiner 1998; Sadiq et al. 2004). For truck delivery to cisterns, it is possible that bacteria enter when a hose is used to fill the truck with water at the water treatment plant, or when a hose is used to fill the cistern at the home with truck water (Baird et al. 2013). In addition, when cisterns are located underground and damaged, bacteria can enter with subsurface water entering through cracks in the cistern wall (Baird et al. 2013), especially in the Spring during periods of snowmelt and heavy rainfall events as this may generate flooding conditions (saturated soils) (Artiola et al. 2012). Well water is typically not treated with chlorine therefore bacteria are detected in well water when groundwater is contaminated (Leal-Bautista et al. 2013).

Components in the water distribution system include three physical phases, including biofilms that can be seen as a biological phase (Liu et al. 2013). The physical phases are bulk water with dissolved nutrients, suspended solids (particles) in that water, and aggregates of suspended solids, which settle within pipes or cisterns (Liu et al. 2013). Nutrients that support bacterial growth, and solids that can act as attachable surfaces for bacteria, can promote biofilm formation on pipeline and cistern walls.

Biofilms are a key player in persistent water contamination. A biofilm is an aggregate of microorganisms, extracellular polymeric substances (EPS) and other substances (e.g., mineral crystals, silt and clay particles). Biofilms attach to surfaces with a tenacity that makes them difficult to remove (Donlan 2002; Wingender and Flemming 2011). It is estimated that 95% of bacteria detected in drinking water distribution systems are contained in biofilms (Flemming et al. 2002), and that most of the free bacterial cells in bulk water were released from such biofilms (Ridgway and Olson 1981; LeChevallier 1987). Thus, the presence of biofilms can result in the continuous presence of bacteria in drinking water distribution systems.

The formation of a biofilm in drinking water distribution systems generally follows three steps (1): Free moving microbial cells attach to inner walls of drinking water networks; (2) Attached microbial cells start to divide resulting in the formation of layers of cells; (3) The biofilm reaches a mature state, at which time motile cells can be released into the bulk water, where they can initiate the formation of biofilms elsewhere (Donlan 2002).

In drinking water distribution systems, biofilms can not only protect bacteria from physical and chemical harm, but they can contribute to bacterial growth by providing nutrient gradients, and by aiding in the exchange of genes, and quorum sensing (Donlan 2002). The formation and growth of biofilms have been frequently studied in model and real drinking water systems. These studies have shown that factors, such as temperature, surface material, disinfect, and nutrient levels all can affect the formation and growth of biofilms and microbial composition in drinking water networks (Lechevallier et al. 1990; Rogers 1994, Rasmus et al. 2002; Codony et al. 2005; Buse et al. 2014; Inkinen et al. 2014; Liu et al. 2014).

**1.3 Water contamination and human health**

Consumption of contaminated water can cause waterborne diseases in humans (WHO 2011). It is estimated that about 1400 species of human pathogens can inhabit drinking water, and of these 538 species are bacteria, 208 are viruses, 317 are fungi, 57 are parasitic protozoa, and 287 are parasitic worms (Woolhouse 2006).

Human and animal feces entering the water distribution system is one way of introducing pathogenic bacteria to drinking water resulting in increased risks to human health. For example, *Vibrio cholera* in drinking water can cause cholera, which results in 100,000 – 120,000 deaths annually mostly in developing countries (WHO 2010). In Canada, *Escherichia coli* (*E. coli*) in the

drinking water distribution system of Walkerton, ON caused an outbreak of gastroenteritis in May 2000, killing seven people and causing 2,300 people to become ill (MOE 2002). In Milwaukee, USA, *Cryptosporidium* infection from contaminated water killed 100 people in 1993 (MacKenzie et al. 1994).

## 1.4 Antibiotic resistance genes in drinking water

Antibiotics are one of the greatest medical achievements in 20th century because they can treat a broad range of bacterial infections in animals, including humans. The widespread use of antibiotics poses public health concerns because it has been shown that antibiotics can be transported into the environment in unchanged forms where they can accelerate the emergence and development of antibiotic resistance in bacteria (Zhang et al. 2009).

Increases in antibiotic resistance means an increased risk of prolonged illnesses and a greater risk of death (Cosgrove 2006). For example, for every 100,000 people in Europe, 5 people die from infections caused by antibiotic resistant bacteria while this is an estimate 3 in every 100,000 people in the United States (Zell and Goldmann 2007).

Development of antibiotic resistance in bacteria can be due to: 1) changes in the permeability of cell walls, thereby restricting the access of antibiotics to target sites in the cell; 2) modifications in the activity of efflux pumps resulting in reduced intracellular concentrations of antibiotics; 3) enzymatic modification of the antibiotic; 4) degradation of antibiotic; 5) alteration of the target metabolic pathway; 6) modification of antibiotic targets; and 7) overproduction of the target enzyme. (Spratt 1994; McDermott et al. 2003; Magnet and Blanchard 2005; Wright 2005).

Antibiotic resistance genes are the key to the antibiotic resistance because they control the mechanisms stated above. In a natural environment with no anthropogenic influence, bacteria acquire antibiotic resistance by longtime evolution/mutation. However, in an environment where

antibiotics are introduced (e.g., sewage from a medical facility being released in a river) there will be significantly more evolution/mutation because of increased selective pressure. In these environments, antibiotic resistance is acquired and spread by mobile genetic elements. Mobile genetic elements (MGE) are DNA fragments that can be translocated from one genome to another or within a genome (Van Hoek et al. 2011). MGE's can be a wide range of genetic elements including plasmids, insertion sequences, insertion sequence common region elements, transposons, integrons, genomic islands, integrating conjugative elements, and bacteriophages (Marti et al. 2014). Mobile genetic elements are transferred between bacteria by horizontal gene transfer. The mechanisms of horizontal gene transfer are: 1) transformation in which bacteria take up free naked DNA from the environment; 2) conjugation in which one bacterium directly transfer mobile genetic elements to another cell by direct contact; and 3) transduction in which bacteriophages (bacterial viruses) pack a mobile genetic element from one bacterium into their phage heads and then inject the mobile genetic element into another bacterium.

Because antibiotics are used to treat bacterial infections in humans and the production of meat and fish for human consumption, municipal sewage plants, as well as livestock and fish farms, are possible contributing sources towards antibiotic resistance (Zhang et al. 2009). For example, several studies have found evidence of antibiotic resistance genes (ARGs) in water and manure samples including *tet* genes (resistant to tetracycline), *cat* genes (resistant to chloramphenicol), and *bla* genes (resistant to beta-lactamase) (Dang et al. 2006; Henriques et al. 2006b; Dang et al. 2007; LaPara et al. 2011; Li et al. 2017). Municipal sewage plants are particularly regarded as an important contributor towards antibiotic resistance (Marti et al. 2014). Rizzo et al. (2013). suggests that municipal sewage provides an ideal environment for horizontal gene transfer because bacteria can easily and directly be in contact with antibiotic resistance genes during the wastewater

treatment process. For example, a metagenomics study showed that 140 antibiotic resistance genes were detected in activated sludge in a municipal sewage (Szczepanowski st al. 2009).

ARGs can be released into various environments such as soils, lakes, and rivers as a result of natural processes such as runoff, or from the failure to remove antibiotic resistance genes from sewage (Marti et al. 2014; Bajaj et al. 2015; Tafoukt et al. 2017; LaPara et al. 2011;).

Lake and river water are commonly used as source water for drinking water treatment plants. If these sources are contaminated, antibiotic resistance genes can enter drinking water distribution systems when water treatment fails to remove them completely (Guo et al. 2014; Su et al. 2018; Zhang et al. 2016). In addition, antibiotic resistance genes also can get into drinking water when soil water or runoff seep into drinking water distribution systems. A prime example of this could be underground cisterns.

**1.5 Methods used to evaluate bacteria and antibiotic resistance genes in drinking water**

It is estimated that one-third of waterborne diseases result from infections with intestinal pathogenic bacteria (Hunter 1997). As a result, *E. coli* and/or coliforms [major genera including *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter* and *Escherichia* (Edberg et al. 2000)] are commonly used as indicator bacteria to evaluate the presence of fecal contamination and waterborne pathogens in drinking water (WHO 2011). While *E. coli* is considered as a more suitable indicator than coliforms bacteria because the *E. coli* is more specific to fecal contamination and other coliforms bacteria can also be found widely in natural environments, therefore, using coliforms as indicator of fecal contamination may give unreliable evaluations (Gruber et al. 2014).

The use of microbial enzyme profiles to detect *E. coli* and coliforms has been applied in drinking water quality monitoring (Feng and Hartman 1982). This method is a culture-based technique and use chromogenic substrate to cleave β-D-glucopyranosiduronic and galactosidase: the former one is the enzyme its productions limited to *E. coli* and can be found in 97% of the strains of this species (Kilian and Bulow 1976), while the latter is an enzyme found in majority of coliforms. One such commercial product is BRILLIANCE E. COLI/COLIFORM AGAR (CM0956, Oxoid): the reaction results in purple *E. coli* colonies as *E. coli* can produce both β-D-glucopyranosiduronic and galactosidase to cleave chromogenic substrates; but pink coliform colonies as coliforms are only able to produce galactosidase to cleave the galactosidase chromogen (Wohlsen 2011).

Although culture-based techniques such as the determination of fecal bacteria (total coliforms and *E. coli*) in treated water provide important information on drinking water safety, it cannot detect all the pathogenic bacteria in drinking water. In addition, many microorganisms present in the drinking water are nonculturable (Ashbolt 2015). Thus, methods such as high-throughput sequencing are required to characterize composition of bacterial communities in water supplies. Several studies have used high-throughput sequencing to provide a more comprehensive illustration of microbial compositions in drinking water (Revetta et al. 2010; Lin et al. 2014; Fernando et al. 2016; Farenhorst et al. 2017; Li et al. 2017; Su et al. 2018).

Methods used to investigate the presence of ARGs in drinking water are polymerase chain reaction (PCR) and quantitative real-time PCR (qPCR). PCR method has been widely used to detect ARGs (Patterson et al. 2007; Taviani et al. 2008). The advantage of PCR method is its sensitivity (Zhang et al. 2009). In order to save time and reduce the cost, some studies used multiplex PCR to detect more than one target ARGs in one reaction (Ramachandran et al. 2007; Fernando et al. 2016). Although this approach has distinct advantages, there are major drawbacks associated with using

multiplex PCR: 1) since more than one target genes are amplified in one reaction, some amplifications may be inhibited, or false-positive results may be obtained; 2) the formation of primer dimer might affect the results (Markoulatos et al. 2002).

qPCR is also commonly used to detect ARGs. While PCR method only test the presence/absence of target ARGs, qPCR can quantify the target ARGs since the initial concentration of genes could be estimated according to the final concentrations of genes and the reaction cycles (Zhang and Fang 2006). Due to its advantages, qPCR is not only used to detect the presence of the target genes but also used to examine the changes of ARGs concentrations in different environments. For example, qPCR has been used to test the removal efficiency of ARGs in a water treatment plant (Su et al. 2018). So far, there are two different types of qPCR: SYBR Green and Taqman probe, which is differentiated by fluorescent reagents.

## 1.6 Objectives and hypothesis

Given by the drinking water issues in Frist Nation communities in Canada and concern of ARGs in the environments, the overall objective of this research was to investigate the microbiological quality of drinking water in First Nation communities in Manitoba. We hypothesized that drinking water in First Nation communities was contaminated by fecal bacteria and ARGs.

In this research, water samples were screened for total and free residual chlorine concentrations, the number of fecal indicator counts (total coliforms and *E. coli*), and the detection of ARGs. The first study, described in Chapter 2, evaluated samples throughout the drinking water distribution systems in three First Nation reserves (referred as communities A, B, and C). The objective of this study was to examine within each community, the impact of type of water distribution on fecal

bacteria and ARGs in the tap water in homes. The hypothesis of this study was drinking water quality from different distribution systems in each community is not the same

The second study, described in Chapter 3, focused on community B only. Community B was selected for further study because of the frequent occurrences of fecal bacteria and ABGs in homes that rely on cisterns. The objectives of this study were to 1) compare the microbiological contamination level (the number of fecal bacterial counts and the presence of ARGs) in tap water of homes with concrete versus plastic cisterns, 2) determine whether the sewage tank is the source of ARGs in concrete cisterns.  The hypothesis of this study was the microbiological contamination level (the number of fecal bacterial counts and the detection frequency of ARGs in tap water of homes with concrete cisterns is greater than tap water of homes with polyethylene.

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## 2. DETECTION OF FECAL BACTERIA AND ANTIBIOTIC RESISTENCE GENES IN DRINKING WATER COLLECTED FROM THREE FIRST NATION COMMUNITIES IN MANITOBA, CANADA

### 2.1 Abstract

This study quantified residual chlorine concentrations, fecal bacteria and antibiotic resistance genes (ARGs) throughout the drinking water distribution systems of three First Nation reserves in Canada with community A relying on an aquifer and community B relying on a lake as source water to their water treatment plants, and community C having no water treatment plant and relying on well water from an aquifer. No fecal bacteria were detected in water samples from community A, but *ampC* was detected in one well sample. Fecal bacteria were detected in cistern water samples of community B and C, but not at other points in the water distribution systems. Cistern samples that tested positive for fecal bacteria had free residual chlorine concentrations less than 0.2 mg/L, the threshold recommended by the World Health Organization to prevent microbial regrowth. *mecA*, *sul1*, SHV, KPC, OXA-48, and NDM genes were detected in cistern water samples in community B. No ARGs were detected in community C. The results suggest that on-reserves residents living in homes with underground cisterns are likely to be exposed to fecal bacteria and ARGs when drinking their tap water that comes from water treatment plants using surface water as source water.

### 2.2 Introduction

Access to clean drinking water is one of the most important determinants of human health (WHO 2011). Consumption of polluted drinking water can result in waterborne infectious diseases, such as gastroenteritis, which has been shown to affect thousands of people, and sometimes lead to

death (MacKenzie et al. 1994; WHO 2010). In urban areas of Canada, people have access to clean and plentiful drinking water because of advanced water treatment technologies, sufficient water operator knowledge, and access to land management decision-making (Patrick 2011). However, many residents living on First Nation reserves lack drinking water security, in part because of unacceptable levels of fecal bacteria in treated water and drinking water supplies.

Chlorination inactivates bacteria and is commonly used to treat water (Cutler and Miller 2005). In order to prevent bacterial regrowth when water is distributed from the treatment plant to the homes, the free residual chlorine concentration in the water distribution systems must remain $\geq 0.2$ mg/L (LeChevallier et al. 1996). In a recent study from a fly-in community in Manitoba, water samples obtained from the water truck and in homes with cisterns all showed free residual chlorine concentrations $< 0.2$ mg/L and unacceptable levels of *E. coli* (1 to 2100 CFUs/100mL) (Farenhorst et al. 2017). In this community that relies on a lake as their source water to the water treatment plant, the water in the cisterns also contained antibiotic resistance genes (ARGs) (Fernando et al. 2016). Surface waters such as lakes and rivers are the main reservoirs of ARGs (Marti et al. 2014). ARGs can enter surface waters through point-sources such as the discharge of municipal and aquacultural wastes, and through non-point sources such as urban and agricultural runoff (Marti et al. 2014). Because lake and river water is used as source water for drinking water treatment plants, ARGs can enter drinking water distribution systems when water treatment plant does not remove them completely (Guo et al. 2014; Su et al. 2018).

Given that we detected fecal bacteria and ARGs in the tap water of cistern homes in a fly-in First Nation community in Manitoba, Canada (Fernando et al. 2016; Farenhorst et al. 2017), the purpose of this study was to examine such water quality parameters in a broader range of First Nation households and communities. The objective of this study was to examine the fecal bacteria and

ARGs in the drinking water distribution systems of three First Nation reserves in Manitoba. Each of the three First Nation reserves included in this study have homes equipped with cisterns but only community A and B have homes with piped water. community C has no water treatment plant and hence utilizes a public well to obtain and deliver water to homes with cisterns.

**2.3 Materials and methods**

**2.3.1 Water distribution systems and sample collection**

A total of 92 water samples were collected across the water distribution systems of three First Nation reserves (Table 2.1) with two sampling rounds per community and all samples being collected between July and November 2016. The sampling points along the drinking water distribution system in each community are illustrated in Figure 2.1. Community A utilizes an aquifer for its source water that is processed through a membrane reverse-osmosis water treatment system and disinfected using chlorine. There are more than 300 homes in community A with the majority of homes receiving well water, and the remaining homes using piped water or aboveground polyethylene cisterns stored in insulated shelters. Community B utilizes lake water that is processed through a conventional water treatment system equipped with a reverse osmosis system and disinfected using chlorine. There are more than 200 homes in community B with about 30% of the homes receiving piped water and 70% of the homes using underground cisterns made of concrete or polyethylene. In both community A and B, a water truck is filled at the water treatment plant to deliver water to the cisterns. Community C has more than 100 homes and no drinking water treatment plant. Most homes (66%) have individual wells (not chlorinated), while 28% of homes use public wells (2-3 households per well, not chlorinated) and 6% of homes use

underground cisterns made of concrete or polyethylene. The water truck is filled up at a public

well and the water is chlorinated in the truck prior to filling the cistern at homes.

**Table 2.1.** The description of water samples collected in this study

| Community | Sample Size and description |
|---|---|
| **Community A** | WTP: finished water at water treatment plant<br>Tr1 and Tr2: water samples collected from water truck 1 and water truck 2<br>P1 to P6: water samples collected from 6 pipeline homes, P1 to P6 are listed in the order of closest to further away from the water treatment plant<br>C1 to C5: water samples collected from 5 cistern (aboveground) homes<br>W1 to W6: water samples collected from 6 well homes |
| **Community B** | WTP: finished water at water treatment plant<br>Tr1 water: water samples collected from water truck 1 and water truck 2<br>P1 to P6: water samples collected from 6 pipeline homes, P1 to P6 are listed in the order of closest to further away from the water treatment plant<br>C1 to C7: water samples collected from 7 cistern (underground) homes |
| **Community C** | Tr: water samples collected from the water truck<br>SW1 to SW5: water samples collected from 5 shared well water<br>PW1 to PW5: water samples collected from 5 private well water<br>C1 to C5: water samples collected from 5 cistern (underground) homes |

Sampling was done following standard method SM 9060A for sample bottle pre-treatment and SM

9060B for sample preservation and storage as described by Rice et al. (2012). Water samples were

transported in coolers with icepacks to the University of Manitoba on the same day as the samples

were collected and immediately processed upon arrival for bacterial count. DNA extraction was

carried out the following day. Communities were located between 200 and 400 km from the

University of Manitoba each sampling round took 1 or 2 days. During sample collection, a Hatch

Chlorine Pocket Colorimeter II (VWR, Mississauga, ON, Canada) was used to determine free residual chlorine according to the adapted USEPA DPD Method 8021 (Hach Company 2002).

**2.3.2 Plate count assay**

Samples were processed in duplicates following SM 9222 for standard membrane filter procedure (Rice et al. 2012). Briefly, 100 mL of water sample and 100 mL of negative control (autoclaved water) were filtered through sterile polyethersulfone membranes (0.45-µm pore size, 47-nm diameter; Pall Corporation, Mississauga, ON, Canada). Filter papers were then placed on agar plates containing Brilliance *E. coli*/coliform medium (Fisher Scientific, Ottawa, ON, Canada) and incubated at 37°C for 24 h. Some samples required dilution because of high bacterial counts. *E. coli* counts in water samples were calculated as CFU/ 100 mL = number of purple colonies/volume of filtered sample (100 mL) × dilution factor. Total coliform bacteria counts in water were calculated as CFU/ mL = number of purple + pink colonies/volume of filtered sample (100 mL) × dilution factor. Brilliance agar can distinguish *E. coli* and coliforms from other bacteria based on β-D-glucuronidase production by *E. coli* resulting in purple *E. coli* colonies; and galactosidase production by a majority of coliforms results in pink coliforms colonies (Wohlsen 2011). Brilliance agar has been widely used for the detection of coliforms and *E. coli* in many studies (Atterbury et al. 2011; Winterbourn et al. 2016; Fernando et al. 2016; Farenhorst et al. 2017). It is possible that hemorrhagic *E. coli* strains may not be identified by this medium because they are not efficient producers of β-D-glucuronidase (Fricker et al. 2010).
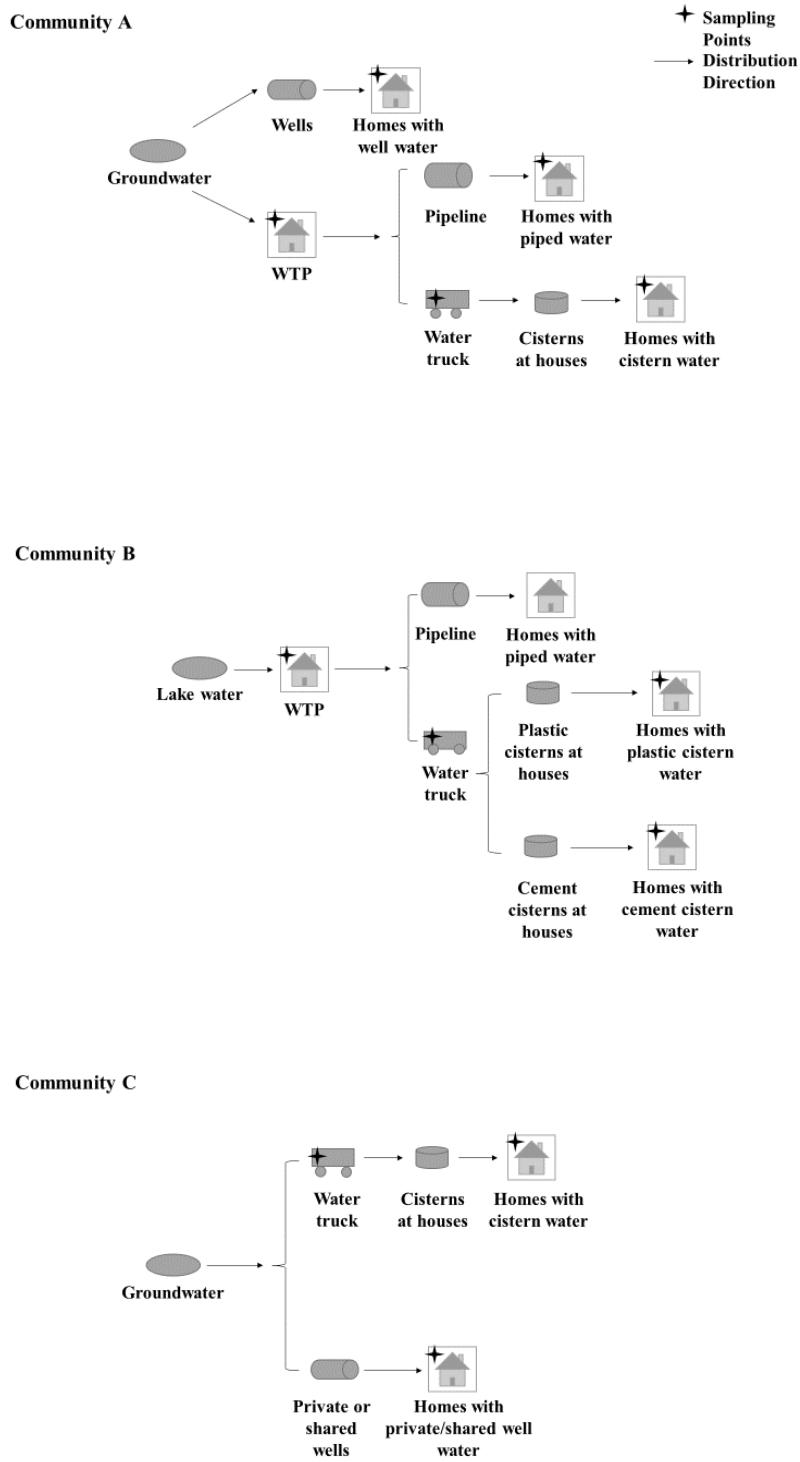
**Figure 2.1.** The drinking water distribution systems in community A, community B, and community C. The black star represents the sampling location in each community.

### 2.3.3 DNA extraction

DNA extraction was conducted for samples collected in the second sampling round. For DNA extraction, 300-500 mL of water sample was filtered through sterile polyethersulfone membranes (0.22-μm pore size; 47-mm diameter; Pall Corporation, Mississauga, ON, Canada). DNA was extracted by using the DNeasy PowerWater Kit (QIAGEN, Germantown, MD, USA) following the manufacturer's instructions. An unused filter membrane was used as the extraction control. DNA concentrations were quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA), and the DNA quality was tested on a 1% agarose gel.

### 2.3.4 Antibiotic resistance genes quantification

Absolute quantitative PCR (qPCR) was used to quantify the abundance of seven different ARGs: *ampC*, *vanA*, *tet(A)*, *mecA*, *sul1*, *sul2*, and *sul3*. *ampC*, *vanA*, *tet(A)*, and *mecA* were quantified for all water samples and *sul1*, *sul2*, and *sul3* were only quantified for cistern samples in community B. All the primers are listed in Table 2.2. For the primers designed in this study, first, positive controls and their sequences were obtained from Agriculture and Agri-Food Canada, London, Canada (Dr. E. Topp Laboratory). Primers were then designed using Custom Primers - OligoPerfect™ Designer at ThermoFisher Scientific (parameters chosen for primers were: 18 – 22 bp for primer length; 55 °C to 65 °C for annealing temperature; 30 % to 40 % for GC content; ~ 120 bp for amplification length). Last, specificity of the primers sequences was also confirmed using NCBI BLAST. Bacterial isolates used for positive controls are listed in Table 2.3. DNA from positive controls was isolated using a DNA extraction kit (Biobasic, Markham, ON, Canada). Following a previously described method with slight modifications (Alexander et al. 2015), the qPCR reaction was conducted on the StepOnePlus real-time PCR system (Life Technologies Inc.,

Burlington, ON, Canada). Reactions were carried out in triplicate. For each reaction, there was a total volume of 8 µL containing a mix of 2.68 µL of DNA (concentrations were all normalized to 0.7 ng/ µL), 9 µM of primers, and 2 × SsoFast EvaGreen Supermix (Bio-Rad Canada, Mississauga, ON, Canada). The amplification cycle used was as follows: 1 cycle of initial denaturation at 95 °C for 3 mins, 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, extension at 95 °C for 15 s, and the final extension at 60 °C for 60 s. The copy number of each gene per nanogram of DNA per 100 mL of water was calculated using a standard curve for each gene as described previously (Fernando et al. 2016). A five-point standard curve was created by using a 10-fold dilution series for the target gene copy number, ranging from 20 to 200,000 copies with three replicates. The square of the related coefficient ($r^2$) of the standard curve for all the genes ranged from 0.998 to 1, except for *vanA* which was 0.977. The copy number of each gene per nanogram of DNA per 100 mL in samples was calculated using the slope of the standard curve, Y-intersection of standard curve, Ct values obtained from each qPCR runs, and with the filtration volume of water samples. The calculation was described as follows: Copy No./ng DNA/ 100 mL

$$= (10^{(Ct-Y\_inter)/slop}) / 1 \text{ ng/ µL of DNA}/ (\frac{V_{filtration}}{100 \ mL}).$$

### 2.3.5 Multiplex PCR detection of β-lactamase and carbapenemase genes

The presence of five different β-lactamase genes: *SHV*, *TEM*, *CTX-M*, *OXA-1*, and *CMY-2* were determined using a multiplex PCR for cistern samples in community B. The specific primers are listed in Table 2.2. Bacterial isolates used for positive controls are listed in Table 2.3. The PCR reaction was conducted using Q5 high-fidelity DNA polymerase (New England BioLabs, Whitby, ON, Canada) with final concentrations of the primers were 0.27 µM. The run parameters for amplification cycle were: 1 cycle of initial denaturation at 95 °C for 15 min, 40 cycles of

denaturation at 94 °C for 30 s, annealing at 63.5 °C for 90 s, extension at 72 °C for 90 s, and final extension at 72 °C for 7 min.

Four different carbapenemase-encoding genes, *KPC*, *NDM*, *GES-5*, and *OXA-48*, were determined using multiplex PCRs for cistern samples in community B. The *IMP*/*VIM* detections were carried out in separate reactions. Specific primers for this assay are listed in Table 2.2. Bacterial isolates used for positive controls are listed in Table 2.3. PCR reactions were also conducted using Q5 high-fidelity DNA polymerase (New England BioLabs, Whitby, ON, Canada) with final concentrations of the primers as follow: 0.27 µM for each gene in multiplex PCR, and 0.27 µM for *VIM*/*IMP* PCR. For the multiplex reactions, the run parameters for amplification cycle steps were: 1 cycle of initial denaturation at 95 °C for 15 min; 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s, extension at 72 °C for 60 s, and final extension at 72 °C for 7 min. For *IMP* and *VIM* PCRs, annealing steps were at 61.7 °C for 90 s and 66 °C for 90 s, respectively. The rest of the steps were the same as in multiplex reaction. PCR products of above reactions were resolved on a 1.3% agarose gel.

**2.3.6 Statistical analysis**

Analysis of variance (ANOVA) was performed using GLIMMIX in SAS 9.4. (SAS Institute, 2013) to test for the effect of the type of water distribution on free residual chlorine concentrations in tap water of homes (i.e., piped versus cisterns versus wells for community A; piped versus cisterns for community B; cisterns versus private wells versus shared wells for community C). Distribution of free chlorine concentrations was considered as lognormal. The Tukey multiple comparison procedure was used to compare least square means for communities A and C. The multiple comparison procedure was set as default to compare least square means for community B. For all statistical analyses, significance was determined at $\alpha = 0.05$.

**Table 2.2.** List of primers used in study

| Primer name | Sequence | Target gene (amplicon size, bp) | Reference or source |
|---|---|---|---|
| **ampC_F1** | TGAGTTAGGTTCGGTCAGCA | *ampC* (98) | Fernando et al. 2016 |
| **ampC_R1** | AGTATTTTGTTGCGGGATCG | | |
| **vanA_F1** | ATAAAGCGCTCGGCTGTAGA | *vanA* (98) | Fernando et al. 2016 |
| **vanA_R1** | GAAACCGGGCAGAGTATTGA | | |
| **tetA_F1** | AGGTGGATGAGGAACGTCAG | *tet(A)* (96) | Fernando et al. 2016 |
| **tetA_R1** | AGATCGCCGTGAAGAGGCG | | |
| **mecA_F1** | CTGATGGTATGCAACAAGTCG | *mecA* (97) | Fernando et al. 2016 |
| **mecA_R1** | TGAGTTCTGCAGTACCGGATT | | |
| **sul1_F1** | TGTTTCAATCGACAGCTTCCAACC | *sul1* (112) | This study |
| **sul1_R1** | AATATCGGGATAGAGCGCAGGG | | |
| **sul2_F1** | GTGTGTGCGGATGAAGTCAG | *sul2* (120) | This study |
| **sul2_R1** | GTTTCGCGCAAATCCTTTC | | |
| **sul3_F1** | TGCGGAGATAATCTGCACCT | *sul3* (119) | This study |
| **sul3_R1** | TGCAGTGTCACGGAAATCAT | | |
| **SHV-UP** | CGCCGGGTTATTCTTATTTGTCGC | *blaSHV* (1,016) | Mulvey et al. 2011 |
| **SHV-LO** | TCTTTCCGATGCCGCCGCCAGTCA | | |
| **TEM-G** | TTGCTCACCCAGAAACGCTGGTG | *blaTEM* (708) | Mulvey et al. 2011 |
| **TEM-H** | TACGATACGGGAGGGCTTACC | | |
| **CTX-U1** | ATGTGCAGYACCAGTAARGTKATGGC | *blaCTX-M* (593) | Mulvey et al. 2011 |
| **CTX-U2** | TGGGTRAARTARGTSACCAGAAYCAGCGG | | |
| **OXA1-F** | CGCAAATGGCACCAGATTCAAC | *blaOXA-1* (464) | Mulvey et al. 2011 |
| **OXA1-R** | TCCTGCACCAGTTTTCCCATACAG | | |
| **CMY2-A** | TGATGCAGGAGCAGGCTATTCC | *blaCMY-2* (323) | Mulvey et al. 2011 |
| **CMY2-B** | CTAACGTCATCGGGGATCTGC | | |
| **KPC-1** | ATGTCACTGTATCGCCGTC | *blaKPC* (863) | Mulvey et al. 2011 |
| **KPC-2** | AATCCCTCGAGCGCGAGT | | |
| **IMP1** | CCWGATTTAAAAATYGARAAGCTTG | *blaIMP* (522) | Mulvey et al. 2011 |
| **IMP2** | TGGCCAHGCTTCWAHATTTGCRTC | | |
| **VIM1** | GTTTGGTCGCATATCGCAAC | *blaVIM* (382) | Mulvey et al. 2011 |
| **VIM2** | AATGCGCAGCACCAGGATAGAA | | |
| **NDM-F** | GGTGCATGCCCGGTGAAATC | *blaNDM* (660) | Mulvey et al. 2011 |
| **NDM-R** | ATGCTGGCCTTGGGGAACG | | |
| **GES-2** | ATCAGCCACCTCTCAATGG | *blaGES* (302) | Mataseje et al. 2012 |
| **GES-3** | TAGCATCGGGACACATGAC | | |
| **OXA-48A** | TTGGTGGCATCGATTATCGG | *blaOXA-48* (744) | Brink et al. 2013 |
| **OXA-48B** | GAGCACTTCTTTTGTGATGGC | | |

**Table 2.3.** List of strains of positive control

| Antibiotic Resistance Genes | Strains |
|---|---|
| *ampC* | *Escherichia coli* 99270 |
| *vanA* | *Enterococcus faecium* ATCC 13048 |
| *tetA* | *E. coli* ER2925 |
| *mecA* | *Staphylococcus aureus* clinical isolate HA-MRSA 100697 |
| *sul1* to *3* | Cloned *E. coli* (StrataClone cloning kit) |
| β-Lactamases genes | *Klebsiella pneumoniae* N09-00080 |
| KPC | *K. pneumoniae* N09-0431 |
| OXA-48 | *E. coli* 12-123T |
| NDM | *E. coli* 10469T |
| VIM | *Pseudomonas aeruginosa* C-10 |
| GES | *E. coli* A44413 |
| IMP | *Acinetobacter baumannii* C-3 |

## 2.4 Results

### 2.4.1 Chlorine concentrations and fecal bacteria counts

For communities A (Figure 2.2) and B (Figure 2.3), the treated water at the water treatment plant (WTP) had free residual chlorine concentrations within the 0.4 to 2.0 mg/L range typically reported in Canada (Health Canada 2009b). For community A, homes with cisterns and piped water always had free residual chlorine concentrations > 0.2 mg/L, except for P1 and P3 in the first sampling round (Figure 2.2). Well water was not chlorinated and therefore, free residual chlorine concentration in tap water of homes with well water was always significantly less than in tap water of pipeline homes and cistern homes. The free residual chlorine concentrations in tap water of pipeline homes and cistern homes showed no significant difference (Table 2.4 and Table 2.5). For community B, homes with cisterns showed significantly less free residual chlorine concentration in tap water that homes with piped water (Table 2.4 and Table 2.5). Regardless of the sampling round, homes with cisterns almost always had free residual chlorine concentrations < 0.2 mg/L (Figure 2.3). In contrast, pipeline homes only showed such low concentrations when located

further away from the WTP (Figure 2.3). In community C, the free residual chlorine concentration in tap water of cistern homes was significantly greater than in tap water of homes with shared wells = private wells (Table 2.4 and Table 2.5) because well water is not chlorinated. Some samples from homes with cisterns had free residual chlorine concentrations < 0.2 mg/L (Figure 2.4).

No *E.coli*/coliforms bacteria were detected in any of the water samples collected in communities A or C, except for home C4 in community C that had numerically the lowest free residual chlorine concentrations among the cistern homes (Figure 2.4) and tested positive for total coliforms (12 ± 4 and 30 ± 0 CFU/ 100 mL for sampling round 1 and 2, respectively) and *E. coli* (8 ± 0 and 12 ± 1 CFU/ 100 mL for sampling round 1 and 2, respectively). In community B, no *E. coli*/coliforms bacteria were detected in water samples from the WTP, water trucks and homes with piped water. However, 6 out of 7 homes with cisterns tested positive for total coliforms and *E. coli* in both sampling rounds (Figure 2.3). There was a large variation across the six homes in the counts of E. coli/coliforms bacteria detected, for example *E. coli* and total coliforms were particularly abundant in tap water of C4 and C6 homes and relative small in tap water of C1 and C7 homes.
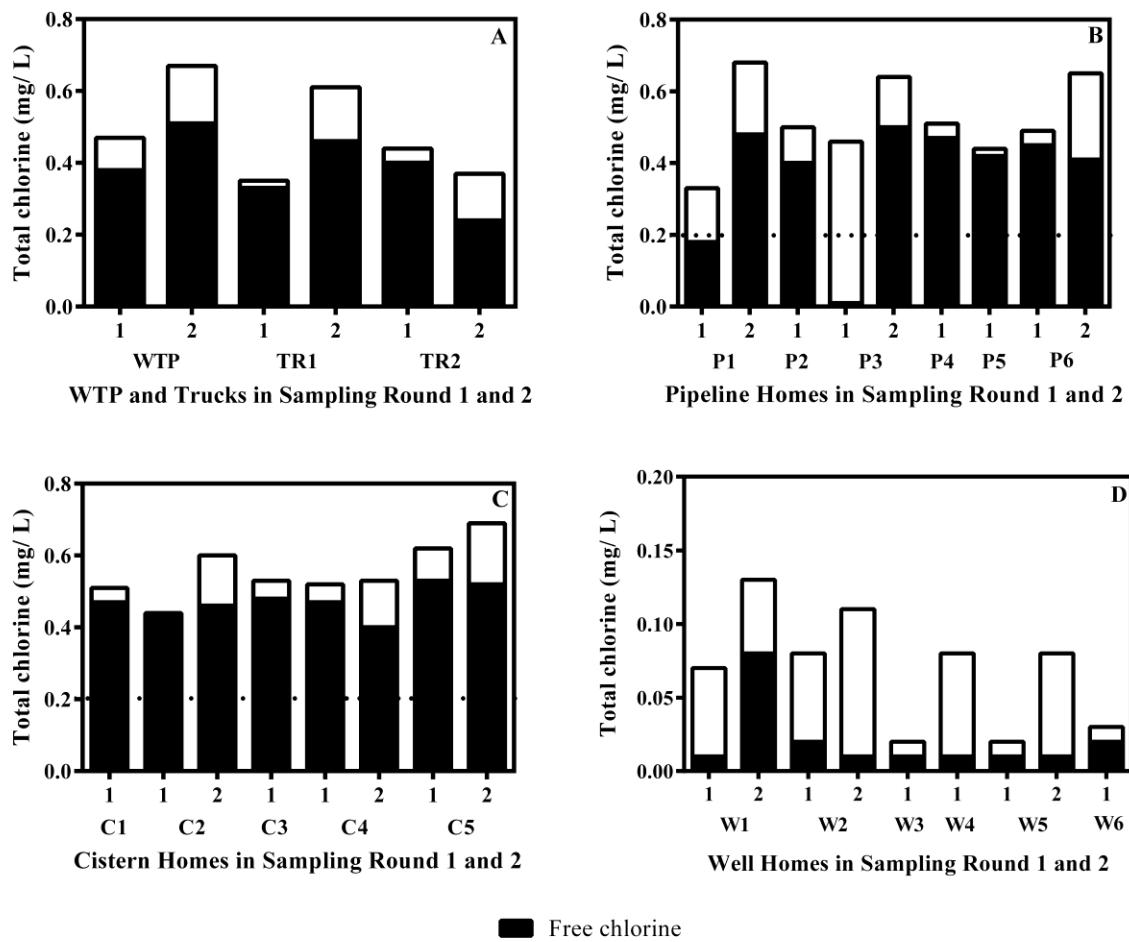
**Figure 2.2.** Total (black + white bar) and free residual (black bar) chlorine concentrations in water samples collected in community A from the water treatment plant and water truck (A), and from homes with piped water (B), cisterns (C), or wells (D). Dashed line is the minimum recommended free residual chlorine concentration (LeChevallier et al. 1996). Description of the samples is provided in Table 2.1.
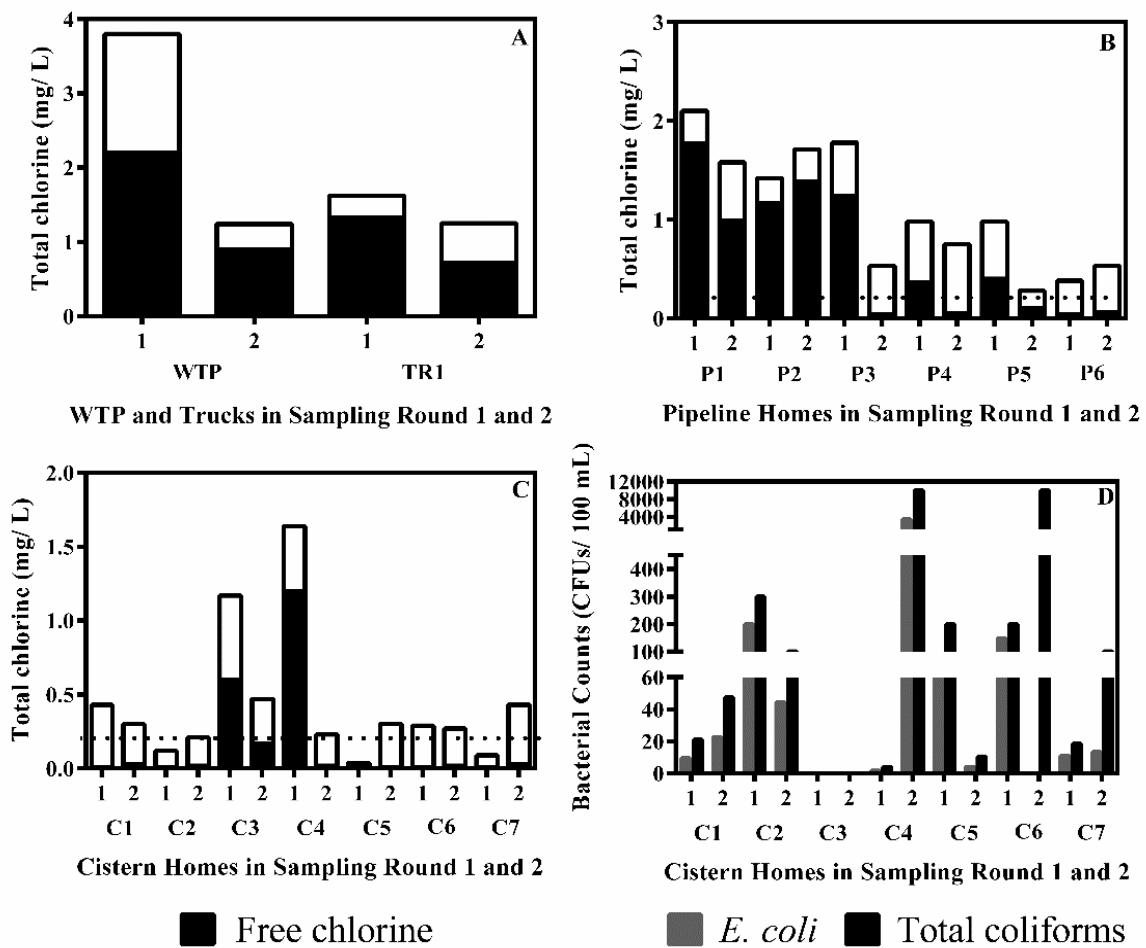
**Figure 2.3.** Total (black + white bar) and free residual (black bar) chlorine concentrations in water samples collected in community B from the water treatment plant and water truck (A), and from homes with piped water (B) or cisterns (C), as well as the colony forming units (CFUs) of *Escherichia coli* and total coliforms detected in cistern samples (D). Dashed line is the minimum recommended free residual chlorine concentration (LeChevallier et al. 1996). Description of the samples is provided in Table 2.1.
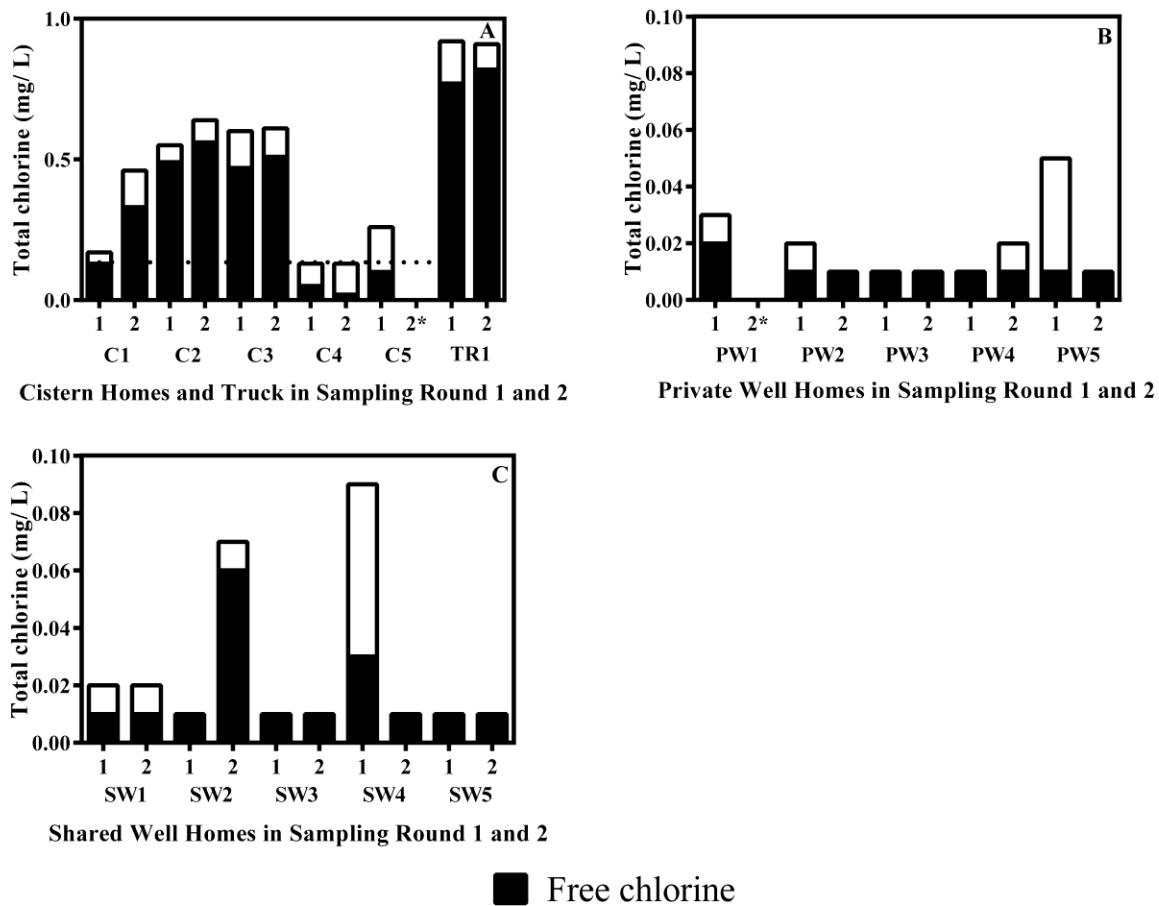
**Figure 2.4.** Total (black + white bar) and free residual (black bar) in water samples collected in community C from the water truck and homes with cisterns (A), and homes with private wells (B) or shared wells (C). * = water sample not collected as family was not home. Dashed line is the minimum recommended free residual chlorine concentration (LeChevallier et al. 1996). Description of the samples is provided in Table 2.1.

**Table 2.4.** Type three test of effect of water distribution system on free chlorine residual concentration

| Community | | | Type III Tests | | |
|---|---|---|---|---|---|
| | Effect | Num DF | Den DF | F Value | Pr > F |
| A | pipe/cistern/well | 2 | 22 | 41.87 | <.0001 |
| B | pipe/cistern | 1 | 22 | 12.2 | 0.0021 |
| C | cistern/shared well/private well | 2 | 24 | 35.42 | <.0001 |

**Table 2.5.** Free chlorine residual concentration (average ± standard error of mean) of water distribution in each community

| Community | Water distribution system | Free chlorine residual (mg/L) |
|:---:|:---:|:---:|
| A | Pipeline | 0.32 ± 0.04 |
|  | Cistern | 0.56 ± 0.12 |
|  | Well | 0.01 ± 0.00 |
| B | Pipeline | 0.37 ± 0.09 |
|  | Cistern | 0.04 ± 0.00 |
| C | Cistern | 0.21 ± 0.01 |
|  | Shared well | 0.01 ± 0.01 |
|  | Private well | 0.01 ± 0.01 |

## 2.4.2 Detection of antibiotic resistance genes

No ARGs were detected in any of the water samples collected in communities A or C, except for home W1 in community A that tested positive by qPCR for the *ampC* gene (41239 ± 3366 copy NO./ ng DNA/ 100 mL). In community B, no ARGs were detected in water samples from WTP, water trucks, and homes with piped water. However, water samples from homes with cistern were observed positive for several ARGs (Figure 2.5): the *sul1* gene was detected in C5 (8 ± 2 copy NO./ng DNA/100 mL) and C6 2 ± 0 copy NO./ng DNA/100 mL). C5 was also positive for the *mecA* gene (25 ± 7 copy NO./ng DNA/100 mL). *sul2*, *sul3*, *ampC*, and *vanA* genes were not detected in any water samples in community B.

Among β-lactamase genes tested, *SHV* gene was detected in three water samples (C4, C5, and C7) (Figure 2.5). Both *CTX-M* and *OXA-1* gene were detected in two cistern water samples: they were C2 and C6, and C3 and C5, respectively. *TEM* gene was only found in one cistern water sample (C6). *CYM-2* was not detected in water samples in community B. As for the carbapenemase genes, *KPC* and *NDM* were the most frequently detected genes: both were detected in five cistern water

samples (C3, C4, C5, C6, and C7). *OXA-48* was detected in three cistern water samples (C2, C4, and C5). *VIM*, *GES*, and *IMP* gene were not detected in water samples in community B.

Among all the cistern samples (Figure 2.5), C5 was positive for 7 ARGs, followed by C6 in which 5 ARGs were detected. C4 was positive for four ARGs. Both C3 and C7 were positive for three ARGs. C2 was positive for two ARGs. C1 was the only cistern water sample in community B for which no ARGs were detected.

| | Cistern Homes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **C1** | **C2** | **C3** | **C4** | **C5** | **C6** | **C7** |
| ***sul1*** | | | | | ▓ | ▓ | |
| ***sul2*** | | | | | | | |
| ***sul3*** | | | | | | | |
| ***ampC*** | | | | | | | |
| ***tetA*** | | | | | | | |
| ***mecA*** | | | | | ▓ | | |
| ***vanA*** | | | | | | | |
| ***SHV*** | | | | ▓ | ▓ | | ▓ |
| ***TEM*** | | | | | | ▓ | |
| ***CTX-M*** | | ▓ | | | | ▓ | |
| ***OXA-1*** | | | ▓ | | ▓ | | |
| ***CYM-2*** | | | | | | | |
| ***KPC*** | | | ▓ | ▓ | ▓ | ▓ | ▓ |
| ***OXA-48*** | | ▓ | | ▓ | ▓ | | |
| ***NDM*** | | | ▓ | ▓ | ▓ | ▓ | ▓ |
| ***VIM*** | | | | | | | |
| ***GES*** | | | | | | | |
| ***IMP*** | | | | | | | |

**Figure 2.5.** The detection of antibiotic resistance genes in cistern water samples in community B. *sul1*, *sul2*, *sul3*, *ampC*, *tetA*, *mecA*, and *vanA* were detected by qPCR. *SHV*, *TEM*, *CTX-M*, *OXA-1*, *CYM-2*, *KPC*, *OXA-48*, *NDM*, and *GES* were detected by multiplex PCR. *VIM* and *IMP* were detected by separate PCRs. The shade represents the presence of the genes.

## 2.5 Discussion

### 2.5.1 Chlorine concentrations and *E. coli*/colifroms counts

The objective of this study was to investigate the microbiological quality of drinking water in three First Nation reserves with community A relying on an aquifer and community B relying on a lake as source water to their water treatment plants, and community C having no water treatment plant and relying on well water from an aquifer for further water distribution. These types of water distribution systems are representative of what is typical First Nation reserves in Manitoba and other provinces in Canada. Treated water at the WTP in community A and B contained adequate levels of free residual chlorine and was free of fecal bacteria, suggesting that the water treatment process in both communities is functioning well. For the pipeline water in community A (Figure 2.2) and B (Figure 2.3), water samples were always negative for fecal bacteria although some of water samples had free residual chlorine concentrations less than the minimum recommended level, particularly pipeline homes that were furthest away from the WTP in community B. The relatively low free residual chlorine concentration in pipeline water further away from the WTP could result from the decay of chlorine which is affected by a) water quality parameters, such as dissolved organic carbon concentrations and inorganic substances (Warton et al. 2006), and b) pipeline system parameters, such as pipe age, pressure variation and flow conditions (LeChevalier 1990). The results of WTP and pipeline in both communities suggest that the drinking water distribution system of WTP + pipeline could provide safe drinking water (free of fecal bacterial contamination) in First Nation reserves. For well water in community A (Figure 2.2) and C (Figure 2.4), no fecal bacteria were detected in any sample, despite water not being chlorinated and the presence of low free residual chlorine concentrations. This observation indicates that groundwater wells can provide safe drinking water to families living on First Nation reserves in Manitoba.

No *E. coli* and total coliforms were detected in cistern water samples in community A (Figure 2.2) which aboveground polyethylene cisterns stored in insulated shelters. None of the underground cisterns in community C (Figure 2.4) showed an indication of fecal bacteria contamination, except for home C4 which had a cracked underground concrete cistern that was prone to contamination. In contrast, community B (Figure 2.3) also utilized underground concrete or polyethylene cisterns, where most cistern water samples were detected positive for fecal bacteria. Furthermore, bacterial levels in cistern water samples ranged from 0 to 3000 CFUs/100 mL for *E. coli* and from 0 to 10000 CFUs/100 mL for total coliforms. This relatively large variation across homes might be due to two reasons. First, some cisterns have been used for a longer time and were cracked. For example, higher fecal bacterial counts were detected in C6 than C5. The cistern in home C6 has been used for 27 years and this house was on the top priority list to have its cistern replaced due to cracking. The cistern in home C5 were used only for 4 years and cracking was not observed. Second, the timing of cistern cleaning varied. For instance, higher fecal bacterial counts were detected in C4 than C3. The cistern in home C4 was not cleaned in the year of sampling (2016), and the cistern in home C3 was just cleaned in July before the sampling trip was conducted. It is worth mentioning that in this study, the presence of fecal bacteria was detected but the pathway of the contamination was not studied. Therefore, the source of fecal contamination is unclear in this study. However, high plate counts samples, such as C4 and C6, were concrete cisterns we suspect that homes relying on underground concrete cisterns have an increased risk of drinking water becoming contaminated when cracked, as well as that the concrete rough surface favors the formation of biofilms (Characklis et al. 1990). Overall, the results demonstrated that cistern cleaning is important to reduce the risk of exposure to fecal bacteria and that older underground

concrete cisterns need to be replaced, preferably with aboveground polyethylene cisterns stored in insulated shelters.

## 2.5.2 Detection of antibiotic resistance genes

The presence of ARGs was investigated by using a qPCR and multiplex PCR method. For qPCR method, seven ARGs: *sul1*, *sul2*, *sul3* (sulfonamides resistance), *tet(A)* (tetracycline resistance), *ampC* (β-lactam resistance), *mecA* (methicillin resistance), and *vanA* (vancomycin resistance) were examined for water samples. *sul2*, *sul3*, *tet(A)*, and *vanA* were not detected in any water samples across the three communities. This is despite them being commonly detected in various environments (Loffler et al. 2016; Suhartono et al. 2016; Zhang et al. 2016). *ampC*, *mecA*, and *sul1* were the three genes that were detected in a few water samples (Figure 5). *ampC* gene belongs to β-lactamase group, whose corresponding antibiotics, β-lactam, are commonly used in the treatment of infections in both humans and animals (Van et al 2011). The *mecA* gene is an indication of the presence of methicillin-resistant *Staphylococcus aureus* (MRSA), although it was also reported in nonstaphylococcal pathogens (Kassem et al. 2008). Sulfonamides are one of the earliest antibiotics introduced in the world and widely used for treating human infections (Van et al. 2011). These genes had been commonly detected in various environments such as drinking water, surface water, and wastewater (Schwartz et al. 2003; Alexander et al. 2015; Suhartono et al. 2016).

For multiplex PCR method, five β-lactamase genes and six carbapenemase genes were detected for cistern samples in community B (Figure 2.5). β-lactamase genes and carbapenemase genes have been detected in many aquatic environments. For example, *OXA-1* and *TEM-1* genes were detected in a water treatment plant in China (Zhang et al. 2016). The *CTX-M* gene was detected in

water samples collected from an Indian river (Bajaj et al. 2015), and the *OXA-48* gene was found in river water in Algeria (Tafoukt et al. 2017). In this study, *SHV*, *CTX-M*, *OXA-1*, *KPC*, *NDM*, and *OXA-48* were the more frequently detected genes in cistern water samples (at least in two out of seven water samples) (Figure 2.5). β-lactams and carbapenems are commonly used to treat infections in human and animals (Van et al. 2011), with carbapenems often being used as the last resort for treating antibiotic-resistant infections. Furthermore, the detections of ARGs are usually associated with pathogenic bacteria (Yong et al. 2009; Robledo et al. 2011; Ben-David. 2012) and hence the detection of ARGs in cistern water in community B could further suggest that the tap water in these homes was unsafe to drink and that steps must be taken to replace the underground cisterns with alternate options for supplying safe drinking water to homes. It is worth mentioning that in multiplex PCR experiment, the presence of antibiotic resistance genes was detected but not the expression.

Overall, based on the results of the cistern samples in community B, the connection between the detection of fecal bacteria and the detection of ARGs is not clear. For example, the tap water in homes C4 and C6 had larger plate counts than the tap water in home C5, but more ARGs were detected in the tap water of home C5 than in the tap water of homes C4 and C6. Moreover, the tap water in home C3 was detected negative for fecal bacteria but positive for ARGs. This observation indicates that ARGs might come from noncoliform organisms.

Both this study and the one by Fernando et al. (2016). detected *ampC*, *mecA*, β-lactamase genes and carbapenemase genes in drinking water of taps in homes, suggesting that the presence of ARGs in drinking water sources of First Nation reserves might not be an isolated case. Although *ampC*, *mecA*, *sul1*, β-lactamase genes and carbapenemase genes were detected, the selection pressure for the presence of these genes in organisms is unclear because the absence of public data on the usage

of antibiotics in different geographical regions of Canada. Moreover, since only drinking water samples were studied, the contamination source of ARGs and the bacteria that may carry these genes are unclear, and this should be a research direction in subsequent studies.

**2.6 Conclusion**

This study investigated drinking water quality in three First Nation communities in Manitoba, Canada by determining free residual chlorine concentrations, and screening for the presence of *E. coli* and total coliforms, as well as ARGs. Regardless of water source to the WTP as ground or surface water, our findings suggest that families living in homes that were connected to the WTP by pipes had tap water free of fecal bacteria and ARGs. Families that had groundwater wells or aboveground polyethylene cisterns also tend to have access to safe drinking water in their home. In contrast, when the source water to the WTP was surface water, families living in homes with underground cisterns were likely to be frequently exposed to fecal bacteria and ARGs, particularly if these cisterns were cracked and/or not recently ($< 7$ months) cleaned. We urge all governments in Canada to decrease the portion of homes on First Nation reserves relying on underground concrete cisterns and retrofit these homes with pipes connected to the WTP or with aboveground polyethylene cisterns stored in insulated shelters.

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# 3. ANTIBIOTIC RESISTANCE GENES IN TAP WATER AND SEWAGE TANK SAMPLES FROM HOMES WITH UNDERGROUND CISTERNS IN A FIRST NATION RESERVE, CANADA

## 3.1 Abstract

In Canada, cisterns are commonly used for providing running tap water in homes on First Nation reserves. Tap water in homes with underground cisterns has shown to be contaminated with fecal bacteria and antibiotic resistance genes (ARGs). For a First Nation reserve in the Province of Manitoba, this study compared homes with concrete versus polyethylene underground cisterns for the presence of coliform bacteria and ARGs in tap water. ARGs were also examined in samples collected from sewage tanks located on the premises of homes with concrete cisterns to determine whether sewage is a possible source of the detection of ARGs in older concrete cisterns that show signs of cracks. The free residual chlorine concentration in tap water was typically less than what is required to suppress microbial growth (0.2 mg/L) and coliforms bacteria were present in tap water of all homes with concrete cisterns and in 75% of homes with polyethylene cisterns. Samples from concrete and polyethylene cisterns, as well as sewage tanks, were found to be positive for *sul1*, *ampC*, β-lactamase genes (TEM-type, CTX-M-type, and OXA-1) and carbapenemase genes (KPC-type, NDM-type, and OXA-48 genes). In addition, other AGRs were detected only in sewage samples (*tetA*, *sul2*, and the carbapenemase gene (GES-type)) or only in the tap water of homes with concrete and polyethylene cisterns (the β-lactamase gene (SHV-type and CYM-2-type). *mecA* gene was detected in sewage samples and tap water of homes with concrete cisterns but not those with polyethylene cisterns. The results suggested that, by drinking their tap water, the residents living in homes with concrete cisterns were more likely to be exposed to higher level of fecal bacteria than homes with polyethylene cisterns; however, the residents were exposed to

the same types of ARGs (except for *mecA)*. In addition, sewage tanks might not be a key source of contamination of ARGs in drinking water because when comparing the types of ARGs present at each location (home), The CYM-2 gene were detected in drinking water but not in sewage and the *tetA*, and GES genes were detected in sewage but not in drinking water, depending on the home. Overall, the results were indicative of the poor drinking water quality in underground cisterns in this First Nation community. We urge governments in Canada to decrease the portion of homes on First Nation reserves relying on underground concrete cisterns. At least, these old cracked concrete cisterns should be given high priority and replaced as soon as possible.

**3.2 Introduction**

The human consumption of drinking water contaminated with pathogenic bacteria has shown to cause waterborne infectious diseases and sometimes death (MacKenzie et al. 1994; WHO 2010; WHO 2011). Access to safe and reliable drinking water is important to population health but many First Nation reserves in Canada have poor drinking water quality (Neegan Burnside Ltd. 2011; Farenhorst et al. 2016; Fernando et al. 2016). About one in every three homes on First Nation reserves in the Province of Manitoba, Canada rely on cisterns that store drinking water delivered by water trucks that fill up at the water treatment plant or a community well (Neegan Burnside Ltd. 2011). Despite cisterns providing water demand, there are specific risks associated with cisterns. Cistern water in First Nation reserves have been shown to contain fecal bacteria and such contamination may result from the inadequate removal of fecal bacteria during water treatment of source water, the introduction of fecal bacteria during truck delivery, and/or the introduction of fecal bacteria through the seepage of subsurface water into underground cisterns (Baird et al. 2013). Concrete and polyethylene are two materials that commonly used to construct cisterns. The use of polyethylene has an advantage over that of concrete because the corrosion of embedded metals in

concrete can leads to concrete rust, and subsequently cracking of concrete walls. Underground concrete cisterns are more likely than aboveground (polyethylene) cisterns to be contaminated with fecal bacteria (described in Chapter2) perhaps because subsurface water seeping through cracks in concrete walls introduces bacteria into cisterns.

Antibiotic resistance genes (ARGs) is a class of emerging contaminants that have become a worldwide concern for population health (Pruden et al. 2006, WHO 2014). Overuse and misuse of antibiotics in hospital environments and livestock production are likely factors in contributing to proliferation of antibiotic resistant bacteria in various environments such as surface water, soil and wastewater water (Jiang et al. 2013; Marti et al. 2014; Tao et al. 2014). Recent studies also detect ARGs in drinking water (Fernando et al. 2016, Xu et al. 2016; Su et al. 2018), suggesting that families in households can be exposed to ARGs by consuming their tap water.

Given that many First Nation communities in Canada experience poor drinking water quality and the use of underground cisterns in these communities is associated with greater risks of microbiological contamination, the purpose of this study was to investigate the numbers of fecal bacteria (*E.coli* and coliforms) and types of ARGs in tap water of homes in a First Nation community relying heavily on underground cisterns. ARGs were also examined for sewage samples from domestic sewage tanks on the premises of homes with concrete cisterns. We hypothesized that: 1) the microbiological contamination level (the number of fecal bacteria count and the detection frequency of ARGs) in tap water of homes with concrete cisterns is greater than tap water of homes with polyethylene cisterns; 2) sewage tank is the source of the detection of ARGs in concrete cisterns. The results of this study can provide better understanding of fecal contamination and the presence of ARGs in underground cisterns. The results may also

recommend better choices of the type of cisterns and constructions used for drinking water storage in First Nation communities.

### 3.3 Materials and Methods

### 3.3.1 Cisterns and sewage tank systems

The selected First Nation reserve is located in North East Manitoba, Canada. The community utilizes lake water as its source water, which is processed through a conventional water treatment system equipped with a reverse osmosis system and disinfected using chlorine. About 30% of the more than 200 homes in the community are connected to the water treatment plant with pipes. Of the remaining homes, about 49% of the homes in the community rely on belowground concrete cisterns and 21% of the homes rely on polyethylene cisterns that are most of which are belowground. Regardless of the cistern material, many cisterns are old (some > 20 years) and annual inspections of cisterns in the community show that some cistern walls are cracked. Since this community has no sewage water treatment plant, each home is equipped with a sewage tank to collect domestic wastewater, with the sewage being transported by a truck to dispose it in a community lagoon. For most cistern homes, the sewage tank is installed underground at about 8 meters distance from the cistern.

### 3.3.2 Water distribution systems and sampling strategy

Water samples were collected from the water treatment plant (WTP), water truck, taps from homes with cisterns, and from sewage treatment tanks (Table 3.1) with two sampling rounds between June and August 2017. The community is located about 400 km from the University of Manitoba, and each sampling round took 1 or 2 days.

Sampling was done following standard method SM 9060A for sample bottle pre-treatment and SM 9060B for sample preservation and storage as described by Rice et al. (2012). Water samples were transported in coolers with icepacks to the University of Manitoba on the same day as the samples were collected and immediately processed in the evening (plate count assay) and the next day (DNA extraction). During sample collection, a Hatch Chlorine Pocket Colorimeter II (VWR, Mississauga, ON, Canada) was used to determine free residual chlorine according to the adapted USEPA DPD Method 8021 (Hach Company 2002).

**Table 3.1.** Description of water samples in the study

| Sample designation | Trip | Number of samples | Description |
|---|---|---|---|
| **Water treatment plant (WTP)** | 1 | 1 | WTP: water samples were collected at the tap (finished water) in WTP |
|  | 2 | 1 |  |
| **Water Truck (Tr)** | 1 | 2 | Tr1 and Tr2: water samples collected from the houses of water delivery trucks. Tr1 was not operating during second sampling round. |
|  | 2 | 1 |  |
| **Concrete cistern (C)** | 1 | 8 | C1 – C8: tap water collected from homes relying on underground concrete cisterns. |
|  | 2 | 8 |  |
| **Polyethylene cistern (P)** | 1 | 8 | P1 – P8: tap water collected from homes relying on underground polyethylene cisterns. P3 was not included in the second sampling round as family was not at home. |
|  | 2 | 7 |  |
| **Sewage tank (S)** | 1 | 8 | S1 – S8: Samples collected from sewage tanks associated with homes relying on cisterns. |
|  | 2 | 8 |  |

### 3.3.3 Plate count assay

Samples were processed in duplicates following SM 9222 for standard membrane filter procedure (Rice et al. 2012). Briefly, 100 mL of water sample and 100 mL of negative control (autoclaved water) were filtered through sterile polyethersulfone membranes (0.45-µm pore size, 47-mm diameter; Pall Corporation, Mississauga, ON, Canada). Filter papers were then placed on agar plates containing Brilliance *E. coli*/coliform medium (Fisher Scientific, Ottawa, ON, Canada) and incubated at 37°C for 24 h. Some samples required dilution because of high bacterial counts. *E. coli* counts in water samples were calculated as CFU/ 100 mL = number of purple colonies/volume of filtered sample (100 mL) × dilution factor. Total coliform bacteria counts in water were calculated as CFU/ mL = number of purple + pink colonies/volume of filtered sample (100 mL) × dilution factor. Brilliance agar was widely used for the detection of coliforms and *E. coli* in many studies (Atterbury et al. 2011; Fernando et al. 2016; Winterbourn et al. 2016; Farenhorst et al. 2017), because it can distinguish *E. coli* and coliforms from other bacteria based on β-D-glucuronidase production by *E. coli* resulting in purple *E. coli* colonies; and galactosidase production by most coliforms results in pink coliforms colonies (Wohlsen 2011). A possible exception is enterohemorrhagic *E. coli* strains, which are not efficient producers of β-D-glucuronidase so that they may not be identified by this medium (Fricker et al. 2010).

### 3.3.4 DNA extraction

For DNA extraction, 200 to 500 mL of drinking/source water sample or 10 to 50 mL of sewage water samples were filtered through sterile polyethersulfone membranes (0.22-µm pore size; 47-mm diameter; Pall Corporation, Mississauga, ON, Canada). Following the manufacturer's instructions, DNA was extracted from filter membranes using the DNeasy PowerWater Kit

(QIAGEN, Germantown, MD, USA). An unused filter membrane was used as the extraction control. DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The yield of DNA by this method ranged from 2 ng/µL to 280 ng/µL (40 to 80 µL in volume).

### 3.3.5 Antibiotic resistance genes quantification

Absolute quantitative PCR (qPCR) was used to quantify the abundance of seven different antibiotic resistance genes (ARGs): *ampC*, *vanA*, *tet(A)*, *mecA*, *sul1*, *sul2*, and *sul3* for all water samples. All the primers are listed in Table 2. For the primers designed in this study, positive controls and their sequences were provided by Agriculture and Agri-Food Canada, London, Canada (Dr. E. Topp Laboratory). Primers were designed using Custom Primers - OligoPerfect™ Designer at ThermoFisher Scientific (parameters chosen for primers were: 18 − 22 bp for primer length; 55 °C to 65 °C for annealing temperature; 30 % to 40 % for GC content; ~ 120 bp for amplification length). Specificity of the primers sequences was also confirmed using NCBI BLAST. Bacterial isolates used for positive controls are listed in Table 2.3. DNA from positive controls was isolated using a DNA extraction kit (Biobasic, Markham, ON, Canada). qPCR reactions were conducted on the StepOnePlus real-time PCR system (Life Technologies Inc., Burlington, ON, Canada). Reactions were carried out in triplicate. Total volume of each reaction was 8 µL that contained of 2.68 µL of template DNA (concentrations were all normalized to 0.7 ng/ µL), 9 µM of primers, and 2 × SsoFast EvaGreen Supermix (Bio-Rad Canada, Mississauga, ON, Canada). The amplification cycle used was as follows: 1 cycle of initial denaturation at 95 °C for 3 mins, 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, extension at 95 °C for 15 s, and the final extension at 60 °C for 60 s. The copy number of each gene per nanogram of DNA per 100 mL of water was calculated using a standard curve for each gene as

described previously (Fernando et al. 2016). A five-point standard curve was created by using a 10-fold dilution series using the positive control for each target gene, with copy numbers ranging from 20 to 200,000 copies in three replicates. The copy number of each gene per nanogram of DNA per 100 mL in samples was calculated using the slope of the standard curve, Y-intersection of standard curve, Ct values obtained from each qPCR runs, and with the filtration volume of water samples. The calculation was described as follows: Copy No./ng DNA/ 100 mL = $(10^{(Ct-Y\_inter)/slop}$ )/ 1 ng/ µL of DNA/ ($\frac{V_{filtration}}{100\ mL}$).

### 3.3.6 Multiplex PCR detection of β-lactamase and carbapenemase genes

The presence of five different β-lactamase genes: *SHV*, *TEM*, *CTX-M*, *OXA-1*, and *CMY-2* were determined using a multiplex PCR for water samples. The specific primers are listed in Table 2. Bacterial isolates used for positive controls for all PCR reactions are listed in Table 2.3. The PCR reaction was conducted using Q5 high-fidelity DNA polymerase (New England BioLabs, Whitby, ON, Canada) with final concentrations of the primers were 0.27 µM. The run parameters for amplification cycle were: 1 cycle of initial denaturation at 95 °C for 15 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 63.5 °C for 90 s, extension at 72 °C for 90 s, and final extension at 72 °C for 7 min.

Six different carbapenemase-encoding genes, *KPC*, *IMP*, *VIM*, *NDM*, *GES-5*, and *OXA-48*, were detected using endpoint PCR. All genes, except for *IMP* and *VIM*, were identified using a multiplex reaction. The *IMP* and *VIM* detections were carried out in separate reactions. PCR reactions were conducted using Q5 high-fidelity DNA polymerase (New England BioLabs, Whitby, ON, Canada) with final concentrations of the primers as follows: 0.27 µM for each gene in multiplex PCR, and 0.27 µM for *VIM*/*IMP* PCR. For the multiplex reactions, the run parameters for amplification cycle

steps were: 1 cycle of initial denaturation at 95 °C for 15 min; 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 90 s, extension at 72 °C for 60 s, and final extension at 72 °C for 7 min. For *IMP* and *VIM* amplification, annealing steps were at 61.7 °C for 90 s and 66 °C for 90 s, respectively. The rest of the steps were the same as in multiplex reaction. PCR products were resolved on a 1.3% agarose gel.

### 3.3.7 Detection of *mcr* genes in water bacteria isolates

PCR reactions were used to detect colistin-resistance genes *mcr-1*, *mcr-2*, *mcr-3*, and *mcr-4*. Primers are listed in Table 2. 10 – 100 mL of water samples were filtered through sterile polyethersulfone membranes (0.45-µm pore size, 47-mm diameter; Pall Corporation, Mississauga, ON, Canada). Filter papers were then placed on MacConkey agar (Sigma-Aldrich, Oakville, ON, Canada) plates supplemented with 2µg/ml colistin (GOlDBIO, St. Louis, MO, USA) and incubated for 24h at 37˚C. All the recovered bacterial colonies on filter papers were then streaked on MacConkey agar plate supplemented with 2 µg/mL colistin and incubate for 24h at 37˚C. Approximately, 800 isolated bacterial colonies (5 – 10 colonies picked from each plate randomly) were subjected to detection of *mcr* genes by PCR. The PCR reaction was performed using Taq polymerase (Froggabio, Toronto, ON, Canada) and the reaction conditions were:1 cycle of initial denaturing at 94 °C for 2 mins, followed by 34 cycles of denaturation at 94 °C for 30 S, annealing at 51, 47, 47, and 45 °C for 30 s for *mcr1*, *mcr2*, *mcr3*, and *mcr4* genes, respectively, extension at 72 °C for 1 min for *mcr1*, *mcr3*, and *mcr4*, and for 2 mins for *mcr2,* respectively, and a final extension at 72 °C for 5 mins. PCR products of reactions were resolved on a 1.0% agarose gel.

## 3.3.8 Statistical analysis

Analysis of variance (ANOVA) was performed using GLIMMIX in SAS 9.4. (SAS Institute, 2013) to test for the effect of concrete versus polyethylene underground cistern on free residual chlorine concentrations, the number of *E. coli* and total coliform forming units, and the detection frequency of ARGs in tap water of homes. Data were considered a lognormal distribution for chlorine concentrations, a negative binomial distribution for bacterial counts and a beta distribution for ARGs frequencies. The multiple comparison procedure was set as default to compare least square means. For the number of *E. coli* and total coliform forming units, data of P8 in the first sampling trip was removed due to the house condition was not suit for this study (septic filed was not functioning). In addition, for the positive detections in tap water, Pearson correlation was used to explore the association between the number of total coliforms forming units and the detection frequency of ARGs. For all statistical analysis, significance was determined at $\alpha = 0.05$.

**Table 3.2.** List of primers used in the study

| Primer name | Sequence | Target gene (amplicon size, bp) | Reference or source |
|---|---|---|---|
| **ampC_F1** | TGAGTTAGGTTCGGTCAGCA | *ampC* (98) | Fernando et al. 2016 |
| **ampC_R1** | AGTATTTTGTTGCGGGATCG | | |
| **vanA_F1** | ATAAAGCGCTCGGCTGTAGA | *vanA* (98) | Fernando et al. 2016 |
| **vanA_R1** | GAAACCGGGCAGAGTATTGA | | |
| **tetA_F1** | AGGTGGATGAGGAACGTCAG | *tet(A)* (96) | Fernando et al. 2016 |
| **tetA_R1** | AGATCGCCGTGAAGAGGCG | | |
| **mecA_F1** | CTGATGGTATGCAACAAGTCG | *mecA* (97) | Fernando et al. 2016 |
| **mecA_R1** | TGAGTTCTGCAGTACCGGATT | | |
| **sul1_F1** | TGTTTCAATCGACAGCTTCCAACC | *sul1* (112) | This study |
| **sul1_R1** | AATATCGGGATAGAGCGCAGGG | | |
| **sul2_F1** | GTGTGTGCGGATGAAGTCAG | *sul2* (120) | This study |
| **sul2_R1** | GTTTCGCGCAAATCCTTTC | | |
| **sul3_F1** | TGCGGAGATAATCTGCACCT | *sul3* (119) | This study |
| **sul3_R1** | TGCAGTGTCACGGAAATCAT | | |
| **SHV-UP** | CGCCGGGTTATTCTTATTTGTCGC | *blaSHV* (1,016) | Mulvey et al. 2011 |
| **SHV-LO** | TCTTTCCGATGCCGCCGCCAGTCA | | |
| **TEM-G** | TTGCTCACCCAGAAACGCTGGTG | *blaTEM* (708) | Mulvey et al. 2011 |
| **TEM-H** | TACGATACGGGAGGGCTTACC | | |
| **CTX-U1** | ATGTGCAGYACCAGTAARGTKATGGC | *blaCTX-M* (593) | Mulvey et al. 2011 |
| **CTX-U2** | TGGGTRAARTARGTSACCAGAAYCAGCGG | | |
| **OXA1-F** | CGCAAATGGCACCAGATTCAAC | *blaOXA-1* (464) | Mulvey et al. 2011 |
| **OXA1-R** | TCCTGCACCAGTTTTCCCATACAG | | |
| **CMY2-A** | TGATGCAGGAGCAGGCTATTCC | *blaCMY-2* (323) | Mulvey et al. 2011 |
| **CMY2-B** | CTAACGTCATCGGGGATCTGC | | |
| **KPC-1** | ATGTCACTGTATCGCCGTC | *blaKPC* (863) | Mulvey et al. 2011 |
| **KPC-2** | AATCCCTCGAGCGCGAGT | | |
| **IMP1** | CCWGATTTAAAAATYGARAAGCTTG | *blaIMP* (522) | Mulvey et al. 2011 |
| **IMP2** | TGGCCAHGCTTCWAHATTTGCRTC | | |
| **VIM1** | GTTTGGTCGCATATCGCAAC | *blaVIM* (382) | Mulvey et al. 2011 |
| **VIM2** | AATGCGCAGCACCAGGATAGAA | | |
| **NDM-F** | GGTGCATGCCCGGTGAAATC | *blaNDM* (660) | Mulvey et al. 2011 |
| **NDM-R** | ATGCTGGCCTTGGGGAACG | | |
| **GES-2** | ATCAGCCACCTCTCAATGG | *blaGES* (302) | Mataseje et al. 2012 |
| **GES-3** | TAGCATCGGGACACATGAC | | |
| **OXA-48A** | TTGGTGGCATCGATTATCGG | *blaOXA-48* (744) | Brink et al. 2013 |
| **OXA-48B** | GAGCACTTCTTTTGTGATGGC | | |
| **mcr-1 F** | CGGTCAGTCCGTTTGTTC | *mcr-1* (308) | Liu et al. 2016 |
| **mcr-1 R** | CTTGGTCGGTCTGTAGGG | | |
| **mcr-2 F** | TGGTACAGCCCCTTTATT | *mcr-2* (1617) | Xavier et al. 2016 |
| **mcr-2 R** | GCTTGAGATTGGGTTATGA | | |
| **mcr-3 F** | TTGGCACTGTATTTTGCATTT | *mcr-3* (542) | Yin et al. 2017 |
| **mcr-3 R** | TTAACGAAATTGGCTGGAACA | | |
| **mcr-4 F** | ATTGGGATAGTCGCCTTTTT | *mcr-4* (487) | Carattoli et al. 2017 |
| **mcr-4 R** | TTACAGCCAGAATCATTATCA | | |

**3.4 Results**

**3.4.1 Chlorine concentrations and fecal bacteria counts**

The free chlorine concentrations for water treatment plant samples (Figure 3.1) were close to or within the range of free chlorine concentration (0.4 to 2.0 mg/L) that is typically reported for water treatment plants in Canada (Health Canada, 2009b). There was no significant difference (Table 3.3 and Table 3.4) in the free residual chlorine concentrations of tap water between homes with concrete (Figure 3.2) and polyethylene (Figure 3.3) cisterns. The tap water in most homes had free residual chlorine concentrations < 0.2 mg/L, which is the minimal level that required to prevent the regrowth of bacteria (LeChevallier et al. 1996), except for C4, C7, P2, and P8 in the second sampling round.

No *E. coli* or coliforms bacteria were detected in WTP and truck water samples. However, *E. coli* and total coliforms were detected in all of the homes with concrete cisterns (Figure 3.2) and most of homes (75%) with polyethylene cisterns (Figure 3.3). There was a large variation across the homes in the counts of fecal bacteria detected. For example, for concrete cistern homes, *E. coli* and total coliforms were particularly abundant in tap water of C5, C6 and C8 homes and relative small in tap water of C4 and C7 homes. For polyethylene cistern homes, *E. coli* and total coliforms were particularly abundant in tap water of P8 home and absent in tap water of P3 and P4 homes. Overall, *E. coli* and total coliforms loads were greater in concrete cisterns than polyethylene cisterns (Table 3.3 and Table 3.4).
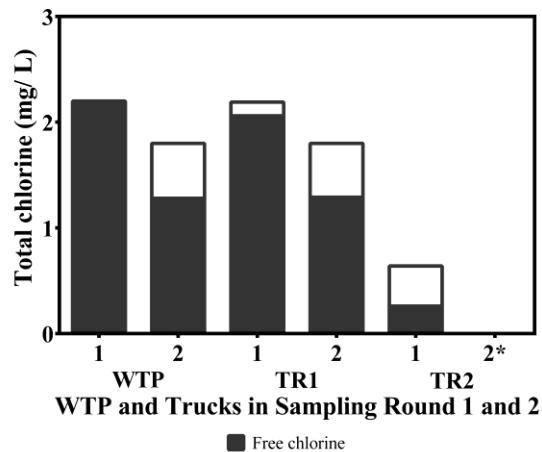
**Figure 3.1.** Total (black + white bar) and free residual (black bar) chlorine concentration in water samples collected from water treatment plant (WTP) and water trucks. * = water sample was not collected as the water truck was not working.
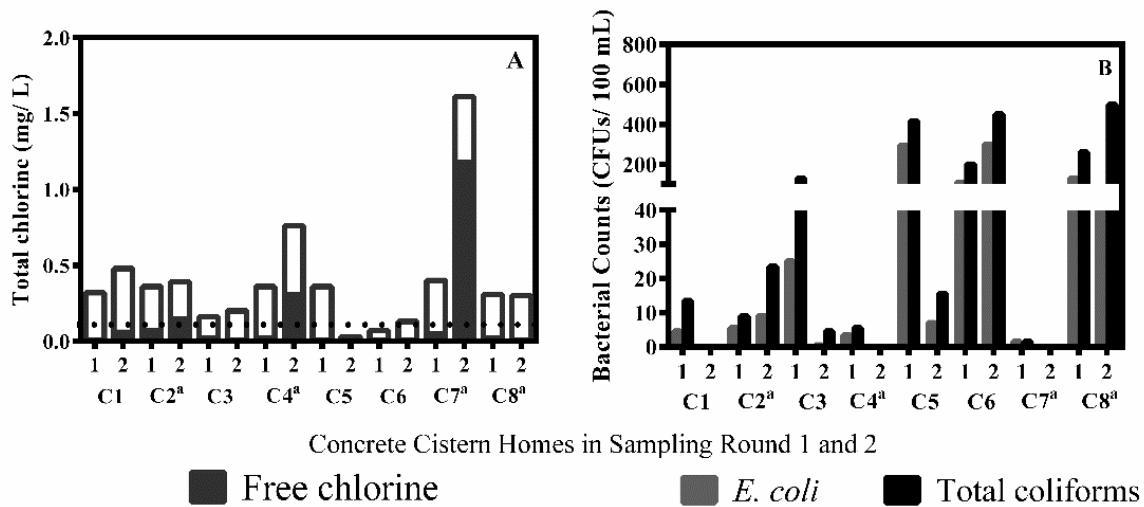


**Figure 3.2.** Total (black + white bar) and free residual (black bar) chlorine concentration in concrete cistern water samples (A) and the colony forming units (CFUs) of *Escherichia coli* and total coliforms detected in concrete cistern water samples (B). Dashed line is minimum recommended free residual chlorine (LeChevallier et al. 1996). For *E. coli* and total coliforms, the error bars represent the standard deviation of duplicates of culturing. a = the cisterns had been cleaned before the second sampling rounds. The sample description is provided in Table 3.1.

60
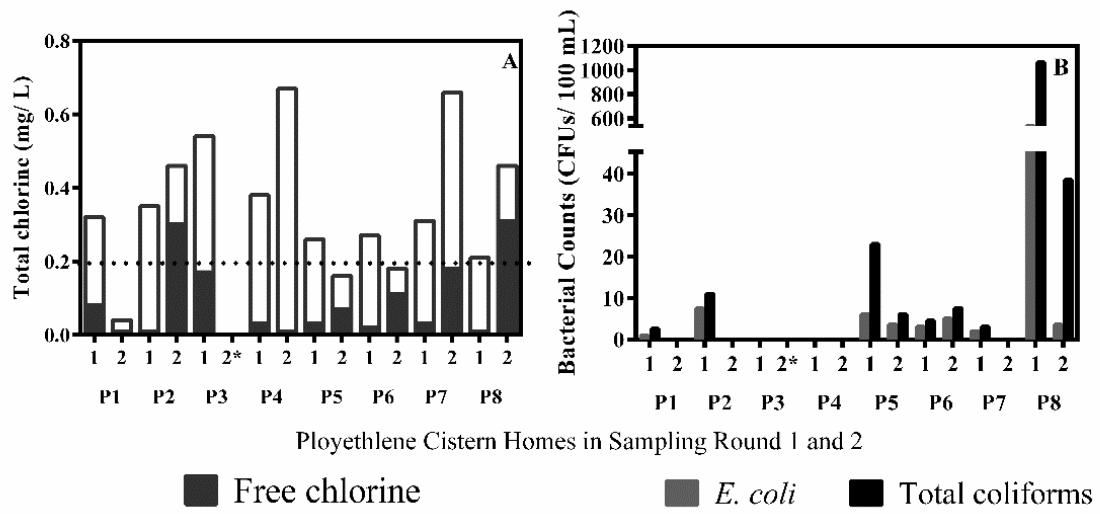
**Figure 3.3.** Total (black + white bar) and free residual (black bar) chlorine concentration in polyethylene cistern water samples (A) and the colony forming units (CFUs) of *Escherichia coli* and total coliforms detected in polyethylene cistern water samples (B). Dashed line is minimum recommended free residual chlorine (LeChevallier et al. 1996). For *E. coli* and total coliforms, the error bars represent the standard deviation of duplicates of culturing. * = water sample not collected as family was not home. The sample description is provided in Table 3.1.

**Table 3.3.** The type three test of effect of cistern types on quality parameters

| Parameters | Type III Tests | | | | |
|---|---|---|---|---|---|
| | Effect | Num DF | Den DF | F Value | Pr > F |
| **Free chlorine** | Polyethylene Concrete | 1 | 28 | 0.74 | 0.3966 |
| ***E. coli*** | Polyethylene Concrete | 1 | 27 | 34.33 | <.0001 |
| **Coliforms** | Polyethylene Concrete | 1 | 27 | 27.54 | <.0001 |
| **ARGs frequency** | Polyethylene Concrete | 1 | 26 | 0.2 | 0.6556 |

61

**Table 3.4.** Water quality parameters (average ± standard error of mean) of water distribution in each community

| Parameters | Water distribution system | Free chlorine residual (mg/L) |
|---|---|---|
| **Free chlorine residual** | Polyethylene | 0.06 ± 0.00 |
| | Concrete | 0.04 ± 0.00 |
| ***E. coli*** | Polyethylene | 2 ± 1 |
| | Concrete | 60 ± 21 |
| **Coliforms** | Polyethylene | 7 ± 2 |
| | Concrete | 126 ± 47 |
| **ARGs frequency** | Polyethylene | 0.21 ± 0.05 |
| | Concrete | 0.19 ± 0.05 |

### 3.4.2 Detection of antibiotic resistance genes

For qPCR detection, samples with Ct value of less than 35 were considered positive for these ARGs. For WTP samples, the *sul1* gene was detected in both sampling rounds (0.004 ± 9.5E-05 and 0.02 ± 0 NO./ng/100mL, respectively). Both Tr1 and Tr2 samples showed detectable levels of the *sul1* in the first sampling round (0.02 ± 0 and 0.09 ± 0 NO./ng/100mL), but the *sul1* was not detected in Tr1 in the second sampling round (Tr2 not sampled).

None of the other genes were detected in WTP and truck water samples. The *sul1* was among the most frequently genes in the tap water of homes, with 87% of homes with concrete cisterns (ranging from 0.006 ± 0 to 2.16 ± 0.48 copy NO./ng/100mL) and 75% of homes with polyethylene cisterns (ranging from 0.007 ± 0 to 0.37 ± 0.09 copy NO./ng/100mL) (Figure 3.4) showing positive detections. In addition, all sewage samples showed positive detection of the *sul1 gene* (ranging from 67.17 ± 6.78 to 21524 ± 472.10 copy NO./ng/100mL) (Figure 3.4). Thus, overall, the *sul1* was the most frequently detected gene by qPCR across all the samples. There was another commonality across samples in that none of the tap water and sewage samples showed positive detections of the *sul3* and *vanA* (Figures 3.4 to 3.6).

For homes with concrete cisterns (Figure 3.4), the *sul2* was detected in one tap water sample, i.e., in home C2 in the second sampling round (0.45 ± 0.2 copy NO./ng/ 100mL). The *sul2* was not detected in the tap water of homes with polyethylene cistern (Figure 3.4). In contrast, *sul2* was detected in every sewage samples (ranging from 1.69 ± 0.61 to 21154.60 ± 759.54 copy NO./ng/100mL) as was the *tetA* (ranging from 405.94 ± 121.05 to 1.6E+07 ± 8.8E+05 copy NO./ng/100mL) (Figure 3.4). Tap water in homes with concrete (Figure 3.4) and polyethylene (Figure 3.4) cisterns never showed detectable levels of the *tetA*.

The *ampC* was detected in tap water of one sample taken in homes with concrete cisterns (C8: 84.46 ± 15.98 copy NO./ng/100 mL) and of one sample taken in homes with polyethylene cistern (P8: 506.33 ± 19.07 copy NO./ng/100mL), both in the first sampling round (Figures 3.4). In contrast, the *ampC* was almost always detected in sewage samples (ranging from 141.76 ± 89.49 to 12426.88 ± 395.11 copy NO./ng/100mL), except for S2 in the second sampling trip (Figure 3.4). In the first sampling round, the C8 home also showed a positive detection of the *mecA* (10.15 ± 0.63 copy NO./ng/100 mL) but this gene was not detected in the tap water of homes with polyethylene cisterns. Again, in contrast, the *mecA* was more often detected in sewage samples (ranging from 0.55 ± 0.1 to 284.87 ± 14.83 copy NO./ng/100mL), rather frequently in the second sampling trip but only one time (S1) in the first sampling trip (Figure 3.4).

None of the β-lactamase and carbapenemase genes examined using multiplex PCRs were detected in the WTP and truck water samples. Among the β-lactamase genes, the *TEM* was the only frequently detected in both tap water and sewage samples. The *TEM* was detected in tap water of seven homes with concrete cisterns (C2 to C8) (Figure 3.4) and in sewage tanks associated with four of these homes (S3 to S5 and S8) (Figure 3.4), as well as seven homes with polyethylene cisterns (P1 and P3 to P7) (Figure 3.4) and one additional sewage tanks (S1) (Figure 3.4). The *SHV*

was frequently detected in tap water of homes with concrete cisterns (C3 to C6 and C8) (Figure 3.4) but only in one of the sewage tanks associated with these homes (S8), as well as an additional one sewage tank (S7) (Figure 3.4). However, the *SHV* was also frequently detected in tap water of homes with polyethylene cisterns (P1, P2, and P4 to P7) (Figure 3.4). Despite the *CYM-2* being frequently detected in tap water of homes with concrete cisterns (C3 to C7) (Figure 3.4), the *CYM-2* was not detected in any sewage tanks (Figure 3.6). However, the *CYM-2* was frequently detected in tap water of homes with polyethylene cisterns (P1, P3 to P8) (Figure 3.4). The *CTX-M* was most frequently detected in tap water of homes with polyethylene cisterns (P1 and P4 to P8) (Figure 3.4). The tap water of homes with concrete cisterns also showed some detections of the *CTX-M* (C3, C7, and C8) (Figure 3.4) of which only one of the sewage tanks associated with these homes (S8) showed detectable levels of the *CTX-M* (Figure 3.4).

For the detection of carbapenemase genes, the *GES* was detected in all sewage samples (Figure 3,6) but never in tap water (Figures 3.4). The *VIM* and *IMP* were not detected in any water samples. In contract, the *KPC* was frequently detected in the tap water of homes with concrete cisterns (C1, C2, C4 to C8) and also in the sewage tanks associated with these homes (S2, S4, S6 and S8). The *KPC* was also frequently detected in the tap water of homes with polyethylene cisterns (P1, P3 to P5, and P7), as well as in sewage tanks S3. The OXA-48 was frequently detected in the tap water of homes with concrete cisterns (C1, C2, C4 to C6, C8) and every sewage tanks associated with these homes. The *OXA-48* was also frequently detected in the tap water of homes with polyethylene cisterns (P1, P3 to P7), and all other sewage samples. In addition, the *NDM* was frequently detected in the tap water of homes with concrete cisterns (C2, C4 to C8) and most of the sewage tanks associated with these homes (S2, S4 to S6 and S8). The *NDM* was also frequently detected in the

tap water of homes with polyethylene cisterns (P1 to P7) and two other sewage samples (S1 and S3).

All five β-lactamase genes were detected in tap water of homes with concrete (Figure 3.4) and polyethylene (Figure 3.4) cisterns. Except for the *CYM-2*, the β-lactamase genes examined for were also detected in sewage (Figure 3.4). The *KPC*, *OXA-48*, and *NDM* were the only carbapenemase genes detected in tap water of homes with concrete (Figure 3.4) and polyethylene (Figure 3.4) cisterns, and these genes were also detected in sewage (Figure 3.4). The *GES* was frequently detected in sewage but was not present in tap water samples. None of the samples showed detections of *mcr* genes.

For a number of homes with concrete cisterns, the tap water contained the same gene as detected on that day in the sewage tank that belongs to that home (Figure 3.4). This was the case for the *sul1* (7 and 6 homes in the first and second sampling round, respectively), the *ampC* gene (1 home in the first sampling round), the *SHV* (1 home in the second sampling round), the *TEM* gene (2 and 1 homes in the first and second sampling round, respevtively), the *OXA-1* (1 home in the second sampling round), the *KPC* (3 and 2 homes in the first and second sampling round, respectively), the *OXA-48* (4 and 1 homes in the first and second sampling round, respectively), and the *NDM* (4 and 5 homes in the first and second sampling round, respectively). Sewage was not the determinative source of contamination of ARGs in the tap water of homes with concrete cisterns because the *tetA*, and *GES* were detected in all eight sewage tanks but never in tap water from the eight concrete cisterns belongs to the same homes. Also, for a number of homes with concrete cisterns, the tap water contained a gene that was not detected on either sampling rounds in the sewage tank that belongs to that home. This was the case for the the SHV (4 homes), the *TEM* (3 homes), the *CTX-M* (2 homes), the *OXA-1* (2 homes), the *CYM-2* (5 homes), the *KPC* (3 homes),

and the *NDM* (1 home). In addition, for a number of homes with concrete cisterns, the sewage tank contained a gene that was not detected on either sampling rounds in the tap water that is derived from the concrete cistern that belongs to that home. This was the case for the *sul1* (1 home), the *sul2* (7 homes), the *ampC* (7 homes), the *mecA* (6 homes), the *TEM* (1 home), the *CTX-M* (1 home), the *KPC* (1 home), the *OXA-48* (2 homes), and the *NDM* (2 homes). Another important observation was that, regardless of homes having concrete or polyethylene cisterns, the same ten ARGs were detected in the tap water (the *sul1*, *ampC*, *SHV*, *TEM*, *CTX-M*, *OXA-1*, *CYM-2*, *KPC*, *OXA-48*, and *NDM*); there was one exception in that the *mecA* was detected ones in tap water of a home with a concrete cistern but never in homes with polyethylene cisterns). Moreover, the frequency of detections of these ten ARGs were the same for tap water from homes with concrete and polyethylene cisterns (Table 3.3 and Table 3.4). The frequency of detection of ARGs was positively associated with total coliforms for the homes with concrete cisterns (r=0.53, P = 0.03), but not for the homes with polyethylene cisterns (P=0.7).
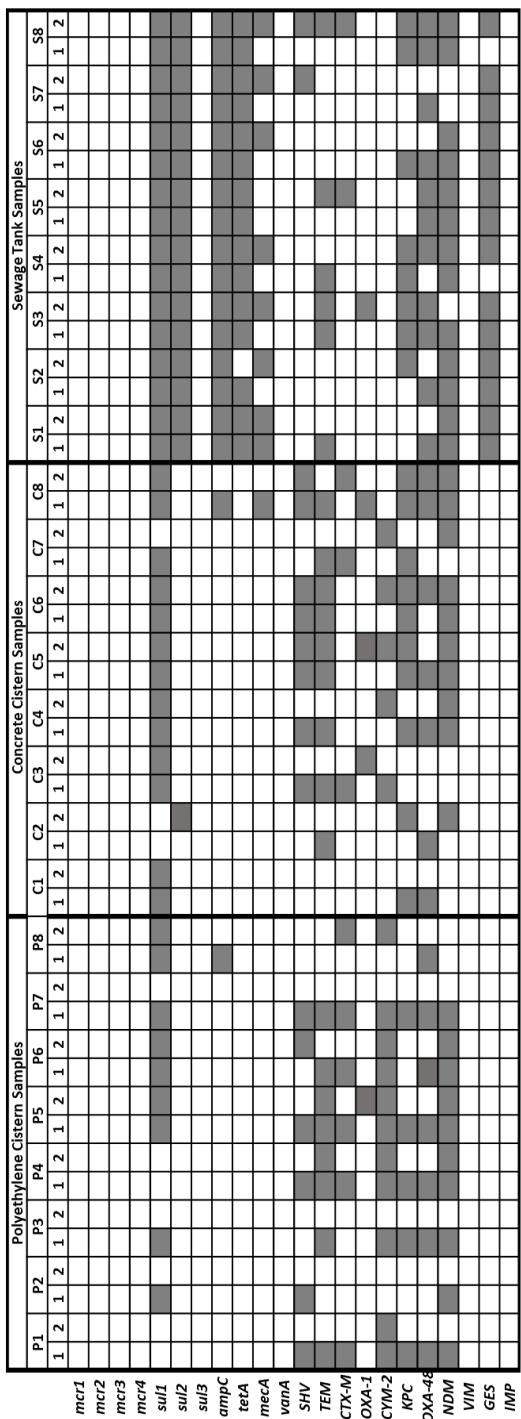
**Figure 3.4.** The detection of antibiotic resistance genes in samples from polyethylene cisterns, concrete cisterns, and sewage tanks. *mcr1*, *mcr2*, *mcr3*, and *mcr4* were detected using isolated bacteria by PCRs. *sul1*, *sul2*, *sul3*, *ampC*, *tetA*, *mecA*, and *vanA* were detected by qPCR. *SHV*, *TEM*, *CTX-M*, *OXA-1*, *CYM-2*, *KPC*, *OXA-48*, *NDM*, and *GES* were detected by multiplex PCR. *VIM* and *IMP* were detected by separate PCRs. The shade represents the presence of the genes. 1 represents the first sampling round. 2 represents the second sampling trip.

67

## 3.5 Discussion

### 3.5.1 Chlorine concentrations and fecal bacterial counts

In this study, we investigated the microbiological quality of drinking water in a First Nation reserve that heavily relied on underground cisterns. Treated water at the WTP contained adequate levels of free residual chlorine and was free of indicator bacteria, suggesting that the water treatment process removed fecal bacteria completely. In contrast, the majority of tap water samples had free residual chlorine < 0.2 mg/L and were detected positive for fecal bacteria. For concrete cistern water, bacterial levels in water samples ranged from 0 to 300 CFUs/ 100 mL for *E. coli* and from 0 to 500 CFUs/ 100 mL for total coliforms. This relatively large variation across concrete cistern homes might be due to two reasons. First, some cisterns have been used for a longer time and were cracked. For example, higher fecal bacteria counts were detected in C5 and C8 than C1 and C4. The cisterns in homes C5 and C8 had been used for 25 and 29 years and these two cisterns were observed having broken manhole covers (i.e., severely cracked at the entry point where the cisterns are filled). The cisterns in both homes C1 and C4 had been used for 5 years and no cracks were observed. Second, the timing of cistern cleaning varied. For instance, higher fecal bacteria counts were detected in C5 than C7. The cistern in home C5 had not been cleaned for two years (2016 and 2017), and the cistern in home C7 had been cleaned in both 2016 and 2017 and the recent cleaning was just before the second sampling round. For C2, C4, C7, and C8, these cisterns had been cleaned before the second sampling round. C4 and C7 were detected negative for fecal bacteria after cleaning while C2 and C8 detected higher or similar level of fecal bacteria comparing to the first sampling round. The difference between these two sets of cisterns is C2 and C8 were cracked and C4 and C7 were not, suggesting that cistern cracking should be the most important factor that causes fecal bacteria contamination. For polyethylene cistern water, excluding P8,

bacterial levels in water samples ranged from 0 to 8 CFUs/ 100 mL for *E. coli* and from 0 to 39 CFUs/100 mL for total coliforms. The relatively small variation across polyethylene cistern homes (relative to concrete cistern homes) might be because the infrastructural condition of the cisterns was consistent across homes, i.e., no cracking was observed in any of these cisterns. The cistern in home P8 had substantial levels of fecal bacteria during the first sampling round. The reason might be that this house has a private septic field that was not functioning well as told by the family during the first sampling round.

Comparing the two types of cisterns, indicator bacterial levels were greater in concrete cisterns than polyethylene cisterns in this community. The detection frequency of fecal bacteria was also higher in concrete cisterns (100%) than polyethylene cisterns (75%). The reason might be: a) concrete cisterns are prone to corrode and rust and then cisterns become cracked; b) concrete cisterns have rougher surface walls favoring by the formation of biofilms (Characklis et al. 1990). Overall, the results thus demonstrate that concrete cistern might be more vulnerable to factors such as long-time usage and infrequent cleaning, which perhaps result in higher level of fecal contamination in water. Therefore, polyethylene cisterns should be a better option than concrete cisterns for First Nation communities to store their drinking water. Moreover, older cracked underground concrete cisterns need to be replaced and cistern cleaning should be frequent to reduce the risk of exposure to fecal bacteria.

### 3.5.2 Detection of ARGs

The presence of antibiotic resistance genes was investigated for water samples by using PCR, qPCR, and multiplex PCR methods. For qPCR method, seven antibiotic resistance genes, namely *sul1*, *sul2*, and *sul3* (sulfonamides resistance), *tet(A)* (tetracycline resistance), *ampC* (β-lactam

resistance), *mecA* (methicillin resistance), and *vanA* (vancomycin resistance), were detected in the tap water of homes with underground cisterns (Figure 3.4). These ARGs are typically detected in the environment, including in surface water, waste water and drinking water sources (Schwartz et al. 2003; Alexander et al. 2015; Loffler et al. 2016; Suhartono et al. 2016; Zhang et al. 2016). The *sul1* was frequently detected in both drinking water samples and sewage samples. It was also detected in the water treatment plant samples suggesting that water treatment plant is perhaps a reservoir (e.g., presence of biofilm on water filters) for bacteria carrying ARGs, or a source of AGRs that detected in drinking water. Sulfonamides are one of the earliest antibiotics introduced in the world and widely used for the treatment of human infections (Van et al. 2011). In addition, the *sul* genes are usually linked to mobile genetic elements, which results in the fast dissemination of the *sul*. In contrast to the *sul1*, the *sul2* was not detected in drinking water and the *sul3* was not detected in drinking water and sewage. This might mean that the bacteria are using the *sul1* gene in their mechanism of resistance to sulfonamides and hence it is perhaps not surprising that the *sul1* gene rather than the *sul2* and *sul3* was widely detected (Antunes et al. 2005). The *ampC* was detected in C8 and P8. The *mecA* was only detected in C8. The *vanA* and *tetA* were not detected in any drinking water samples. In contrast, these four ARGs were frequently detected in sewage samples. Since concrete cistern samples and sewage samples were collected (8 meters apart) at the same location, there is no clear indication that the domestic sewage tanks are contaminating cistern water with ARGs.

For multiplex PCR method, five β-lactamase genes and six carbapenemase genes were detected for water samples (Figure 3.4). β-lactamase genes and carbapenemase genes have been found in many aquatic environments. For example, *OXA-1* and *TEM-1* were found in a water treatment plant in China (Zhang et al. 2016). The *CTX-M* was found in water samples collected from a river

in India (Bajaj et al. 2015), and the *OXA-48* gene was found in river water in Algeria (Tafoukt et al. 2017). In this study, *SHV*, *TEM*, *CYM-2*, *KPC*, *OXA-48*, and *NDM* genes were frequently detected in concrete cistern samples. *SHV*, *TEM*, *CTM-2*, *CTX-M*, *KPC*, *OXA-48* and *NDM* were frequently detected in polyethylene cistern samples. Only *TEM*, *OXA-48*, *NDM*, and *GES* were frequently detected in sewage samples, suggesting that something other than the sewage tanks is the reason for the persistent or reoccurring contamination of cisterns with ARGs. The detection frequency of AGRs in tap water was similar for concrete and polyethylene cisterns. Total coliform counts were greater in the tap water of cement cisterns than in the tap water of polyethylene cisterns and, for this tap water of cement cisterns, the frequency of AGRs was significantly positively correlated with total coliform counts. This suggests that coliform bacteria might be carrying ARGs.

None of the *mcr* genes were detected in water or sewage samples. This is an important finding because *mcr* genes cause resistance to the antibiotic colistin, which is the last-resort antibiotic used for infections caused by pathogens. In addition, since *mcr* genes are located on plasmid, they are able to rapidly spread or transfer in the environment. For example, the plasmid-mediated colistin resistance gene, *mcr-1*, was first detected in *E. coli* isolates from raw meet and animals in China (Liu et al. 2016). Since then, many regions in the world have reported on the presence of *mcr* genes in bacteria isolates obtained from animals, including humans (Abuoun et al. 2017; Chan et al. 2018; Chen et al. 2018; Haenni et al. 2018). In recent studies, the *mcr-1* gene was detected in hospital sewage water and river water (Yang et al. 2017; Jin et al. 2018). Although *mcr* genes were not detected in water samples collected in the First Nation community, bacteria isolated from these water samples were growing on the colistin-supplemented agar. This observation suggests that these bacteria might have other mechanisms to resist colistin. For instance, mutations in the PmrAB is associated with colistin-resistance in *Acinetobacter baumannii* (Adams et al. 2009).

Fernando et al. (2016). also detected ARGs in water samples collected in a First Nation Community in Manitoba, suggesting that the presence of ARGs in the tap water of First Nation homes might not be an isolated case. Although the *sul1*, *sul2, ampC*, *mecA*, β-lactamase genes and carbapenemase genes were detected, the selection pressure for the presence of these genes in bacteria is unclear because the absence of public data on the usage of antibiotics across geographical regions of Canada.

**3.6 Conclusion**

This study compared the drinking water quality of samples taken from tap water in First Nation homes relying on concrete and polyethylene underground cisterns, and examined whether sewage tanks at the home were responsible for contaminating cistern water with antibiotic resistance genes. Our findings suggest that, by drinking their tap water a) the residents living in homes with concrete underground cisterns are likely to be exposed to higher level of fecal bacteria than residents living in homes with polyethylene underground cisterns. This is particularly the case when the concrete cisterns show signs of cracking and/or are not frequently cleaned (< 1 year); and b) residents living in homes with concrete or polyethylene underground cisterns are exposed to similar ARGs and at the same frequency. In addition, some ARGs were detected in sewage tanks but not in drinking water, suggesting that the presence of ARGs in concrete cisterns are unlikely to be influenced by sewage tanks that have been installed near these cisterns. Overall, for this First Nation community, the observed poor drinking water quality in homes relying on underground cisterns is alarming, particular in a country that is considered to be among the most advanced economies in the world. We urge provincial and federal governments in Canada to immediately decrease the portion of homes on First Nation reserves relying on underground concrete cisterns.

## 3.7 Acknowledgements

## 3.8 References

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# 4. OVERALL SYNTHESIS

## 4.1. Summary of Research

Safe drinking water is an important resource for human beings. Residents of Canada's urban areas have access to safe drinking water because of advanced water treatment technologies, knowledgeable water operators, and access to land management decision-making (Patrick 2011). Many residents of Canada's First Nation's communities do not have the luxury of safe drinking water, and this has been the case for decades. According to Health Canada (https://www.sac-isc.gc.ca/eng/1100100034879/1521124927588), in May 2018, there were still more than 100 First Nation Communities in Canada under drinking water advisories many of which were the result of bacterial contamination.

In a recent study, Farenhorst et al. (2016) detected *E. coli* and total coliforms as indicators of water contamination with fecal materials. Other bacteria, such as a member of TM6 group and *Betaproteobacteria* were in the drinking water samples from a First Nation community in Manitoba. In addition, various antibiotic resistance genes were detected in the drinking water from the same community (Fernando et al. 2016). These findings suggest that residents living in this community, and those living under similar conditions, are at a higher risk of being exposed to pathogenic bacteria through their drinking water and some of these may be resistant to antibiotics. As these earlier studies only examined the water quality in one community, water quality needed to be evaluated in more First Nation communities.

The study described in Chapter 2 investigated drinking water quality in three First Nation communities (referred to as communities A, B and C) in Manitoba. Analysis consisted of free residual chlorine concentration, and for the screening for the presence/abundance of *E. coli*, total

coliforms, and antibiotic resistance genes. In this study, regardless of whether the water treatment plant water source was groundwater or surface water, or whether the water treatment systems were conventional or advanced membrane filtration systems, drinking water sampled at the treatment plants always had an acceptable free chlorine residual concentration (ranged from 0.4 to 2 mg/L), and had no indicator bacteria or ARGs. This finding suggests that the water treatment plants in these three communities were functioning well and producing safe water. Regardless of the community, free chlorine residual concentrations of greater than 0.2 mg/L were measured in most of the samples taken from pipelines. As well, no indicator bacteria and ARGs were detected in these samples. This indicates that water distribution system of WTP + pipeline provided safe drinking water to residents on reserves.

Safe water was also provided when the water distribution was WTP + aboveground polyethylene cisterns or wells, such as in communities A and C. The chlorine concentration in community A's cistern water was greater than 0.2 mg/L and ARGs were not detected. The reason for the low free chlorine concentration in well water was the well water in community A was not chlorinated. Community C's well water also had low chlorine concentrations for the same reason. Despite the low chlorine concentrations, no indicator bacteria or ARGs were detected, suggesting that residents were not at risk of being exposed to microbial contaminants when drinking their tap water connected with well.

When the drinking water distribution system was WTP + underground cisterns, such as in community B, free chlorine residual concentrations of less than 0.2 mg/L were observed in most of the drinking water samples and indicator bacteria and ARGs were detected. This observation suggests that residents living in homes with underground cisterns are at risk of being exposed to fecal bacteria and ARGs.

The study described in Chapter 3 further investigated the underground cisterns in community B because of the level of contamination of cistern water in that community. The effect of cistern design on water quality was considered by comparing water quality in polyethylene cisterns to that in concrete cisterns. The ARGs in sewage were compared to those in drinking water to determine if sewage could be the source of the fecal contamination.

The results show that the majority of cistern water, regardless of types of cisterns, had free chlorine residual less than 0.2 mg/L. *E. coli* and total coliforms were detected in 100% of concrete cistern water and 75% of polyethylene cistern water. In addition, the bacterial counts suggested that residents living in homes with concrete underground cisterns are likely to be exposed to higher level of fecal bacteria than residents living in homes with polyethylene underground cisterns. The reasons for this might be a) some concrete cisterns were cracked but no cracking was observed in any of the polyethylene cisterns; and b) concrete has a rougher surface which is more favorable for the development of biofilms. Various antibiotic resistance genes were detected in both the concrete and polyethylene cisterns which suggests that exposure to antibiotic resistance genes did not depend on the cistern type.

The *GES* and *tetA* genes were detected in sewage tanks but not in concrete cistern water. The *CYM-2* gene was detected in concrete cistern water but not in sewage tanks, indicating that the presence of AGRs in concrete cisterns was unlikely to be influenced by sewage tanks that had been installed near these cisterns.

Overall, the results from both Chapter 2 and Chapter 3 illustrated that cistern water in community B was contaminated with *E. coli*/total coliforms and ARGs, indicating that when surface water is used as source water, the water distribution system of WTP + underground cisterns might not provide safe drinking water for residents living in First Nation communities.

## 4.2. Practical Implications

Each First Nation Community should have a water treatment plant. Drinking water at the water treatment plant always had an acceptable level of free chlorine residuals and no indicator bacteria. Water distribution by pipelines or storage in above ground polyethylene cisterns is the preferred way to store water at the household as suggested by the absence of indicator bacteria in these water distribution systems. The poor drinking water quality in homes relying on underground cisterns in community B suggested that the number of First Nation households relying on these cisterns should be reduced.

Chlorine concentrations should also be measured in water coming from the tap because some pipeline water samples had free chlorine residual concentrations less than 0.2 mg/L. This is particularly important for the homes located towards the end of the pipeline. Second, since some cisterns in community B had high level of indicator bacteria, the government of Canada should fund the community to replace these cisterns immediately. Although indicator bacteria and ARGs were detected in several cisterns in community B, no drinking water advisories had been issued in this community. The results from this study suggest that the drinking water in community B should be tested again and that these results be used to determine if drinking water advisories need to be issued.

There is now increased evidence that replacing underground concrete cisterns will improve public health. Residents using water from underground cisterns appear to be at increased risk of being exposed to antibiotic resistance pathogenic bacteria, which could explain a higher than normal incidence of infections like bacterial gastroenteritis and impetigo (Murdocca 2010; NACI 2010).

84

**4.3 Recommendations for Further Study**

There are several limitations to this study that could be used to determine the direction of future studies.

First, in this study, indicator bacteria and ARGs were only examined for water samples collected in the summer. Seasonal variation of microbiological quality of drinking water could be important because it will show if water quality varies through the year. This information could help communities to improve their management plans such as in setting the cleaning schedule at the most appropriate time and/or in setting the cleaning frequency.

Second, the study suggests that sewage was not the source of ARGs in concrete cisterns. Because bacteria can enter with subsurface water through cracks into the cistern wall (Baird et al. 2013), the contamination source might be identified by examining the presence of ARGs in the snowmelt and soils of community B, especially in the spring during periods of snowmelt and heavy rainfall events as this could generate flooding conditions or saturated soils (Artiola et al. 2012)

Third, although various ARGs were detected, the bacteria carrying these ARGs were not identified. If these bacteria are pathogenic, their resistance to antibiotics could make treatment more difficult. For examples, the *SHV* gene is prevalent in *K. pneumoniae* (Shaikh et al. 2015), which is an important pathogenic bacterium in infections originating in hospitals, and the *CTX* type genes which are mainly found in pathogenic bacteria such as *Salmonella enterica serovar*, *Typhimurium*, *E. coli*, and other species of *Enterobacteriaceae* (Gazouli et al. 1998; Knothe et al. 1983). Thus, to further understand the risk associated with antibiotic resistance in the water supply the identity of the bacteria carrying the ARGs needs to be determined.

Fourth, more work needs to be done on the relationship between the presence of ARGs in the communities studied and the presence of its corresponding antibiotic in the community's environments. In this study, the selective pressure towards the development of antibiotic resistance could not be identified because of the lack of data on antibiotic usage in Canada. There is information on the relationship between the presence of antibiotics and the presence of ARGs in the environment. For instance, Wu et al. (2015). quantified five groups of antibiotics (sulfonamide, quinolone, tetracycline, macrolide, and chloramphenicol) and six ARGs (*sul1*, *sul2*, *tetQ*, *tetM*, *ermB*, and *mefA*) in municipal solid waste leachates by using high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) and qPCR, respectively. They found that the abundance of *tetQ* and *tetM* genes were strongly correlated with the level of tetracycline antibiotic. A similar result was observed in the study conducted by Chen et al. (2017). In this study, they examined antibiotics and ARGs in water and sediments samples collected from mariculture sites: they quantified sulfonamides antibiotics (sulfadiazine, sulfapyridine, sulfamethoxazole, sulfathiazole, sulfaquinoxaline, sulfamethazine, and sulfamerazine) and tetracyclines antibiotics (tetracycline, oxytetracycline, doxycycline, and chlortetracycline) by using HPLC-MS/MS and they also quantified sulfonamide resistance genes (*sul1*, *sul2*, *sul3*), tetracycline resistant genes (*tetA*, *tetB*, *tetM*, *tetO*, *tetQ*, *tetW*) by using qPCR. They found that the abundance of *sul3* was significantly correlated with the level of sulfamerazine in water samples, while the abundance of *sul2* and *tetM* were significantly correlated with sulfadiazine and oxytetracycline levels in the sediment samples, respectively. Thus, to further understand the reason of the presence of a certain group of ARGs, the future study could determine the selective pressure of these antibiotic resistance genes by examining their corresponding antibiotic using the methods that described in above studies.

Finally, although the study examined indicator bacteria and ARGs in bulk water, it did not examine them in other phases in water distribution systems. Of particular interest would be biofilms in First Nation water distribution systems. It is estimated that most free bacterial cells in bulk water are released from biofilms attached to the solid surfaces of the distribution system (Ridgway and Olson 1981; LeChevallier 1987), and 95 % of bacteria in drinking water networks are in biofilms (Flemming et al. 2002). Moreover, biofilms provide an ideal habitat for horizontal gene transfer (HGT) due to the high density of bacteria cells and disinfectants cannot penetrate (Balcázar et al. 2015).

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# 5. APPENDICES
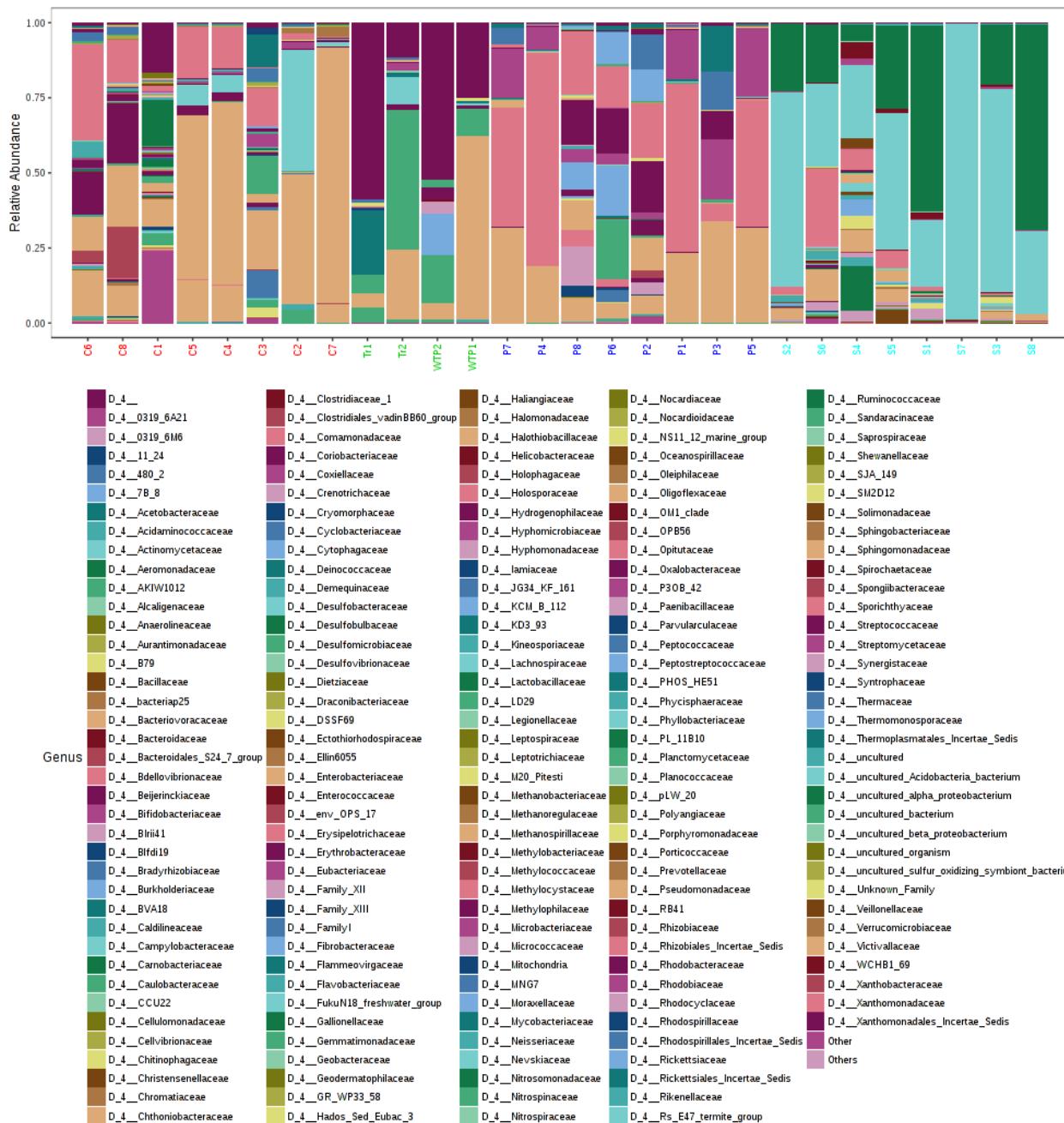


**Figure 5.1.** The bacteria community of water samples from concrete and polyethylene cisterns, and sewage tanks in community B. The data refers to the water samples collected in the first sampling round. The samples are listed according to the similarity of bacteria community.