From Source Water to Drinking Water: Microbiological Quality Analysis of Water Collected from Two First Nation Communities in Manitoba, Canada

by

Sabrin Bashar

A Thesis Submitted to the Faculty of Graduate Studies of

The University of Manitoba

in Partial Fulfilment of the Requirements of the Degree of

Master of Science

Department of Microbiology

University of Manitoba

Winnipeg

TRADITIONAL TERRITORIES ACKNOWLEDGEMENT

The University of Manitoba campuses are located on original lands of Anishinaabeg, Cree, Oji-Cree, Dakota, and Dene peoples, and on the homeland of the Métis Nation.

We respect the Treaties that were made on these territories, we acknowledge the harms and mistakes of the past, and we dedicate ourselves to move forward in partnership with Indigenous communities in a spirit of reconciliation and collaboration.

Abstract

This study determined the microbiological quality of drinking water from various water distribution systems of two First Nation communities in Manitoba, Canada. *E. coli*, other coliforms as well as different types of antibiotic resistance genes (ARGs) were more often detected in water from homes with cisterns than homes with the direct-lined piped system. Cistern water showed the presence of bacteria, as well as ARGs, and had free chlorine concentrations less than the World Health Organization (WHO) standard recommended level that is 0.2 mg/L. Also, environmental changes affect the fecal bacterial counts as warmer months showed higher counts than the colder months. Moreover, this study also showed the presence of *mecA* gene carrying *Mammaliicoccus fleurettii* in lake water which is used as source water and recreational purposes in a First Nation community in Manitoba, Canada. Our study suggests that both drinking water and recreational water should be monitored for taking further steps to protect the community residents' health from waterborne illness.

Preface

This dissertation is written in manuscripts style which have not yet been submitted to any journal.

The manuscripts are:

Manuscript 1: Investigation of the presence of fecal bacteria and antibiotic resistance genes in drinking water collected from two First Nation communities in Manitoba, Canada across different months.

Manuscript 2: Characterization of methicillin-resistant gene, *mecA*, harboring *Mammaliicoccus fleurettii* isolated from lake water in a First Nation community, Manitoba, Canada by whole genome sequencing approach.

Acknowledgments

This study is supported by the Canadian Institutes of Health Research under its Project Grant program, and by the Natural Science and Engineering Research Council of Canada under its Collaborative Research and Training Experience Program. Firstly, I would like to thank two First Nation communities for their partnership. I want to say thank to Chief and Council from each community and the community members whose support made this research possible.

I am grateful to my supervisor, Dr. Ayush Kumar, and co-supervisor, Dr. Annemieke Farenhorst for their guidance and supervision throughout my research. I am also grateful to my advisory committee Dr. Deborah Court and Dr. Gregg Tomy for their constructive comments towards my research. I want to acknowledge Dr. George Golding for helping us characterization of *Mammaliicoccus fleurettii* isolates.

I am also thankful to Rakesh Patidar, Anita Murdock, Kristy Anderson, and Ruidong Mi for fieldwork as well as laboratory work. I would like to give special thanks to Alvan Wai for helping me in computational analysis and Geethani Eragoda Arachchilage, Mostafizur Rahman as well as Sirajum Munira Rumi for guiding me in statistical analysis. Finally, I want to thank my all-lab members (Yane Lis Acebo, Soumya Dao, Yanqi Li, Vanessa Kornelsen, Ellen Sykes, Tajinder Kainth, Debjyoti Thakur) and my beloved husband, Md. Deen Islam, for continuously supporting me during this period.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

1.0 Introduction

1.1 Water is a basic human right

Water is called 'life' because it is indispensable for the maintenance of life. This natural resource covers approximately two-thirds of the globe's surface of which merely 2.6% is freshwater.[1] However, accessible surface water is only 0.3% of all freshwater on this planet. As a result, there is a critical shortage of accessible water across the world and a considerable amount of available freshwater is not suitable for use because of poor quality.[1]

Many countries face a lack of adequate water for necessary demands whereas many others have sufficient water but a large proportion is unsuitable for drinking.[2] Both situations are considered violations of human rights as the United Nations declared in 2010 that safe and clean running drinking water is a fundamental human right.[3] Above all, poor quality water or insufficient amounts of water not only affects the health but also the social status of a community. Therefore, access to water in an adequate amount and good quality is critical to ensure the quality of life in the form of improvement in living conditions and health benefits to individuals and their communities.

According to the World Health Organization (WHO), the basic needs of clean water per person per day are between 50 – 100 liters.[4] Also, the United Nations Development Program recommends that household water costs should not go beyond 3% of a household's yearly income.[4] However, many First Nation communities in Canada spend significantly more for household water costs. For example, a previous study showed that 10% of people living in eight First Nation reserves in the Province of Saskatchewan spent high monthly expenses on bottled water (more than $50 per month), as well as that members of some communities in the Province of Manitoba are unable to pay for the expense of refilling their water cistern (approximately $25 per refill).[5,6] Human

Rights Watch (2016)[7] continues to convey concerns about the lack of access to adequate water and sanitation systems in First Nation reserves of Canada.

1.2 Colonial history and Indigenous water law in Canada

The great mass of immigrants' settlement into Canada was opened by the agreement between the Royal Proclamation issued by the British Crown and Indigenous peoples in Canada in 1763. This agreement involved mentioning the rights and protection of Indigenous peoples but also enabled European Settlers to occupy land while establishing Treaties between Indigenous Nations and the British Crown.[8] Moreover, such actions led to the forced settlement of Indigenous Peoples on small reserves and European Settlers to occupy prime agricultural lands. In 1876, the *Indian Act* was created by the Federal government through which undesirable political and education systems were imposed for Indigenous Peoples[9], causing prolonged negative consequences for Indigenous people such as carrying the trauma of residential schools from generation to generation.[10] In 1991, the *Royal Commission on Aboriginal Peoples* (RCAP) established by the Federal government examined solutions for improving relationships between the government and Indigenous Peoples.[12] More recently, *the United Nations Declaration on the Rights of Indigenous Peoples* (UNDRIP) was accepted by the Federal Government in 2017 to recognize the Rights of Indigenous Peoples, and respect the need for renewed cooperative partnerships with Indigenous Peoples. As well, the Truth and Reconciliation Commission of Canada (TRC) resulted in 94 Calls to Actions to address the legacy of residential schools as well as advance the process of reconciliation between Settlers and Indigenous Peoples.[12,13]

Manitoba is situated on the lands of the Ojibwe (Anishinaabe), Cree, Oji-Cree, Dakota, and Dene Peoples.[14] Individual Nations had their own laws as well as legal systems. For example, according to the principle of Anishinaabe water law, water has a spirit, women are responsible for water and

people must respect the water.[15] Canada has the responsibility to respect the Rights of Indigenous Peoples to maintain and improve their spiritual relationship with water and other resources, and to maintain their duties to future generations in this concern.[16] However, due to colonialism, First Nations have long been severely restricted from practicing Indigenous Laws and in act faced penalties while exercising traditional laws.

In 1919, the City of Winnipeg implemented a project to withdraw water from Shoal lake and transport it to Deacons Reservoir from where the water could be distributed into the City of Winnipeg to provide for clean, running drinking water for its residents. This project occupied 3,000 acres from Shoal Lake First Nation to build an aqueduct on First Nation land, as well as caused flooding to First Nation land thereby forced the relocation of Shoal Lake First Nation to a human-made island without facilities such as a drinking water treatment plant.[17,18] Consequently, Shoal Lake 40 First Nation has been under a boil water advisory for 23 years.[19] A substantial number of other First Nations reserves in Canada are dealing with insufficient and unsafe drinking water.[20]

1.3 Waterborne disease and public health

About one-third of waterborne diseases result from infections with intestinal pathogenic bacteria.[21] For that reason, *E. coli* and/or total coliforms are commonly used as indicator bacteria to evaluate the presence of fecal contamination and waterborne pathogens in drinking water.[3,22] *E. coli* is considered a more suitable indicator than other coliforms as they are widely dispersed in different natural environments whereas *E. coli* is more specifically associated with fecal contamination.[23] According to “Guidelines for Canadian Drinking Water Quality” by Health Canada, the count for total coliform and *E. coli* should be 0 per 100 mL for treated water.[24]

According to WHO (2011),[3] pathogenic microorganisms causing infectious diseases are most frequently associated with health risks associated with drinking water. It is reported that waterborne pathogenic bacteria with high (negative) health significance in water supplies are *Campylobacter*, *E. coli*, *Legionella*, *Salmonella*, *Shigella*, *Yersinia enterocolitica* as well as *Vibrio cholera*e. Typical waterborne pathogens can persist in drinking water, but most are not able to grow or proliferate in water. For example, *E. coli* and *Campylobacter* can persist in water by multiplying in sediments and mobilizing when the water stream raises.[3]

Pathogenic bacteria can enter the water distribution networks through human as well as other animal feces contamination which can result in increased health risks to humans. For instance, *Vibrio cholerae* contamination in drinking water, whose incidence is mostly limited to resource-poor countries, causes 21,000 to 143,000 deaths annually worldwide.[25] However, water-borne illnesses can occur in developed countries as well. For example, in Canada, *E. coli* contamination in drinking water distribution systems of Walkerton, Ontario, caused an outbreak of gastroenteritis with several fatal cases.[26,27]

The insufficient access to clean drinking water on First Nations reserves is believed to be a crucial factor in several health conditions such as respiratory, gastrointestinal, and skin infections.[5,28,29] Lack of running water in First Nations homes in the Island Lake regions accelerated the 2009 influenza A (H1N1) epidemic in northern Manitoba.[30,31] The health inequalities First Nations Peoples experience in Canada are the outcome of colonial and discriminatory practices,[32] including the lack of addressing the drinking water crisis in many First Nations reserves in Canada.

1.4 Drinking water contamination at different water distribution points

There are several barriers to preventing clean, running drinking water in homes, and could include the quality of source water and the distribution system used to provide the final water supply to the consumers. The protection of these various barriers in drinking water supplies is important because it secures the microbiological quality of drinking water. Protecting drinking water supplies include the protection of water sources such as river water, lake water, etc., and having sufficient resources for maintenance of various operation steps throughout the water treatment process, and for the distribution system of treated water.

Feces are considered as a source of different pathogenic microorganisms, and the feces of domestic and wild animals can contaminate source water, as well as land surfaces, and enhance the microbial risk to humans.[3] The intrusion of bacterial contaminants in a pipeline may happen during repair or maintenance, through broken pipes and pipe joints known as a gasket, and cross-connections with non-portable water systems.[33,34] According to Geldreich (1990),[35] broken gaskets can be a pathway for a variety of heterotrophic bacteria in the distribution network. Moreover, natural disasters may cause the invasion of microbial contaminants into the water distribution points.[33]

According to Health Canada, the majority of Drinking Water Advisories (DWAs) in Canada are Boil Water Advisories indicating the contamination of water with fecal bacteria.[36] According to Health Canada, DWA is a precautionary measure executed to shield public health when drinking water could be contaminated. On basis of the severity and nature of the problem, DWAs can be categorized into three groups:

- *Boil water advisory*: Water needs to be boiled for drinking purpose.

- *Do not consume advisory*: Water cannot be consumed.
- *Do not use advisory*: Water cannot be used or consumed.

There are several standard methods to reduce or remove the microbial load in water. Among these, chlorination is the most frequently used on First Nation reserves. It is an effective disinfection method to treat source water and kill pathogenic bacteria to provide safe drinking water for the end-user.[37] In previous studies it was observed that bacteria from source water can be effectively eliminated at the water treatment plants (WTPs) but the drinking water from taps in homes was found to be of poorer quality than the treated water leaving the WTPs.[38,39] This indicates that there is a likelihood of bacterial invasion or re-growth of bacteria during water distribution. The reason behind pathogen ingress in water distribution systems may be inadequate amounts of disinfectant residuals to overcome contamination.[3]

Water quality failure at water distribution points is alarming as the quality of drinking water is directly linked to human health. As a preventative measure, filtering devices can be used by consumers, but such filters are only effective for certain contaminants. In First Nation communities in Canada, the common distribution systems to homes include piped water from the WTP, or water truck delivery to an underground or above ground cistern associated with the home. In some communities, some homes depend on well water or have no running water.

For truck delivery to cisterns, microbial contamination may take place at various points throughout the transportation process.[4] Thus daily water testing and weekly disinfection is needed for water delivery truck to avoid contamination.[4] Also, during the filling of cisterns by trucks, microbial contamination could occur when the hoses of the water truck may have come in contact with the ground.[40,41] As well, cisterns can be contaminated by bacteria by entering through cracks in the cistern wall, improperly fitted materials, or through the entrance of insects into the lids.[40]

Moreover, heavy rainfall or snowmelts during spring can generate flooding causing contamination of water in cracked underground cisterns.[42]

Although the pipeline networks or cisterns are deficient in nutrients and have residual chlorine concentration, bacteria can grow both in water and as a biofilm.[39,43] The bacteria in a biofilm can aggregate themselves on various surfaces (such as pipe and cistern wall) and grow together to high densities, which then gives them better protection from the adverse environment.[44,45] There are three factors that aid to biofilm formation in water distribution systems. Firstly, when bulk water contains dissolved nutrients, this can support bacteria growth; secondly, suspended solids in water can act as attachable surfaces for bacteria; and finally, suspended solids can form aggregates that settle in pipes or cisterns.[39] Biofilms can cause the constant presence of bacteria in drinking water distribution systems and release free bacterial cells.[46,47] According to Flemming *et al*. (2002),[48] 95% of bacteria identified in drinking water distribution systems are in biofilms. Therefore, biofilm on surfaces within water distribution networks may result in microbiological contamination in drinking water and ultimately deterioration of hygienic drinking water quality.

1.5 Surface or source water contamination

Several reports are focusing on the inequitable opportunity to safe drinking water for many First Nation reserves in Canada but there are very few that draw attention to the importance of Source Water Protection (SWP) to advance access to clean and safe drinking water for First Nation communities.[49] The Canadian Council of Ministers of the Environment (CCME) delineates a multi-barrier strategy to check or decrease the contamination level of drinking water to protect public health.[50] CCME illustrates three basic components in this strategy that begins with source water protection such as protection of water of rivers and lakes, followed by monitoring the continual steps of drinking water treatment, and attention towards the drinking water distribution

systems that could be either piped or other systems. SWP not only focuses on reducing the risk of microbial contamination at the water source[37] but also creates cost-effective senses by reducing the costs and challenges.[51-54] According to British Columbia Drinking Water Protection Act, source water evaluations are aimed to detect possible risks to drinking water quality followed by developing response plans to diminish possible risks associated with public health.[55]

According to Center for Disease Control and Prevention (CDC) report, 79% of water source outbreak-related cases are associated with surface water (i.e., river, lake, etc.).[56] The surface water is not only used as a source of drinking water but also as recreational water and many other things such as commercial and subsistence fishing. The bacterial quality of recreational waters is assessed by the same indicators recommended for drinking water, i.e., *E. coli* and total coliforms that indicate the presence of enteric bacteria in drinking water. But infections from recreational waters are not restricted to enteric diseases but extend to different organs resulting from microbes that are not necessarily the residents of the intestinal tract of animals. The predominated illnesses by recreational outbreaks are acute gastrointestinal illness, acute respiratory illness, skin diseases, etc.[57] Majority of swimming-related illnesses are transmitted by contact rather than ingestion.[58]

Several studies have shown a relationship between bacterial load and anthropogenic actions. One prominent bacterium is *Staphylococcus* spp. that have been reported both in wastewater and recreational water. S. *aureus* is an important cause of skin infections from recreational waters such as swimming pools, lakes, or rivers.[59-61] Moreover, Plano *et al*. (2013),[62] demonstrated a correlation between the number of bathers and density of *S. aureus* in a sub-tropical recreational water indicating people as a possible source of staphylococci in the water reservoir.

1.6 Antibiotic-resistant genes (ARGs) and common mechanisms of their spread in water bodies

Antibiotics are commonly employed to prevent or treat pathogenic bacterial infection in humans and animals and most of these antibiotics are excreted unmodified from the animal or human body in the environment. 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.[5] According to Rysz and Alvarez (2004),[64] ARGs are considered as 'pollutants' for the environment as they are extensively dispensed throughout diverse compartments of the environment such as wastewater, surface water, groundwater, drinking water, etc. There are several mechanisms for resistance to antibiotics. The environment can act as an antibiotic resistance pool under the selective pressure of antibiotics by the bacterial acquisition of resistance genes through different mobile genetic elements or rising mutation in their genes to adapt to the environment.[65] A summary of several bacterial antibiotic resistance mechanisms arising in water bodies are provided below:

1.6.1 Mutations causing the emergence of antibiotic resistance in water bodies

Mutations are regarded as the most significant strategy in the perspective of bacterial antibiotic resistance mechanism in water habitats and there is a possibility for selection of mutations at a higher rate because of the residuals of antibiotics in water bodies. For example, mutations in the genes encoding penicillin-binding proteins can cause a reduced affinity towards β-lactam antibiotics conferring bacterial β-lactam resistance.[66] Moreover, diverse levels of point mutations in ribosomal proteins give resistance to different antibiotics such as aminoglycosides, tetracyclines, and macrolides.[67] It has been previously reported that even lower concentrations of antibiotics can render less susceptible bacteria into resistant microorganisms resulting in a high frequency of resistant microorganisms in environmental habitats.[68]

1.6.1.1 Chromosomally Encoded Cephalosporinase

Gram-negative bacteria can resist β-lactam antibiotics by producing hydrolytic enzymes, β-lactamases. Jacoby (2009)[69] has described one of the most promising β-lactamases encoding genes, *ampC* are localized on the chromosome of bacteria are broadly dispensed in environmental bacterial species, for example, *Aeromonas* spp., various *Pseudomonas* spp. and numerous Enterobacteriaceae.

1.6.1.2 DNA Gyrase and Topoisomerase

Bacterial resistance to fluoroquinolones results from mutations in the quinolone resistance determining region (QRDR) within DNA gyrase encoding genes, *gyrA* and *gyrB* as well as topo-isomerase IV encoding genes, *parC*, and *parE*. Figueira *et al*. (2011)[70] reported mutations in *gyrA* and *parC* associated with *Aeromonas* spp. isolated from an urban effluent as well as *E. coli* collected from a wastewater effluent responsible for ciprofloxacin resistance.

1.6.1.3 Efflux pumps

There are five major families of efflux pumps that are linked to drug resistance, from which the resistance nodulation division (RND) families are found only in Gram-negative bacteria. Point mutations in a regulator or the promoter sequence of the RND efflux operon can be the reason for the over-expression of the pump and the development of antibiotic resistance. Hernandez *et al.* (2011)[71] demonstrated a connecting relationship between water pollution by antibiotics or different contaminants such as metal, could select the bacterial expression or over-expression of efflux pumps. The author demonstrated the over-expression of SmeDEF pump in aquatic species, *Stenotrophomonas maltophilia*, leading to multi-drug resistance and might cause nosocomial infections.

1.6.2 Acquisition of genes in water habitats responsible for antibiotic resistance

Acquisition of antibiotic resistance genes is facilitated under strong selective pressures of antibiotics. Exchange of this foreign DNA in bacteria may be conducted through mobile genetic elements such as plasmids, phages, transposons, or incorporating of free DNA by the transformation. Plasmids carrying resistance gene can be transferred from cell to cell at a lower rate in non-clinical environments such as surface water.[72]

1.6.2.1 Integron

Integrons are bacterial genetic elements that work as platforms for capturing or excising genes in or from gene cassettes and localizing the gene cassettes on mobile genetic elements, for example, plasmids and/or transposons which help to disseminate numerous antibiotic resistance genes leading to multidrug-resistant. Gillings *et al.* (2008)[73] proved that clinical class 1 integrons originated from environmental bacterial communities by localizing on a Tn402-like transposon followed by dispensing in commensal and pathogenic bacteria. Moreover, Wright *et al.* (2008)[74] demonstrated that loads of integrons in bacterial communities from water bodies are possibly linked to the level of antibiotic pollutions in water habitats.

1.6.2.2 Phages

The abundance of phages is higher in the water body than bacterial abundance and phages are likely to perform a vital part in horizontal gene transfer.[75,76] Colomer-Lluch *et al.* (2011)[77] reported the occurrence of bla_{TEM} and $bla_{CTX\text{-}M}$, the most widespread β-lactamase genes responsible for resistance towards β-lactams in Enterobacteriaceae and *mecA*, responsible for methicillin resistance in *Staphylococcus* spp., in phage DNA obtained from a wastewater treatment plant and a river. However, the presence of *mecA* in the phage present in freshwater is of enormous concern

because methicillin-resistant *Staphylococcus aureus* (MRSA) infections are considered a public health threat to the population.[78]

Several studies have found evidence of ARGs in water samples including *bla* genes (encoding β-lactamase and responsible for bacterial resistance to beta-lactam antibiotic).[79,80] 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.[80,81]

β-lactamase and carbapenemase encoding genes have been observed in various aquatic reservoirs across the world.[82] β-lactams and carbapenems are widely used to treat infections in the human and veterinary field,[65,83] with carbapenems mostly being considered as the last resort for treating antibiotic-resistant pathogens. The presence of ARGs mentioned above in pathogenic bacteria can cause a major threat to the health of humans or animals exposed to such bacteria.[84,85] Therefore, a lower number of environmental bacteria containing β-lactamase and carbapenemase genes is concerning.

Moreover, methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant concern worldwide as a nosocomial pathogen, but knowledge regarding its frequency in natural environments such as surface water is limited, and little is known about the role surface water plays in the spreading and development of MRSA. In most strains, *mec*A is normally located in the chromosome of *Staphylococci* spp. and is associated with a mobile genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) which is capable of moving horizontally among staphylococcal species.[86] SCC*mec* elements are not restricted to MRSA and have also been reported in several Coagulase-negative methicillin-resistant staphylococcal species (MRS).[87] MRS strains have evolved autonomously by transporting the *mec*A gene into diverse strains of methicillin-resistant *Staphylococcus* spp. Though several incidences of clinical MRS have been

reported, there is still a study gap on the role of the aquatic environment as a pool of *mecA* carrying staphylococci. Also, our previous study observed a high copy number of *mecA* gene in lake water from a First Nation community in Manitoba.[88] For that reason investigation regarding the occurrence of MRS and *mec*A in environmental water needs to be more studied.

1.7 Cultural and molecular techniques are used for the detection and characterization of bacteria in water habitats

Pollution of water habitat with water-borne pathogens poses a major health risk to humans using this water for drinking or recreational purposes. *E. coli* or other coliforms such as *Klebsiella, Citrobacter,* and *Enterobacter* are known as fecal bacteria are found in the intestine of humans and animals and are discharged into the environment through faces. That is why these bacteria are commonly used as indicators of fecal contamination which can influence water bodies. For health risk accessing and monitoring acceptable bacterial limits for fecal coliform is important to measure.

Feng and Hartman (1982)[89] described a culture-based method that includes the use of microbial enzyme profiles to detect *E. coli* and coliforms for monitoring drinking water quality. This involves chromogenic substrates that can be cleaved by β-D-glucopyranosiduronic and galactosidase. This enzyme, β-D-glucopyranosiduronic, is present in more than 97% of the strains of *E. coli* species whereas galactosidase enzyme is found in the majority of coliforms.[90] Commercial product, BRILLIANCE E. COLI/COLIFORM AGAR (CM0956, Oxoid), based on this method giving purple reaction results in *E. coli* colonies as it can produce both β-D-glucopyranosiduronic and galactosidase to cleave chromogenic substrates (X-Glu and Rose-Gal) and pink coliform colonies as coliforms are only able to produce galactosidase to cleave the galactosidase chromogen (Rose-Gal).[91]

Though historically culturing cells on defined media is a common approach to detect specific bacteria in the field of food microbiology and environmental microbiology, it cannot detect stressed cells or viable but non-culturable cells.[92] Bacteria become stressed during mobilization from the intestinal habitat to the natural environment which reduces their ability to be cultured. Therefore, molecular approaches are superior to cultural approaches for identifying bacteria from environmental samples. For example, nowadays next-generation sequencing (NGS) is being broadly used not only for detecting bacterial taxa but also for whole metagenomics study. Another culture-independent method is the quantitative real-time PCR (qPCR) method targeting 16S rDNA and/or 23S rDNA for the detection of bacterial pathogens from environmental habitat. This method was first granted by EPA to evaluate microbiological water quality targeting *Enterococcus* spp.[93] While qPCR assay for water quality evaluation is suitable for quantifying contact to biological threats, application of NGS or high-throughput sequencing can be an initial footstep to spotlight on additional explicit exposure evaluation of appropriate objects. Thus, for getting a more comprehensive illustration of microbial compositions in drinking water, several studies have used high-throughput sequencing.[88,94-98]

1.8 Molecular approaches for the detection and characterization of ARGs from the environment

PCR assays have been extensively utilized in different water samples for revealing definite ARGs. One obstacle to work with water samples is a low concentration of DNA or RNA of interest. This can be overcome by using PCR-based methods which amplify nucleic acids although a false-positive outcome is not uncommon in this method.

The cost-effective multiplex PCR is regarded as a rapid method for the identification of multiple ARGs from target DNA. The main advantages of using multiplex PCR are: 1) it saves time; 2) it

reduces the cost; 3) it can amplify the isolated DNA target containing multiple ARGs by using different primer pairs at the same time in a single reaction.[99] Several studies have used multiplex PCR to detect more than one target ARGs from environmental samples.[88,100] Despite the advantages pointed out above, there are several drawbacks regarding this method such as 1) generation of false-negative results because of inhibition of DNA amplification; 2) reduced sensitivity due to primer-dimer formation .[101]

The hurdle of cultural and molecular assays has been overcome by the advances of sequence-based metagenomics and functional metagenomics which give an enhanced understanding of the categories of ARGs in specific bacteria or bacterial communities in environmental samples and their co-occurrence with various mobile genetic elements including plasmids or different mobile jumping elements. In this sequence-based approach, whole DNA from a water sample is directly extracted and arbitrarily sequenced. The NGS datasets offer a vital step to promote in assisting extensive environmental studies to evaluate the emerging threat enforced by antibiotic resistance. Several metagenomic analysis tools have been enlarged to extract bigger and additional open reference sequence databases for ARG detection such as Comprehensive Antibiotic Resistance Database (CARD).[102]

1.9 Drinking water advisory in First Nation communities, Canada

Lack of access to clean drinking water among First Nation communities living on reserves is not uncommon in Canada which indicates a violation of basic Human Rights to safe, running drinking water. Although many Canadians have sufficient access to clean and safe potable water, there is several evidence that the tap water of households in First Nations reserves contain contaminants, particularly in homes without running water (and need to utilize buckets to store household water)

or having cisterns.[88,96,103-105] The Canadian media has reported on the lack of clean tap water in homes on First Nations reserves.[106,107]

Neegan Burnside Ltd. (2011)[108] reported that 72% of First Nation community homes in Canada have piped water systems that are connected to a water treatment plants (WTPs) whereas 13.5% of the cistern-based homes depend on drinking water delivery by trucks. The other 14.5% of homes either rely on individual well water or do not have running water. Neegan Burnside Ltd (2011)[108] also reported that 39% of water systems on reserves are at high overall risk. According to Indigenous and Northern Affairs Canada Risk Level Evaluation Guidelines (INAC), which is now known as Indigenous Services Canada (ISC) and Crown-Indigenous Relations and Northern Affairs Canada (CIRNAC), high overall risk means the water systems have major deficiencies leading to water quality parameters to exceed the maximum acceptable concentrations of substances as defined by the Guidelines for Canadian Drinking Water Quality and that immediate counteractive step must be taken to minimize the deficiencies to avoid human health risks or environmental risks.[108] First Nations reserves situated south of 60º N, drinking water responsibilities are shared among the Federal government (through Health Canada, as well as ISC) and First Nation Band Councils. For example, water treatment plants and distribution systems are expected to be maintained by Band councils whereas funding for the construction or advancement of water treatment plants, as well as some of the maintenance and operating costs are regulated by ISC. Health Canada is liable for water quality monitoring programs on First Nations reserves that ultimately decide whether a community will be under drinking water advisory or not. Unlike provinces and territories, reserves do not have legally binding drinking water regulations. First Nation reserves do not have legally drinking water regulations, which are common in provinces.[109]

Based on the water quality of a water treatment plant in a community, DWAs are categorized as short-term DWAs and long-term DWAs. A short-term DWAs represent a temporarily water quality issue on a particular water system whereas long-term DWAs are in place for more than 1 year. As of June 17, 2020, there were about 24 short-term DWAs in place including short-term boil water advisories in 4 First Nation communities in Manitoba.[19,110] As of March 9, 2021, 58 long-term DWAs remained in First Nation reserves in place because of the unsafe water quality issues across Canada.[19] In Manitoba, there are 3 long-term boil water advisories active in First Nation communities. Although the Trudeau government had committed termination of these long-term water advisories by March 2021 it has become clear that this promise will not be met.[19]

Although a drinking water advisory is most commonly based on the quality of water in WTP, multiple studies showed that water contamination happens after water released from WTP.[88,96,111] Survey data from different studies reported that individuals in First Nations reserves have less expectation of having safe water in homes, relative to individuals living in non-Indigenous communities.[28,112] In another survey completed in a fly-in First Nation reserve in Manitoba, individuals in homes with cisterns expressed concerns about both the safety and the accessibility of their tap water.[6] Moreover, Anderson *et al.* (2020)[113] did a door-to-door survey on three First Nation communities in Manitoba that showed that the perceptions of community members regarding the safety of their household water were dependent on the type of water distribution systems available in the homes. This study reported that homes with cisterns had considerably greater water safety concerns than homes with piped water.

1.10 Objectives and hypothesis

Concerning bacterial and ARGs contamination in drinking water of First Nation communities in Canada, our research group previously studied fecal coliforms and ARGs in drinking water in

different First Nation communities in Manitoba, Canada.[88,96,111] In our present research, the purpose is to evaluate the microbiological quality of drinking water in a wider range of First Nation homes in different communities in Manitoba, Canada. We hypothesized that the microbiological quality of the source and drinking water in First Nation communities shows the contamination of fecal bacteria and antibiotic resistance genes (ARGs) and that this contamination can vary between months, as well as between water distribution systems (piped versus cistern).

In the first study (Chapter 2), water samples from different water distribution systems in two First Nation reserves (referred to as communities B and D) were screened for free residual chlorine concentrations, the number of fecal bacteria (total coliforms, *E. coli*), and the presence of beta-lactamase and carbapenemase genes in drinking water. The objective of this study was to examine within each community, the impact of the type of water distribution systems and temporal effect on fecal bacteria and ARGs in the tap water homes. The hypothesis was that the microbiological quality of drinking water differs among different water distribution systems.

The second study (Chapter 3) focused on community D only, which is in Island Lake Region of Manitoba, and for which a previous study detected the presence of *mecA* in lake water.[88] Expression of *mecA* is usually associated with methicillin-resistant *Staphylococcus* spp. which represents a significant clinical problem. That is why the objective of this study was to find out the occurrence of *mecA* carrying *Staphylococcus* spp. from lake water and to carry out molecular and antimicrobial resistance characterization of the isolated isolates by whole genome sequencing approach. The hypothesis of this study was *mecA* gene contamination in lake water is originated from methicillin-resistant *Staphylococcus* spp.

1.11 References

1. Maran de Oliveira, C. Sustainable access to safe drinking water: fundamental human right in the international and national scene. *An Interdiscip. J. Appl. Sci.* **12**, 985–1000 (2017).

2. Gómez, P. *et al.* Characterization of staphylococci in urban wastewater treatment plants in Spain, with detection of methicillin resistant *Staphylococcus aureus* ST398. *Environ. Pollut.* **212**, 71–76 (2016)..

3. World Health Organization. *Guidelines for drinking-water quality, fourth edition*. (2011).

4. United Nations. *The Right to Water Fact Sheet No.35. Geneva, Switzerland: Office of the United Nations High Commisioner for Human Rights*. Retrieved February 22, 2021, from United Nations: https://www.ohchr.org/Documents/Publications/FactSheet35en.pdf. (2010)

5. Waldner, C. L. *et al.* Self-reported Effects of Water on Health in First Nations Communities in Saskatchewan, Canada: Results From Community-Based Participatory Research. *Environ. Health Insights* **11**, (2017).

6. O'Gorman, M. & Penner, S. Water infrastructure and well-being among First Nations, Métis and Inuit individuals in Canada: what does the data tell us? *Environ. Sci. Pollut. Res.* **25**, 33038–33055 (2018)

7. Human Right Watch. Canada's Obligation to End the First Nations Water Crisis. https://www.hrw.org/report/2016/06/07/make-it-safe/canadas-obligation-end-first-nations-water-crisis (2016).

8. Daschuk, J. *Clearing the Plains*. *University of Regina press,* 11-77 (2013).

9. Henderson, W. B. Indian Act - The Canadian Encyclopedia. *Historica Canada* https://www.thecanadianencyclopedia.ca/en/article/indian-act (2006)

10. Aguiar, W., & Halseth, R. Aboriginal peoples and historic trauma: the process of intergenerational transmission. *Prince George, BC: National Collaborating Centre for Aboriginal Health*.(2015)

11. National Inquiry into Missing and Murdered Indigenous Women and Girls. Reclaiming Power and Place. Gatineau, QC. Retrieved from https://www.mmiwg-ffada.ca/final-report/.(2019)

12. Canada. *Royal Commission on Aboriginal Peoples*. https://www.thecanadianencyclopedia.ca/en/article/royal-commission-on-aboriginal-peoples (1991).

13. United Nations. *Resolution Adopted by the General Assembly on 25th September*. *General Assembley 70 session* https://www.justice.gc.ca/eng/declaration/decl_doc.html (2015).

14. Aboriginal Affairs and Northern Development Canada. First Nations in Manitoba. https://www.aadnc-aandc.gc.ca/eng/1100100020400/1100100020404 (2012).

15. Craft, A. Ki'inaakonigewin: reclaiming space for Indigenous laws. Retrieved from https://www.cerp.gouv.qc.ca/fileadmin/Fichiers_clients/Documents_deposes_a_la_Commission/P-317.pdf (2015**)**

16. United Nation. *United Nations Declaration on the Rights of Indigenous Peoples*. https://www.un.org/development/desa/indigenouspeoples/wp-content /uploads /sites/19/2018/11/ UNDRIP_E_web.pdf (2011).

17. Green, C. and Paul, A. So near, so far. *Winnipeg Free Press* https://www.winnipegfreepress.com/local/so-near-so-far-113126539.html (2011)

18. Edwards, K. 'We've been stranded on this man-made island for the past 100 years'. *Maclean's.* https://www.macleans.ca/news/canada/shoal-lake-40-first-nation-freedom-road/ (2019).

19. Government of Canada. Ending long-term drinking water advisories. *Indig. Serv.* 1–10 (2019).

20. McClearn, M. Risky water systems pose health threat to one-third of people on First Nation reserves - The Globe and Mail. *The Globe and Mail* https://www.theglobeandmail.com/news/national/indigenous-water/article31589755/ (2016).

21. Hunter, P. Waterborne disease epidemiology and ecology. *Public Health* **112**, 203 (1998).

22. Edberg, S. C., Rice, E. W., Karlin, R. J. & Allen, M. J. Escherichia coli: The best biological drinking water indicator for public health protection. *Journal of Applied Microbiology Symposium Supplement* vol. 88 (2000).

23. Gruber, J. S., Ercumen, A. & Colford, J. M. Coliform bacteria as indicators of diarrheal risk in household drinking water: Systematic review and meta-analysis. *PLoS One* **9**, (2014).

24. Health Canada. *Guidance for Providing Safe Drinking Water in Areas of Federal Jurisdiction*. (2013).

25. Ali, M., Nelson, A. R., Lopez, A. L. & Sack, D. A. Updated global burden of cholera in

endemic countries. *PLoS Negl. Trop. Dis.* **9**, (2015).

26. Clark, C. G. *et al.* Characterization of waterborne outbreak-associated *Campylobacter jejuni*, Walkerton, Ontario. *Emerg. Infect. Dis.* **9**, 1232–1241 (2003).

27. Bruce-Grey-Owen. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May-June 2000. *Can. Commun. Dis. Rep.* **26**, 170–173 (2000).

28. First Nations Information Governance Centre. National report of the First Nations regional health survey phase 3: Volume 1. Ottawa. (2018)

29. Thomas, T. K. *et al.* Impact of providing in-home water service on the rates of infectious diseases: Results from four communities in Western Alaska. *J. Water Health* **14**, 132–141 (2016).

30. Embree, J. Pandemic 2009 (a)H1N1 influenza (swine flu) - the Manitoba experience. *Biochemistry and Cell Biology* vol. 88 589–593 (2010).

31. Godbout, A. 'We're in a war zone': chief. *Winnipeg Free Press* https://www.winnipegfreepress.com/special/flu/were-in-a-war-zone-chief-47760982.html (2009).

32. Adelson, N. The embodiment of inequity: Health disparities in Aboriginal Canada. *Canadian Journal of Public Health* vol. 96 S45 (2005).

33. Sadiq, R., Kleiner, Y. & Rajani, B. Aggregative risk analysis for water quality failure in distribution networks. *J. Water Supply Res. Technol. - AQUA* **53**, 241–261 (2004).

34. Kleiner, V. Risk factors in water distribution systems, British Columbia Water and Waste Association 26th Annual Conference. *Whistler. British Columbia* 26–29 https://www.scielo.br/pdf/ea/v22n63/v22n63a02.pdf (1998).

35. Geldreich, E. E. Microbiological Quality Control in Distribution Systems. *American Water Works Association, McGraw-Hill, New York* https://www.ccme.ca/files/Resources/water/source_tap/mba_guidance_doc_e.pdf (1990) doi:10.1002/047147844x.wq491.

36. Government of Canada. About drinking water advisories. *www.sac-isc.gc.ca/* https://www.canada.ca/en/environment-climate-change/services/environmental-indicators/drinking-water-advisories.html (2019).

37. Davies, J. M. & Mazumder, A. Health and environmental policy issues in Canada: The role of watershed management in sustaining clean drinking water quality at surface sources. *J. Environ. Manage.* **68**, 273–286 (2003).

38. Verberk, J. Q. J. C., O'Halloran, K. J., Hamilton, L. A., Vreeburg, J. H. G. & Van Dijk, J. C. Measuring particles in drinking water transportation systems with particle counters. *J. Water Supply Res. Technol. - AQUA* **56**, 345–355 (2007).

39. Liu, G., Verberk, J. Q. J. C. & Van Dijk, J. C. Bacteriology of drinking water distribution systems: An integral and multidimensional review. *Appl. Microbiol. Biotechnol.* **97**, 9265–9276 (2013).

40. Baird, J. M., Summers, R. & Plummer, R. Cisterns and safe drinking water in Canada. *Can. Water Resour. J.* **38**, 121–134 (2013).

41. Duncan, L. & Bowden, M. A. A Legal guide to Aboriginal Drinking Water: A prairie Province Perspective. (2009).

42. Artiola, J., Rock, C. & Hix, G. Water Storage tank Disinfection, testing, and Maintenance. *The University of Arizona Cooperative Extension* (2012).

43. Prest, E. I., Hammes, F., van Loosdrecht, M. C. M. & Vrouwenvelder, J. S. Biological stability of drinking water: Controlling factors, methods, and challenges. *Frontiers in Microbiology* vol. 7 45 (2016).

44. Donlan, R. M. Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases* vol. 8 881–890 (2002).

45. Wingender, J. & Flemming, H. C. Biofilms in drinking water and their role as reservoir for pathogens. *Int. J. Hyg. Environ. Health* **214**, 417–423 (2011).

46. Ridgway, H. F. & Olson, B. H. Scanning electron microscope evidence for bacterial colonization of a drinking-water distribution system. *Appl. Environ. Microbiol.* **41**, 274–287 (1981).

47. LeChevallier, M. W., Babcock, T. M. & Lee, R. G. Examination and characterization of distribution system biofilms. *Appl. Environ. Microbiol.* **53**, 2714–2724 (1987).

48. Flemming, H.-C., Percival, S. L. & Walker, J. T. Contamination potential of biofilms in water distribution systems. (2002).

49. Patrick, R. J. Uneven access to safe drinking water for First Nations in Canada: Connecting health and place through source water protection. *Health Place* **17**, 386–389 (2011).

50. CCME. From Source to Tap: Guidance on the Multi-Barrier Approach to Safe Drinking Water Produced jointly by the Federal-Provincial-Territorial Committee on Drinking Water and the CCME Water Quality Task Group. www.ccme.ca (2004).

51. Chichilnisky, G. & Heal, G. Economic returns from the biosphere. *Nature* vol. 391 629–630 (1998).

52. Gullick, R. W. AWWA's Source Water Protection Committee Outlines How To Maintain The Highest Quality Source Water. *J. Am. Water Works Assoc.* **95**, 36–42 (2003).

53. Job, C. A. Benefits and Costs of Wellhead Protection. *Groundw. Monit. Remediat.* **16**, 65–68 (1996).

54. Council, N. R. Watershed Management for Potable Water Supply. Watershed Management for Potable Water Supply.*National Academies Press*.2000 doi:10.17226/9677.

55. BC Ministry of Health. Drinking Water Protection Act. (2001).

56. Liang, J. L., E. J. Dziuban, G. F. Craun, V. Hill, M. R. Moore, R. J. Gelting, R. L. Calderon, M. B. Beach, and S. L. R. Surveillance for Waterborne Disease and Outbreaks Associated with Drinking Water and Water not Intended for Drinking --- United States, 2003--2004. *MMWR Surveil. Summ.* 31–65 https://www.cdc.gov/mmwr/preview/mmwrhtml/ss5512a4.htm (2006).

57. Stevenson, A. H. Studies of bathing water quality and health. *Am. J. Public Health* **43**, 529–538 (1953).

58. Seyfried, P. L., Tobin, R. S., Brown, N. E. & Ness, P. F. *A Prospective Study of*

Swimming-Related Illness II. Morbidity and the Microbiological Quality of Water.

59. Alexander, J., Bollmann, A., Seitz, W. & Schwartz, T. Microbiological characterization of aquatic microbiomes targeting taxonomical marker genes and antibiotic resistance genes of opportunistic bacteria. *Sci. Total Environ.* **512**–**513**, 316–325 (2015).

60. Gómez, P. *et al.* Characterization of staphylococci in urban wastewater treatment plants in Spain, with detection of methicillin resistant *Staphylococcus aureus* ST398. *Environ. Pollut.* **212**, 71–76 (2016).

61. Charoenca, N. & S. Fujioka, R. Association of staphylococcal skin infections and swimming. *Water Sci. Technol.* **31**, 11–17 (1995).

62. Plano, L. R. W. *et al.* Human-Associated Methicillin-Resistant *Staphylococcus aureus* from a Subtropical Recreational Marine Beach. *Microb. Ecol.* **65**, 1039–1051 (2013).

63. Zhang, X. X., Zhang, T. & Fang, H. H. P. Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology* vol. 82 397–414 (2009).

64. Rysz, M. & Alvarez, P. J. J. Amplification and attenuation of tetracycline resistance in soil bacteria: Aquifer column experiments. *Water Res.* **38**, 3705–3712 (2004).

65. Van Hoek, A. H. A. M. *et al.* Acquired antibiotic resistance genes: An overview. *Frontiers in Microbiology* vol. 2 (2011).

66. Lambert, P. A. Bacterial resistance to antibiotics: Modified target sites. *Advanced Drug Delivery Reviews* vol. 57 1471–1485 (2005).

67. Lupo, A., Coyne, S. & Berendonk, T. U. Origin and evolution of antibiotic resistance: The

common mechanisms of emergence and spread in water bodies. *Frontiers in Microbiology* vol. 3 (2012).

68. Gullberg, E. *et al.* Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. *PLoS Pathog.* **7**, e1002158 (2011).

69. Jacoby, G. A. AmpC B-Lactamases. *Clinical Microbiology Reviews* vol. 22 161–182 (2009).

70. Figueira, V., Vaz-Moreira, I., Silva, M. & Manaia, C. M. Diversity and antibiotic resistance of *Aeromonas* spp. in drinking and waste water treatment plants. *Water Res.* **45**, 5599–5611 (2011).

71. Hernández, A., Ruiz, F. M., Romero, A. & Martínez, J. L. The binding of triclosan to SmeT, the repressor of the multidrug efflux pump smeDEF, induces antibiotic resistance in *Stenotrophomonas maltophilia*. *PLoS Pathog.* **7**, (2011).

72. Sengeløv, G. & Sørensen, S. J. Methods for detection of conjugative plasmid transfer in aquatic environments. *Curr. Microbiol.* **37**, 274–280 (1998).

73. Gillings, M. *et al.* The evolution of class 1 integrons and the rise of antibiotic resistance. *J. Bacteriol.* **190**, 5095–5100 (2008).

74. Wright, M. S. *et al.* Influence of industrial contamination on mobile genetic elements: Class 1 integron abundance and gene cassette structure in aquatic bacterial communities. *ISME J.* **2**, 417–428 (2008).

75. Weinbauer, M. G. Ecology of prokaryotic viruses. *FEMS Microbiology Reviews* vol. 28 127–181 (2004).

76. Srinivasiah, S. *et al.* Phages across the biosphere: contrasts of viruses in soil and aquatic environments. *Res. Microbiol.* **159**, 349–357 (2008).

77. Colomer-Lluch, M., Jofre, J. & Muniesa, M. Antibiotic Resistance Genes in the Bacteriophage DNA Fraction of Environmental Samples. *PLoS One* **6**, e17549 (2011).

78. Campanile, F. *et al.* Changing Italian nosocomial-community trends and heteroresistance in *Staphylococcus aureus* from bacteremia and endocarditis. *Eur. J. Clin. Microbiol. Infect. Dis.* **31**, 739–745 (2012).

79. Henriques, I. S., Fonseca, F., Alves, A., Saavedra, M. J. & Correia, A. Occurrence and diversity of integrons and β-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Res. Microbiol.* **157**, 938–947 (2006).

80. Lapara, T. M. *et al.* Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environ. Sci. Technol.* **45**, 9543–9549 (2011).

81. Marti, E., Jofre, J. & Balcazar, J. L. Prevalence of Antibiotic Resistance Genes and Bacterial Community Composition in a River Influenced by a Wastewater Treatment Plant. *PLoS One* **8**, e78906 (2013).

82. Zhang, S., Lin, W. & Yu, X. Effects of full-scale advanced water treatment on antibiotic resistance genes in the Yangtze Delta area in China. *FEMS Microbiol. Ecol.* **92**, (2016).

83. Roberts, M. C., Schwarz, S. & Aarts, H. J. M. Erratum: Acquired antibiotic resistance genes: an overview. *Front. Microbiol.* **3**, 203 (2012).

84. Yong, D. *et al.* Characterization of a new metallo-β-lactamase gene, bla NDM-1, and a

novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. *Antimicrob. Agents Chemother.* **53**, 5046–5054 (2009).

85. Ben-David, D. *et al.* Outcome of carbapenem resistant *Klebsiella pneumoniae* bloodstream infections. *Clin. Microbiol. Infect.* **18**, 54–60 (2012).

86. Ito, T. *et al.* Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **45**, 1323–1336 (2001).

87. Garza-González, E. *et al.* Diversity of staphylococcal cassette chromosome mec structures in coagulase-negative staphylococci and relationship to drug resistance. *J. Med. Microbiol.* **59**, 323–329 (2010).

88. Fernando, D. M. *et al.* Detection of antibiotic resistance genes in source and drinking water samples from a first nations community in Canada. *Appl. Environ. Microbiol.* **82**, 4767–4775 (2016).

89. Feng, P. C. S. & Hartman, P. A. Fluorogenic assays for immediate confirmation of Escherichia coli. *Appl. Environ. Microbiol.* **43**, 1320–1329 (1982).

90. Kilian, M. & Bülo, P. Rapid diagnosis of Enterobacteriaceae I. Detection of Bacterial Glycosidases. *Acta Pathol. Microbiol. Scand. Sect. B Microbiol.* **84 B**, 245–251 (1976).

91. Wohlsen, T. D. Comparative evaluation of chromogenic agar CM1046 and mFC agar for detection of *E. coli* and thermotolerant coliform bacteria from water samples. *Lett. Appl. Microbiol.* **53**, 155–160 (2011).

92. Oliver, J. D., Dagher, M. & Linden, K. Induction of *Escherichia coli* and *Salmonella typhimurium* into the viable but nonculturable state following chlorination of wastewater. *J. Water Health* **3**, 249–257 (2005).

93. Haugland, R. A. *et al.* Standardization of enterococci density estimates by EPA qPCR methods and comparison of beach action value exceedances in river waters with culture methods. *J. Microbiol. Methods* **105**, 59–66 (2014).

94. Revetta, R. P., Pemberton, A., Lamendella, R., Iker, B. & Santo Domingo, J. W. Identification of bacterial populations in drinking water using 16S rRNA-based sequence analyses. *Water Res.* **44**, 1353–1360 (2010).

95. Lin, W., Yu, Z., Zhang, H. & Thompson, I. P. Diversity and dynamics of microbial communities at each step of treatment plant for potable water generation. *Water Res.* **52**, 218–230 (2014).

96. Farenhorst, A. *et al.* Bacteria in drinking water sources of a First Nation reserve in Canada. *Sci. Total Environ.* **575**, 813–819 (2017).

97. Li, L. G., Xia, Y. & Zhang, T. Co-occurrence of antibiotic and metal resistance genes revealed in complete genome collection. *ISME J.* **11**, 651–662 (2017).

98. Su, H. C. *et al.* Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: From drinking water source to tap water. *Sci. Total Environ.* **616**–**617**, 453–461 (2018).

99. Gilbride, K. A., Lee, D. Y. & Beaudette, L. A. Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control.

Journal of Microbiological Methods vol. 66 1–20 (2006).

100. Ramachandran, D., Bhanumathi, R. & Singh, D. V. Multiplex PCR for detection of antibiotic resistance genes and the SXT element: Application in the characterization of *Vibrio cholerae*. *J. Med. Microbiol.* **56**, 346–351 (2007).

101. Markoulatos, P., Siafakas, N. & Moncany, M. Multiplex polymerase chain reaction: A practical approach. *J. Clin. Lab. Anal.* **16**, 47–51 (2002).

102. McArthur, A. G. *et al.* The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* **57**, 3348–3357 (2013).

103. McKeown, I. *et al. Helicobacter Pylori* in The Canadian Arctic: Seroprevalence and Detection in Community Water Samples. *Am. J. Gastroenterol.* **94**, 1823–1829 (1999).

104. Bernstein, C. N. *et al.* Seroprevalence of *Helicobacter pylori*, incidence of gastric cancer, and peptic ulcer-associated hospitalizations in a Canadian Indian population. *Dig. Dis. Sci.* **44**, 668–674 (1999)

105. Jones, N. L. *et al. Helicobacter pylori* in First Nations and recent immigrant populations in Canada. *Canadian Journal of Gastroenterology* vol. 26 97–103 (2012).

106. Stastna, K. Clean running water still a luxury on many native reserves. *CBC News* https://www.cbc.ca/news/canada/clean-running-water-still-a-luxury-on-many-native-reserves-1.1081705 (2011).

107. Gerster, J. & Hessey, K. Why some First Nations still don't have clean drinking water — despite Trudeau's promise - National | Globalnews.ca. *Global News* https://globalnews.ca/news/5887716/first-nations-boil-water-advisories/ (2019).

108. National Assessment of First Nations Water and Wastewater Systems National Roll-Up Report FINAL *Department of Indian Affairs and Northern Development*. www.neeganburnside.com (2011).

109. Dunn, G., Bakker, K. & Harris, L. Drinking Water Quality Guidelines across Canadian provinces and territories: Jurisdictional variation in the context of decentralized water governance. *Int. J. Environ. Res. Public Health* **11**, 4634–4651 (2014).

110. Government of Canada. Short-term drinking water advisories. *Indigenous Services Canada* https://www.sac-isc.gc.ca/eng/1562856509704/1562856530304 (2019).

111. Mi, R. *et al.* Detection of fecal bacteria and antibiotic resistance genes in drinking water collected from three First Nations communities in Manitoba, Canada. *FEMS Microbiol. Lett.* **366**, (2019).

112. White, J. P., Murphy, L. & Spence, N. Water and indigenous peoples: Canada's paradox. *Int. Indig. Policy J.* **3**, (2012).

113. Anderson, K. L. Perception of household drinking water across a variety of water distribution systems in three First Nations in Manitoba. *University of Manitoba.* (2020).

Chapter 2: Investigation of the presence of fecal bacteria and antibiotic resistance genes in drinking water collected from two First Nation communities in Manitoba, Canada, across different months.

2.0 Investigation of the presence of fecal bacteria and antibiotic resistance genes in drinking water collected from two First Nation communities in Manitoba, Canada, across different months.

2.1 Abstract

Water distribution systems are considered as the final linkage for guaranteed high-quality drinking water for consumers but bacterial as well as antibiotic resistance genes (ARGs) contamination in water may pose a significant human health risk. As there are few studies conducted regarding bacterial and ARG contamination in water distribution systems of First Nation reserves in Canada, we targeted to measure free chlorine concentration, fecal bacteria, and ARGs throughout water distribution systems of two First Nation reserves in Manitoba, Canada. In community B, a high count of fecal bacteria with free chlorine concentration below the recommended level of 0.2 mg/L, were observed in cistern samples whereas this was not observed in samples from the pipeline. Fecal bacterial counts were higher in warmer months than colder months. Fecal bacteria were observed in only one cistern sample from community D, but not at other water distribution points. *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{OXA-1}$, *bla*$_{CMY-2,}$ and *bla*$_{OXA-48}$ were detected in cistern samples but not in pipeline samples in community B. In community D, *bla*$_{TEM}$ has been detected both in cistern and pipeline samples which had <0.2mg/L of free chlorine concentration. This observation concludes that families living in homes with cisterns are more likely to be exposed to fecal bacteria and ARGs than homes directly connected with water treatment plant by pipeline and maintaining a recommended level of free chlorine concentration in water distribution systems is required to prevent bacterial and ARGs contamination.

2.2 Introduction

Household access to sources of safe and reliable drinking water crucial to human health and it is considered a basic human right.[3] For many developing countries one of the most urgent problems is inaccessibility to safe and clean drinking water. Unfortunately, lack of access to safe drinking water also remains a problem for many Indigenous communities in North America. Drinking water advisories (DWAs) are common in First Nations communities across Canada. The majority 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.[9]

About one-third of waterborne diseases result from infections with intestinal pathogenic bacteria.[10] *E. coli* and/or coliforms are commonly used as indicator bacteria to evaluate the presence of fecal contamination and waterborne pathogens in drinking water.[3,11] According to Health Canada's "Guidelines for Canadian Drinking Water Quality", treated water is safe for drinking if it contains 'zero' *E. coli* and/ coliforms count per 100 mL of water.[12]

Chlorine is an effective disinfection method because it kills pathogenic bacteria and prevents their growth. According to LeChevallier *et al.* (1996), treated water released from the water treatment plant (WTP) should maintain ≥ 0.2 mg/L of free residual chlorine concentration throughout the water distribution systems to prevent bacterial re-growth.[13] In a previous study from our group, samples were collected from various water distribution points of different First Nations communities in Manitoba that use surface or groundwater as their source water for the drinking WTP.[6–8] Most of the water samples collected from homes with cisterns showed the presence of coliforms and *E. coli* with free residual chlorine concentrations below the recommended level of 0.2 mg/L. Moreover, different types of β-lactamase and carbapenemase genes were also observed

in these samples. Surface water is considered the pool of antibiotic resistance genes (ARGs) and failure of ARGs carrying bacteria during WTP can lead to the entrance of ARGs in drinking water distribution systems.[14,15]

Based on the findings from our previous studies,[6–8] the focus of this research was to measure above mentioned water quality parameters in two different First Nation communities in Manitoba. The principal goal of this research was to examine the *E. coli* and total coliforms counts and the presence of multiple types of β-lactamase genes and carbapenemase genes in the drinking water distribution systems of two First Nation reserves in Manitoba at various months. Each of the two First Nation reserves has access to a WTP. The homes in these communities receive their water through pipes or from water delivery trucks where water is stored in cisterns.

2.3 Methods

2.3.1 Drinking water sample collection from different water distribution systems

Overall 104 drinking water samples were collected throughout the different point of water distribution systems from two First Nations communities in Manitoba namely community B and community D (**Table 2.1**, **Table 2.2**), with four sampling rounds for community B and two sampling rounds for community D. Community B is 410 km away from Winnipeg and can be accessed through road whereas community D is a fly-in community 600 km away from Winnipeg Community D is unreachable by road during summer but accessible by ice roads during winter. Sampling periods for each community are described in **Table 2.1**.

Table 2.1: Description of sampling periods in the study.

Community	Sampling periods
B	April 2018
	May 2018
	August 2018
	October 2018
D	October 2018
	June 2019

Table 2.2: Details of collected water samples used in this research.

Community	Sample name	Details of collected samples
B	S	S1 and S2: plant source water (lake water) is collected on the same day
	WTP	WTP1 and WTP2: finished water at the water treatment plant
	T	T1, T2, T3: water samples collected from water truck 1, water truck 2, and water truck 3
	P	P1 to P6: water samples collected from 6 pipeline homes
	PL	PL1 to PL6: water samples collected from 6 polyethylene cistern-based homes
	C	C1 to C6: water samples collected from 6 concrete cistern-based homes
D	L	L: water is collected from the lake at a location where kids frequently swim. This sample was only collected during June 2019.
	S	S1 and S2: plant source water (lake water) is collected on two consecutive days
	WTP	WTP1 and WTP2: finished water at the water treatment plant is collected on two consecutive days.
	T	T1, T2, T3: water samples collected from water truck 1, water truck 2, and water truck 3
	SP	SP: water sample collected from community standpipe
	P	P1 to P7: water samples collected from 7 pipeline homes
	PL	PL1 to PL7: water samples collected from 7 polyethylene cistern-based homes
	FG	FG: water samples collected from one fiber-glass cistern-based home

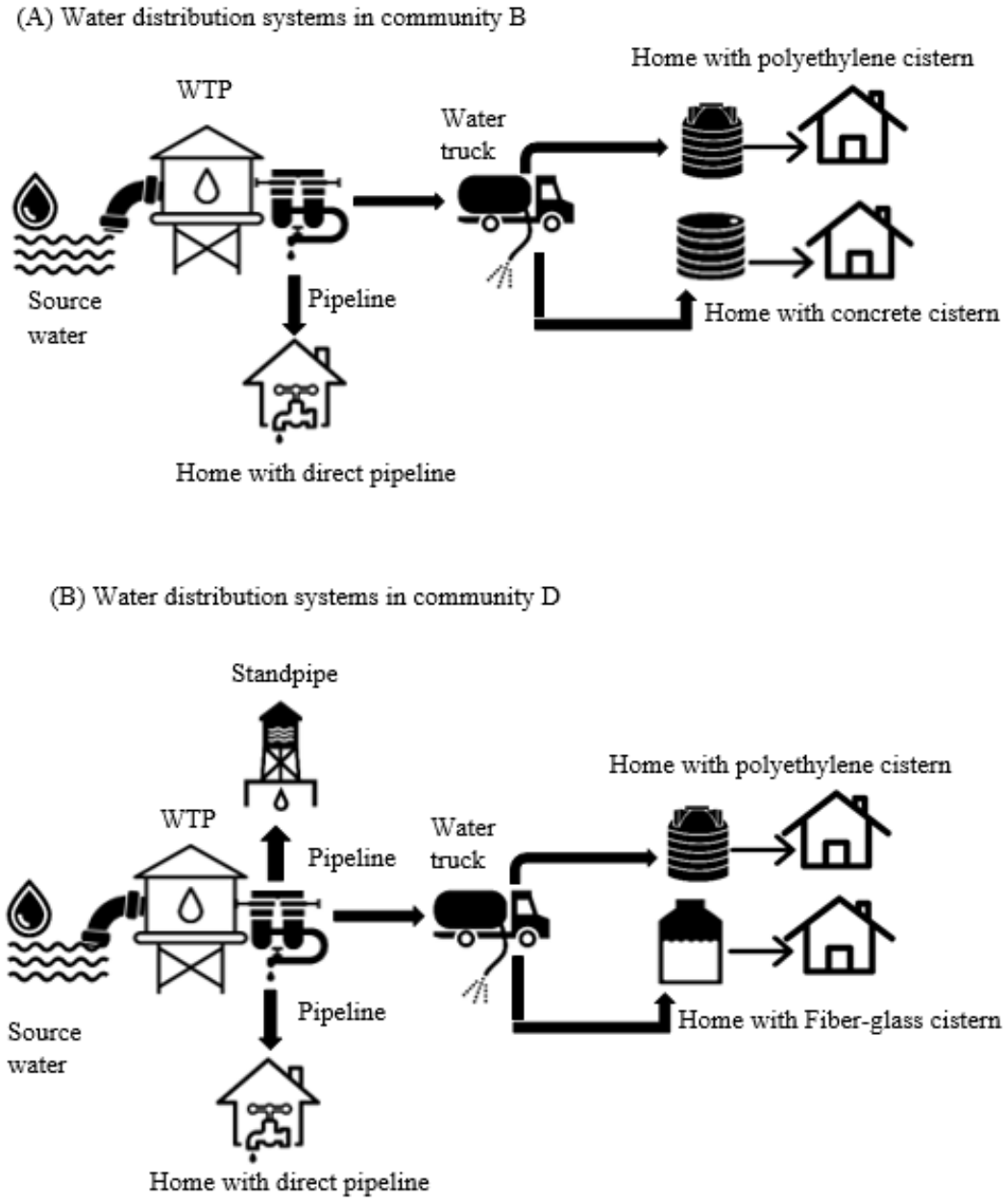

Figure 2.1: Schematic diagram of water distribution systems of community B (A) and community D (B).

The sample collection points across the drinking water distribution networks in each community are elucidated in **Figure 2.1**. Both communities utilize lake water as their source water (S) that is treated through a water treatment plant (WTP) 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 direct lined piped water (P) and 70% of the homes use cisterns made of concrete or polyethylene. In community D, there are more than 200 homes where approximately half of the homes get water through pipes and the other half uses water-storage cisterns which are mostly made of polyethylene cisterns. Community D also has a standpipe (SP) that is directly connected with WTP and families that live in homes without running water collect water from SP. In both communities B and D, water trucks (T) are filled at the WTP to deliver water to the cisterns. It is noteworthy to mention that neither of the communities is under any boil water advisories as WTP is working well in each of this community.

Two to four biological replicates of water samples were collected using the standard method as described by Rice *et al*. (2012): SM 9060A for sample bottle pre-treatment and SM 9060B for sample preservation and storage.[16] In addition, water sample was collected from a tap of University of Manitoba which was autoclaved to use as field water blank. From water samples collection to receiving samples from the University of Manitoba, the water samples were maintained in standard conditions. For example, during transportation, the water bottles were stored in coolers with enough icepacks to prevent bacterial growth. The water samples were instantly processed after receiving them at our laboratory for the fecal coliform count. Each of the water samples was also processed for DNA extraction on consecutive days. For both communities, each sampling round took 2-3 days. For fly-in community D, collected water samples were stored in coolers with icepacks and transported to Winnipeg by air on the same day after collection. If the flight was not

available, samples were transported the following morning. These water samples were stored in a refrigerator until transportation to Winnipeg. Free residual chlorine concentration (mg/L) and total chlorine concentration (mg/L) of each water sample were measured using a Hach Chlorine Pocket Colorimeter II (VWR, Mississauga, ON, Canada) along with the adapted USEPA DPD Method 8021 (Hach Company 2002) during sample collection. Also, autoclaved tap water collected from the University of Manitoba Fort Garry campus was used as a negative control.

2.3.2 *E. coli* and total coliform counts

Water samples were processed in duplicates (April and May samples from community B) or triplicates (August and October samples from community B; October and June samples from community D) to determine *E. coli* (CFU/100 mL) and total coliform counts (CFU/100 mL). The counts were calculated following the "Standard Methods for the Examination of Water and Wastewater" of the American Public Health Association/American Water Works Association/ Water Environment Federation as outlined in SM 9222.[16] In brief, 100 mL of collected water sample and 100 mL of negative control sample were filtered through sterile polyethersulfone membranes (0.45-µm pore size, 47-nm diameter; Pall Corporation, Mississauga, ON, Canada) followed by placing the filter papers on agar plates having Brilliance *E. coli*/coliform medium (Fisher Scientific, Ottawa, ON, Canada) and incubated at 37°C for 24 h. Purple colonies were measured to quantify *E. coli* and pink colonies were measured to quantify other coliforms. Brilliance agar, widely used for detecting *E. coli* and coliform counts, can distinguish *E. coli* from other coliforms based on two chromogenic agents, Rose-Gal and X-Glu.

β-D-glucuronidase and galactosidase production by *E. coli* breaks down X-Glu and Rose-Gal, respectively, and results in purple colonies. Whereas other coliforms have only galactosidase activity resulting in pink colonies by active on Rose-Gal. However, hemorrhagic *E. coli* strains

may not be identified by this medium because they are not efficient producers of β-D-glucuronidase and may result in pink colonies.[17]

2.3.3 DNA extraction

For extraction of DNA, 500-700 mL of each sample was filtered through sterile polyethersulfone membranes (0.22-μm pore size; 47-mm diameter; Pall Corporation, Mississauga, ON, Canada). Following filtration, DNeasy PowerWater Kit (QIAGEN, Germantown, MD, USA) was used according to the manufacturer's instructions to extract DNA. NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) was employed for quantifying DNA concentration and the quality of DNA was tested on a 1% agarose gel.

2.3.4 Detection of β-lactamase and carbapenemase genes by multiplex PCR

To detect five different β-lactamase genes, namely, SHV-type, TEM-type, CTX-M-type, OXA-1, and CMY-2-type, a multiplex PCR was carried out with specific primers listed in Table 2.3. The PCR reaction was validated using *Klebsiella pneumoniae* N09-00080 carrying five different β-lactamase genes mentioned above as positive control (Supplementary figure S1). This PCR was carried out using Q5 high-fidelity DNA polymerase (New England BioLabs, Whitby, ON, Canada) with the final concentrations of each primer were 0.27 μM. The PCR amplification cycle consisted of the following steps: 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, and extension at 72°C for 90 s; and a final extension step at 72°C for 7 min.[18]

Six different carbapenemase encoding genes, namely, bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{IMP}, $bla_{VIM,}$ and bla_{GES}, were determined using endpoint PCRs with defined primers listed in Table 2.3. This PCR was validated using different strains as positive controls such as *K. pneumoniae* N09-0431 for KPC-2; *E. coli* 12-123T for OXA-48; *E. coli* A44413 for GES-5; *E. coli* 10469T for NDM;

Pseudomonas aeruginosa VIM-2 for VIM-2 and *Pseudomonas aeruginosa* IMP-1 for IMP-1 (Supplementary figure S2). All genes, except bla_{IMP}, were detected by multiplex PCR and bla_{IMP} was determined by a separate reaction. PCR was carried out with Qiagen Multiplex PCR Kit (Qiagen, Germany) containing HotStarTaq DNA polymerase. The final concentrations of each primer pairs are 0.27 µM. The parameter for the multiplex PCR includes the following steps: 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 a final extension at 72 °C for 7 min.[18] For bla_{IMP}, the annealing parameter was at 61.7 °C for 90 s. All PCR products of the above reactions were analyzed using UV transilluminator after resolving on 1.3% agarose gel.

2.3.5 Statistical analysis

Analysis of variance (ANOVA) was carried out using GLIMMIX in 'SAS® University Edition' to examine for the effect of different water distribution systems and sampling months on free residual chlorine concentrations in community B and community D, the count of *E. coli* and total coliforms in community B, and the detection of ARGs in the drinking water of different homes in community B and community D. Data distribution were considered a lognormal distribution for free residual chlorine concentration; negative binomial distribution for *E. coli* and total coliforms counts; and binomial distribution for the occurrence of ARGs in water. The Tukey multiple comparison procedure was used to compare least square means for community B and D. All the above statistical analysis was carried out using 'SAS® University Edition' and significance was determined at $\alpha = 0.05$.

Table 2.3: List of primers was applied in multiplex PCR.

Primer name	Sequences (5' → 3')	Target gene	Amplicon size (bp)	Reference
SHV-UP	CGCCGGGTTATTCTTATTTGTCGC	*bla*$_{SHV}$	1016	[18]
SHV-LO	TCTTTCCGATGCCGCCGCCAGTCA			
TEM-G	TTGCTCACCCAGAAACGCTGGTG	*bla*$_{TEM}$	708	[18]
TEM-H	TACGATACGGGAGGGCTTACC			
CTX-U1	ATGTGCAGYACCAGTAARGTKATGGC	*bla*$_{CTX-M}$	593	[18]
CTX-U2	TGGGTRAARTARGTSACCAGAAYCAGCGG			
OXA1-F	CGCAAATGGCACCAGATTCAAC	*bla*$_{OXA-1}$	464	[18]
OXA1-R	TCCTGCACCAGTTTTCCCATACAG			
CMY2-A	TGATGCAGGAGCAGGCTATTCC	*bla*$_{CMY-2}$	323	[18]
CMY2-B	CTAACGTCATCGGGGATCTGC			
KPC-1	ATGTCACTGTATCGCCGTC	*bla*$_{KPC}$	863	[18]
KPC-2	AATCCCTCGAGCGCGAGT			
IMP1	CCWGATTTAAAAATYGARAAGCTTG	*bla*$_{IMP}$	522	[18]
IMP2	TGGCCAHGCTTCWAHATTTGCRTC			
VIM-1	GTTTGGTCGCATATCGCAAC	*bla*$_{VIM}$	382	[18]
VIM-2	AATGCGCAGCACCAGGATAGAA			
NDM-F	GGTGCATGCCCGGTGAAATC	*bla*$_{NDM}$	660	[18]
NDM-R	ATGCTGGCCTTGGGGAACG			
GES-2	ATCAGCCACCTCTCAATGG	*bla*$_{GES}$	302	[19]
GES-3	TAGCATCGGGACACATGAC			
OXA-48A	TTGGTGGCATCGATTATCGG	*bla*$_{OXA-48}$	744	[20]
OXA-48B	GAGCACTTCTTTTGTGATGGC			

2.4 Results

2.4.1 Free chlorine concentration and fecal bacterial count

ANOVA was performed to test the effect of water distribution and sampling months on free residual chlorine concentration and fecal bacteria for community B and community D. In community B, piped water showed significantly higher free residual chlorine concentration than the homes with polyethylene or concrete cisterns (**Figure 2.2 (A)**, **Table 2.4**). Also, the Tukey multiple comparison test showed that there was no significant difference of free residual chlorine concentrations between polyethylene and concrete cisterns (**Table 2.4**). During four sampling rounds of community B, cistern water constantly showed free chlorine concentration <0.2 mg/L whereas this was >0.2 mg/L in piped water (**Figure 2.2 (A), Table 2.4**). Also, no effect of months was observed on free residual chlorine concentration in community B (**Table 2.4**). The water samples collected from the WTP of community D showed that the average free residual chlorine concentrations were <0.2 mg/L in two sampling months. As a result, most of the water distribution systems from community D showed <0.2 mg/L free chlorine concentration and water samples from homes with polyethylene cistern, and those with pipeline showed no significant difference (**Figure 2.2 (B)**, **Table 2.5**). As water sample was collected from one fiber-glass cistern home, it was not compared with seven homes of piped or polyethylene cistern homes for statistical analysis. In community D, the average free residual chlorine concentration from WTP and standpipe showed <0.2 mg/L in both the sampling period (**Figure 2.2 (B)**). In contrast, samples from community B from all four trips showed that the WTP maintained the recommended free chlorine concentration (**Figure 2.2 (A)**).

In community B, water samples collected from WTP, water delivery trucks, and piped water homes were negative for *E. coli* or total coliforms (**Figure 2.3**). The cistern samples showed a lower level

of free residual chlorine concentration than piped water and the effect of lower chlorine concentration was reflected in the presence of bacterial counts (**Figure 2.3**). There was a variation across the six homes having cisterns in the counts of *E. coli*/total coliform bacteria detected. *E. coli* and total coliform showed significantly higher in concrete cisterns than polyethylene cisterns in community B (**Table 2.4**). Also, *E. coli* counts were higher in water samples collected during August and October than water samples collected during April and May (**Table 2.4**). It is noteworthy to mention that water samples from P5 during the August sampling round and from P5, PL4, PL5, C2, C3 during the October sampling round were not collected due to the unavailability of owners at homes. These samples were excluded during statistical analysis.

In community D, no *E. coli*/coliforms were detected in any of the water samples from WTP and piped homes (**Figure 2.4**) whereas polyethylene sample, PL3, collected during October and June tested positive for total coliforms and *E. coli*. Polyethylene cistern samples, PL6 and PL7, collected during October showed a positive result for total coliform but no fecal bacteria were detected in samples collected in June. Moreover, water delivery truck samples were free from *E. coli*/coliforms except for T1 from October showed a positive result for coliforms.

2.4.2 Detection of antibiotic resistance genes

Among the five β-lactamase genes tested, bla_{TEM} has been observed frequently in water samples in this study. In community B, no WTP and Truck samples were positive for ARGs except for WTP and T1 samples collected in May. None of the β-lactamases and carbapenemases genes were detected in samples from piped homes. However, cistern water showed positive results for multiple types of β-lactamase genes (**Figure: 2.5**). Both concrete and polyethylene cisterns showed bla_{TEM} most frequently. All concrete cisterns (C1 to C6) were positive for bla_{TEM} and three polyethylene cisterns (PL1, PL2, and PL6) showed positive for the bla_{TEM}. However, bla_{SHV} was also repeatedly

observed in concrete cisterns (except for C2 and C6) whereas no *bla*$_{SHV}$ was detected in water samples from polyethylene cistern. *bla*$_{CTX-M}$ was frequently detected in water samples of polyethylene cisterns. Water samples from three polyethylene cisterns (PL1, PL2, and PL3) were positive for *bla*$_{CTX-M}$. This gene was also detected in samples from concrete cisterns (C1 and C5). *bla*$_{OXA-1}$ was detected from only one polyethylene cistern (PL3) and one concrete cistern (C5). Two polyethylene cisterns (PL1 and PL3) and one concrete cistern (C1) were also positive for *bla*$_{CMY-2}$. Among carbapenemase genes, only *bla*$_{OXA-48}$ was detected in the samples from concrete cisterns (C1, C2, C3, and C4). Samples from the polyethylene cistern did not show the presence of any carbapenemase genes. Overall, *bla*$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{OXA-1}$, and *bla*$_{CMY-2}$ were detected in both polyethylene as well as concrete cisterns, but no statistically significant difference was observed among these distribution systems in various months regarding the presence of ARGs (**Figure 2.5**, **Table 2.6**)

In community D, *bla*$_{TEM}$ was frequently detected in samples from polyethylene cisterns (**Figure 2.6**). This gene was detected in samples from all polyethylene cisterns except PL6. *bla*$_{TEM}$ has also been detected in piped water (P4 and P5). However, no ARGs were detected in samples from WTP, trucks, standpipe, and fiberglass cistern. Also, none of the carbapenemase genes were detected in the water samples collected from this community.

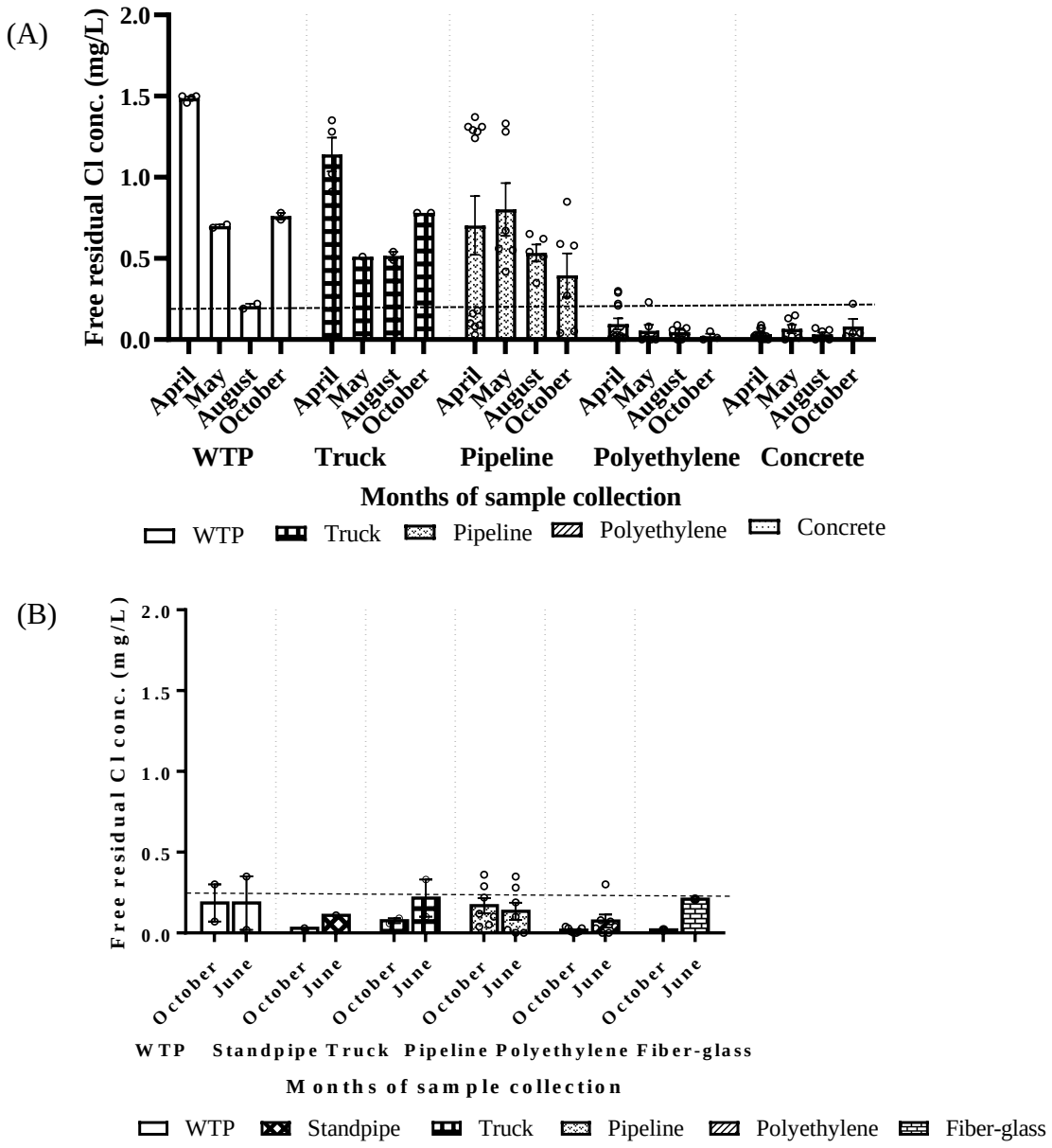

Figure 2.2: Free residual chlorine concentration (mg/L) in water samples collected from different water distribution points with various sampling months in community B (A) and community D (B). Dashed line indicates 0.2 mg/L free chlorine concentration. Each bar represents mean value of free residual chlorine concentration (mg/L) of defined sample and error bar represents standard error mean (SEM).

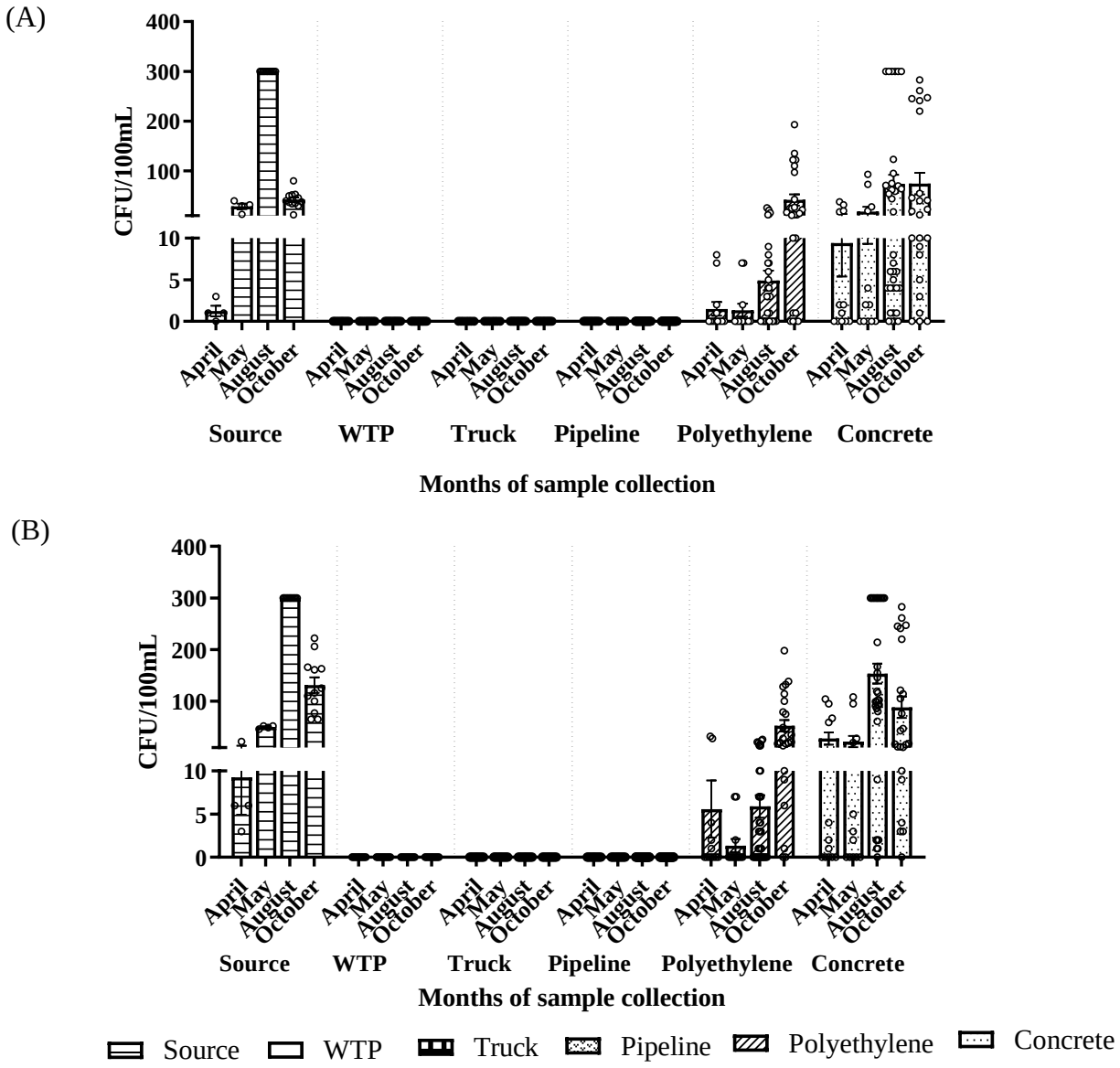

Figure 2.3: Fecal bacterial counts in water samples collected from different water distribution points with four sampling months in community B. *E. coli* (A) and total coliform (B) counts in colony forming units (CFU) were detected in water samples collected from source but not in WTP and truck. No *E. coli* (D) and total coliform (E) counts (CFU/ 100 mL) was observed in homes with pipeline whereas high numbers of CFUs were observed in homes with polyethylene or concrete cisterns. Each bar represents mean value of bacterial count (CFU/100mL) of defined sample and error bar represents standard error mean (SEM).

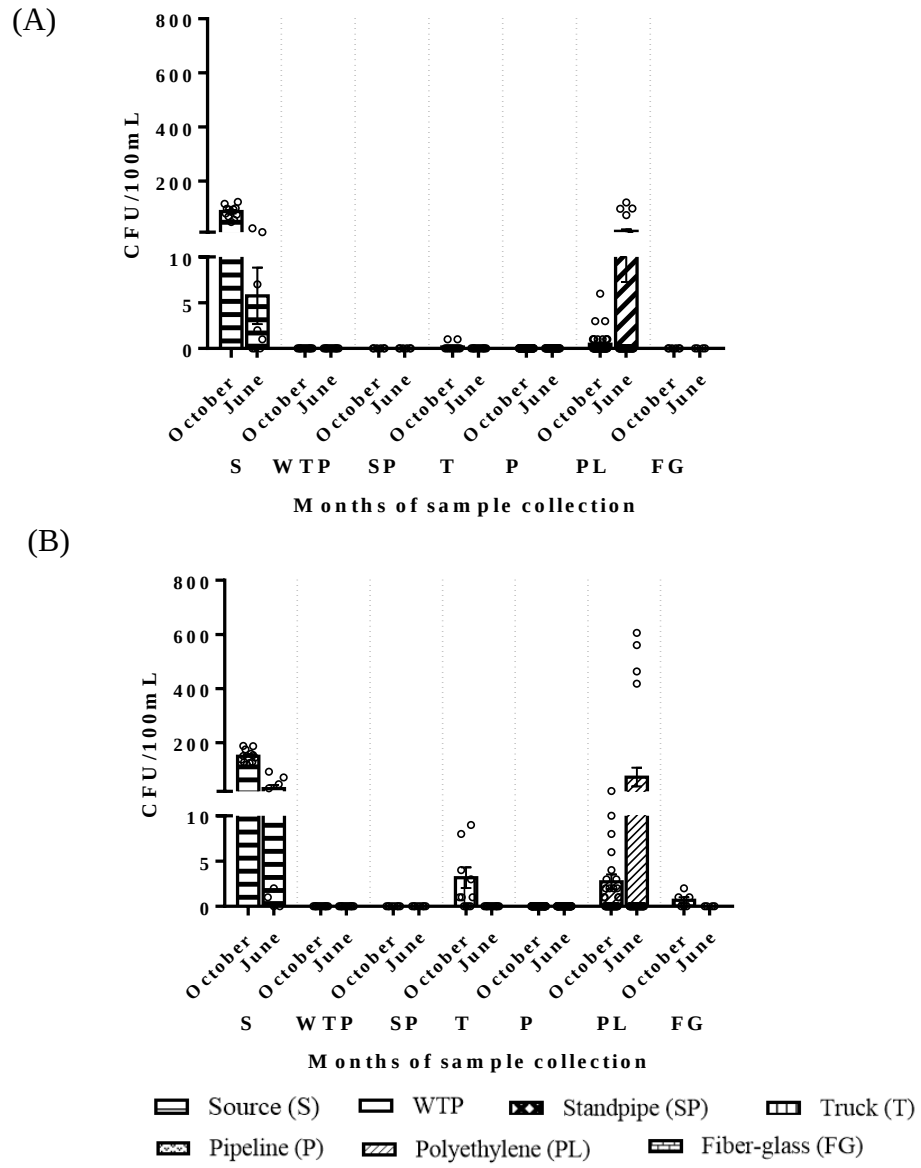

Figure 2.4: Fecal bacterial counts in water samples collected from different water distribution points with two sampling months in community D. *E. coli* (A) and total coliform (B) counts (CFU/100 mL) were detected in water samples collected from homes with polyethylene cisterns (A and B). Each bar represents mean value of bacterial count (CFU/100mL) of defined sample and error bar represents standard error mean (SEM).

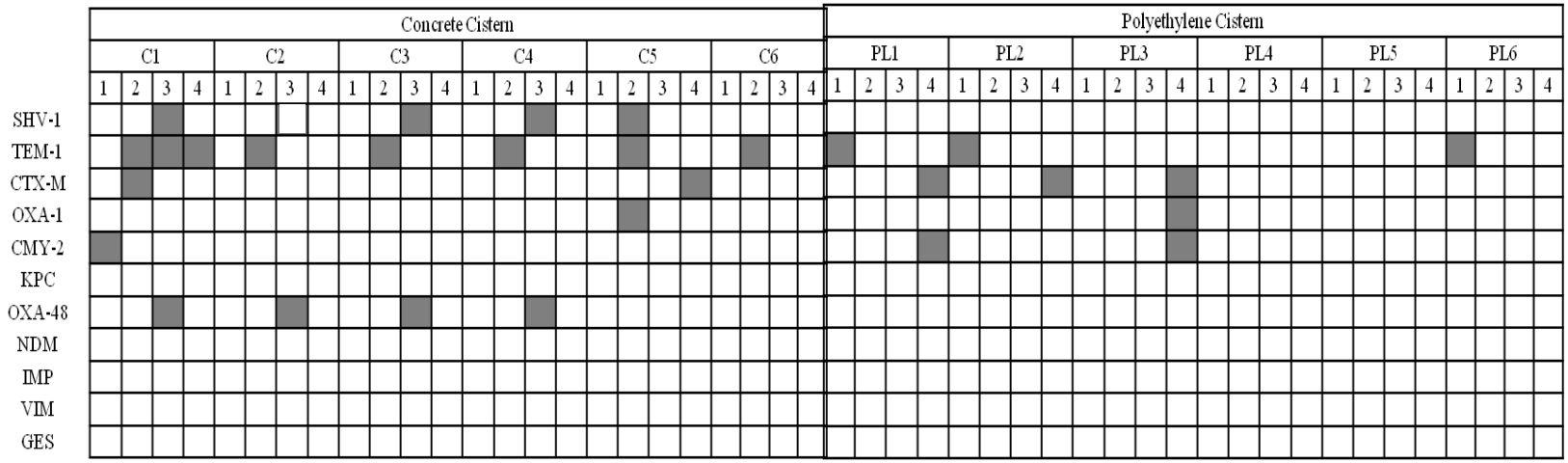

Figure 2.5: The detection of β-lactamase and carbapenemase genes in water samples collected from concrete and polyethylene cistern homes in community B. Both β-lactamase genes (*SHV-1*, *TEM-1*, *CTX-M*, *OXA-1*, and *CMY-2)* as well as carbapenemase genes (*KPC*, *OXA-48*, *NDM*, *VIM*, *IMP*, and *GES*) were detected by multiplex PCR. The positive results are represented by shades. The numbers are representative of sampling months such as 1=April, 2=May, 3=August and 4=October.

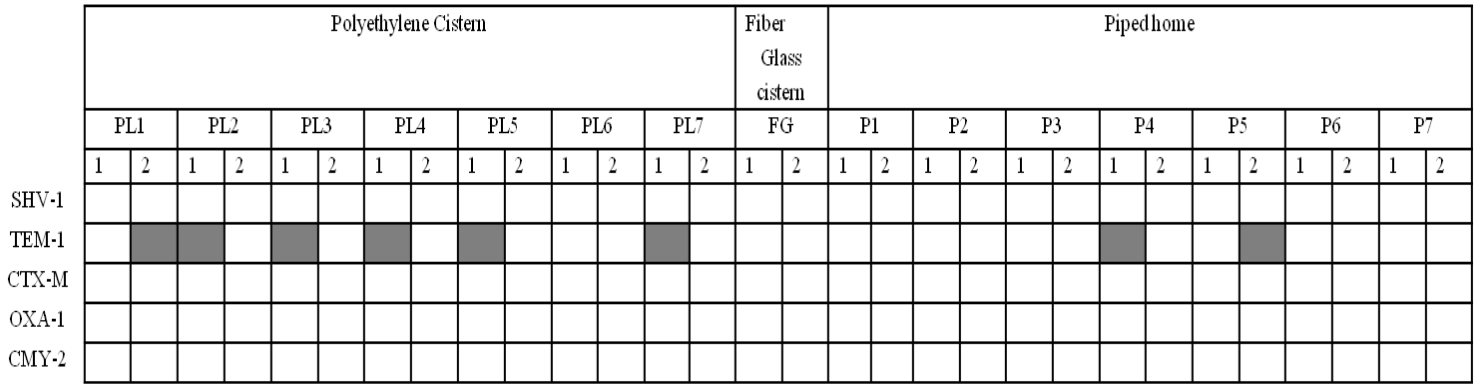

Figure 2.6: The detection of β-lactamase and carbapenemase genes in water samples collected from concrete and polyethylene cistern homes in community D. Both β-lactamase genes (*SHV-1*, *TEM-1*, *CTX-M*, *OXA-1*, and *CMY-2)* as well as carbapenemase genes (*KPC*, *OXA-48*, *NDM*, *VIM*, *IMP*, and *GES*) were detected by multiplex PCR. The positive results are represented by shades. The numbers are representative of sampling months such as 1=October, and 2=June.

Table 2.4: Least square means of free residual chlorine concentrations, *E. coli* counts and total coliform counts in community B as affected by water distribution system and sampling months.

Effect		**Free residual chlorine (mg/L)[a]**	***E. coli* (CFU/100mL)[a]**	**Total coliform (CFU/100mL)[a]**
Distribution system	Piped	0.64a	---[b]	---[b]
	Polyethylene	0.07b	5b	7b
	Concrete	0.05b	31a	53a
Sampling month	April	0.28a	4b	12ab
	May	0.32a	5b	5b
	August	0.18a	19ab	30ab
	October	0.23a	56a	68a
		***p*-value**		
Distribution system		<0.0001	0.0010	0.0003
Sampling month		0.2081	0.0055	0.0120
Distribution system x sampling month		0.9262	0.5080	0.2768

[a] Means within the same column and effect followed by the same letter are not significantly different according to the Tukey multiple comparison procedure (p <0.05).

[b] No *E. coli* / total coliform was observed in piped homes.

Table 2.5: Least square means of free residual chlorine concentration in community D as affected by water distribution system and sampling months.

Effect		**Free residual chlorine (mg/L)[a]**
Distribution system	Piped	0.15a
	Polyethylene	0.05b
Sampling month	October	0.09a
	June	0.10a
	***p*-value**	
Distribution system		0.016
Sampling month		0.7818
Distribution system x sampling month		0.2735

[a] Means within the same column and effect followed by the same letter are not significantly different according to the Tukey multiple comparison procedure ($p < 0.05$).

Table 2.6: Least square means of ARGs in community B as affected by water distribution system and sampling months.

Effect		**ARGs**				
		***bla*$_{SHV}$**[a]	***bla*$_{TEM}$**[a]	***bla*$_{CTX-M}$**[a]	***bla*$_{OXA-1}$**[a]	***bla*$_{OXA-48}$**[a]
Distribution system	Polyethylene	0.04a	0.09a	0.11a	0.05a	0.11a
	Concrete	0.11a	0.31a	0.099a	0.05a	0.12a
Sampling month	April	0.03a	0.16a	0.11a	0.04a	0.07a
	May	0.69a	0.23a	0.13a	0.06a	0.07a
	August	0.17a	0.09a	0.11a	0.04a	0.44a
	October	0.04a	0.23a	0.26a	0.06a	0.08a
		***p*-value**				
Distribution system		0.4719	0.2368	0.8775	0.9719	0.9573
Sampling month		0.7909	0.9130	0.4929	0.9909	0.1743
Distribution system x sampling month		0.9026	0.1983	0.8859	0.9883	0.9984

[a] Means within the same column and effect followed by the same letter are not significantly different according to the Tukey multiple comparison procedure (p <0.05).

Table 2.7: Least square means of bla_{TEM} in community D as affected by water distribution system and sampling months.

Effect		$\boldsymbol{bla_{TEM}}$[a]
Distribution system	Piped	0.14a
	Polyethylene	0.39a
Sampling month	October	0.39a
	June	0.14a
	***p*-value**	
Distribution system		0.1988
Sampling month		0.1988
Distribution system x sampling month		0.1988

[a] Means within the same column and effect followed by the same letter are not significantly different according to the Tukey multiple comparison procedure (p <0.05).

2.5 Discussion

2.5.1 Concentrations of free chlorine and counts of *E. coli* as well as total coliforms

The focus of this research was to investigate the microbiological contamination of drinking water from different water distribution systems in two First Nation reserves that are not under any boil water advisory. Both communities rely on lake water as source water which is treated before the distribution. Treated water from WTP in community B had standard levels of free chlorine concentration that were ≥0.2 mg/L was negative for *E. coli* or other coliforms. No fecal bacteria were observed in treated water from WTP in community D, despite the presence of free chlorine concentration less than the minimum recommended level in the water.

In community B and community D, no fecal bacteria were observed in water samples of piped water although most of the piped water samples from community D and some of the piped water samples from community B were the recommended level of free chlorine concentration. The reason behind low free chlorine concentration in piped water of community B could be the longer distance of those homes from WTP which could result in the disappearance of chlorine. However, other parameters could cause low free chlorine concentration such as the age of the pipes, conditions of water flow, a variation of pressure inside the pipe, etc.[21] Thus, the above results suggest that the WTP system and direct lined pipe-based homes have safe drinking water for these two First Nation reserves in Manitoba and the water is negative for *E. coli* or other coliforms. Similar results were also observed in our previous studies in different communities.[6,8]

In community B, water sampling has been done in four different months and no significant effect of sampling months on free residual chlorine concentration in different water distribution systems was observed (**Table 2.4**). In this community, there are two types of cisterns (i.e., polyethylene or

concrete) used by the community to store their water. During the four sampling periods, the free chlorine concentration was <0.2 mg/L in both types of cisterns except the PL2 water sample collected in April. The reason could be that the PL2 cistern was newly installed that year and the cleaner internal surface of this cistern might prevent the decay of chlorine. Whereas the concrete cisterns were older with most of the concrete cisterns age between 15 to 28 years old. However, no statistically significant difference was observed between polyethylene and concrete cistern on the effect of free chlorine concentration (**Table 2.4**), but a significant difference was found between piped water and cistern water (**Table 2.4**). In community B, most of the water samples from cistern homes were positive for *E. coli*/ coliforms but there were large variations from home to home. The reason for the bacterial count variation could be the infrastructure of cistern: concrete cisterns showed a higher number of the coliform count than polyethylene cistern (**Table 2.4**). According to Characklis *et al.* (1990),[22] the concrete surface is rough which is favorable for biofilm formation. Moreover, concrete cisterns were in use for a much longer duration and some cisterns have visible cracks.[22] For example, C2 (age= 20 years) and C6 (age=28 years) had cracks and not surprisingly showed a high number of the coliform count with <0.2 mg/L free chlorine concentration. An additional vital reason could be the cleaning time of cistern which varied home to home. The cisterns that were cleaned (less than 6 to 7 months) before sampling showed less bacterial count than the cisterns not recently cleaned. For instance, C2 and C3 cisterns were cleaned before May sampling and no *E. coli* found despite low chlorine concentration. A similar cleaning effect is also observed in polyethylene cisterns. Lastly, we also observed the cisterns that are recently filled (within 1 to 2 days) had low bacterial count and high chlorine concentration than those filled earlier. Also, the effect of the month was observed on bacterial counts such as cistern

samples collected during August and October showed higher *E. coli* counts than the water samples collected during April and May (**Table 2.4**) indicating the temporal effect on microbial growth.[23]

In community D, the sampling was done in two different months. Though the chlorine concentrations were below the recommended level, PL3 showed a high number of *E. coli/* total coliform counts. The owner of PL3 did not know about the condition or age of the cistern but in this community, most cisterns were cleaned recently before the sampling. Most of the cisterns were 2 to 4 years old and in good condition.

In summary, the above results demonstrated that piped water is much safer for drinking purposes than cistern water and cisterns should be replaced with pipeline systems. If not possible then frequent cleaning of the cistern should be maintained to lower the risk of exposure to coliforms and older or cracked concrete cisterns should be substituted with polyethylene cisterns, which also requires additional resources.

2.5.2 Detection of antibiotic resistance genes

The presence of β-lactamase and carbapenemase genes was investigated for collected water samples by using multiplex PCR targeting five genes for β-lactamases and six genes for carbapenemases. Detection of clinically significant β-lactamase genes and carbapenemase genes are not uncommon in natural aquatic environments that receive minimal anthropogenic activities. For example, $bla_{CTX\text{-}M}$, $bla_{OXA,}$ and bla_{TEM} have been detected in water samples collected from rivers, lakes, and sea waters.[24–26] Besides that, these genes have also been detected in water treatment plants such as $bla_{OXA\text{-}1}$ and $bla_{TEM\text{-}1}$ detected in water collected from a water treatment plant in China.[27] In community B of this study, $bla_{CTX\text{-}M}$, $bla_{OXA\text{-}1}$, bla_{TEM}, $bla_{SHV\text{-}1}$, $bla_{CMY\text{-}2,}$ and $bla_{OXA\text{-}48}$ were detected in water samples collected from concrete cisterns, and $bla_{CTX\text{-}M}$, $bla_{OXA\text{-}1}$,

bla$_{TEM,}$ and *bla*$_{CMY-2}$ were detected in water samples collected from polyethylene cisterns. Though total coliform counts were high in cistern homes, the correlation between the frequency of coliforms and ARGs are unclear. For instance, concrete sample C4 collected in May showed no coliform but the presence of *bla*$_{TEM}$ whereas concrete sample C6 collected in August showed total coliform counts more than 300 CFU/100mL without the presence of any ARGs. For community D, ARGs have been detected in cistern water and piped water despite the low abundance of total coliforms. These results indicate that ARGs might originate from different organisms other than coliform. For instance, opportunistic pathogen *Pseudomonas aeruginosa* has been found in different water environments showing β-lactam and carbapenem resistance.[28,29]

In community D, the frequency of detection of ARGs was much higher in water collected from polyethylene cisterns than from piped home. *bla*$_{TEM}$ was observed in both polyethylene cistern and piped water. As the water treatment plant did not show any of the examined ARGs, the contamination of piped water could be the result of low chlorine concentration and biofilm growth in the pipe wall.[27,30] It was previously reported that bacteria in biofilm are 1000 times more likely to be associated with antibiotic resistance than the planktonic bacteria in water. As well, particulates entering in water distribution network supports the re-growth of microorganism with ARG contamination.[27,31,32]

Overall, the results from this study and the studies done by Fernando *et al.* (2016) as well as Mi *et al.* (2019) suggest that the detection of β-lactamase genes and carbapenemase genes in the drinking water of First Nation communities are not uncommon albeit the results to differ from community to community.[6,8] Moreover, both our current and previous studies prove that water in some cistern-based homes was relatively unsafe to drink and steps must be taken to replace these cisterns. However, the selection pressure for these antibiotic genes was not determined because of the

unavailability of public data on antibiotic usage in Canadian First Nation communities. The future direction of this study should be the tracking of the source of ARGs contamination and the type of bacteria carrying these ARGs.

2.6 Conclusion

This study compared the drinking water quality collected from different homes in two First Nation communities in Manitoba relying on cisterns or direct lined pipes. The parameters considered for comparing the water quality were free residual chlorine concentration, presence of *E. coli,* and total coliforms as well as ARGs. Regardless of various periods, residents living in homes with cisterns are more likely to be exposed to fecal bacteria and antibiotic resistance genes than those living in homes with direct lined pipe with WTP. Also, environmental changes affect the fecal bacterial counts as warmer months showed higher counts than the colder months. In general, many First Nations reserves lack the resources to improve on the condition of cisterns, including replacement or cleaning, as there has been a systematic underfunding of First Nations reserves in the colonial Canadian system. Overall, we observed poor quality of drinking water in our two collaborating communities as it applies to homes relying on cisterns and the Federal Government of Canada should take necessary steps to overcome this situation.

2.7 Acknowledgments

We thank the two First Nation communities of Manitoba for contributing to this research partnership. This research is funded by the Canadian Institutes of Health Research under its Project Grant program and the Natural Science and Engineering Research Council of Canada under its Collaborative Research and Training Experience Program.

2.8 References

1. Maran de Oliveira, C. Sustainable access to safe drinking water: fundamental human right in the international and national scene. *An Interdiscip. J. Appl. Sci.* **12**, 985–1000 (2017).

2. Côrtes, P. L. *et al.* Crise de abastecimento de água em São Paulo e falta de planejamento estratégico. *Estud. Avancados* **29**, 7–26 (2015).

3. World Health Organization. *Guidelines for drinking-water quality, fourth edition*. (2011).

4. Health Canada. *Guidance for Providing Safe Drinking Water in Areas of Federal Jurisdiction*. (2013).

5. Zhang, X. X., Zhang, T. & Fang, H. H. P. Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology* vol. 82 397–414 (2009).

6. Fernando, D. M. *et al.* Detection of antibiotic resistance genes in source and drinking water samples from a first nations community in Canada. *Appl. Environ. Microbiol.* **82**, 4767–4775 (2016).

7. Farenhorst, A. *et al.* Bacteria in drinking water sources of a First Nation reserve in Canada. *Sci. Total Environ.* **575**, 813–819 (2017).

8. Mi, R. *et al.* Detection of fecal bacteria and antibiotic resistance genes in drinking water collected from three First Nations communities in Manitoba, Canada. *FEMS Microbiol. Lett.* **366**, (2019).

9. Government of Canada. About drinking water advisories. *www.sac-isc.gc.ca/* https://www.sac-isc.gc.ca/eng/1538160229321/1538160276874 (2019).

10. Hunter, P. Waterborne disease epidemiology and ecology. *Public Health* **112**, 203 (1998).

11. Edberg, S. C., Rice, E. W., Karlin, R. J. & Allen, M. J. *Escherichia coli*: The best biological drinking water indicator for public health protection. *Journal of Applied Microbiology Symposium Supplement* vol. 88 (2000).

12. Health Canada. Guidelines for Canadian Drinking Water Quality- Summary Table. *Water, Air Clim. Chang. Bur. Environ. Consum. Saf. Branch* 16 (2006).

13. Lechevallier, M. W., Welch, N. J. & Smith, D. B. Full-scale studies of factors related to coliform regrowth in drinking water. *Appl. Environ. Microbiol.* **62**, 2201–2211 (1996).

14. Marti, E., Variatza, E. & Balcazar, J. L. The role of aquatic ecosystems as reservoirs of antibiotic resistance. *Trends in Microbiology* vol. 22 36–41 (2014).

15. Su, H. C. *et al.* Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: From drinking water source to tap water. *Sci. Total Environ.* **616**–**617**, 453–461 (2018).

16. Rice EW, Baird RB, Eaton AB, C. L. Standard methods for the examination of water and wastewater, 22nd ed. *Am. Public Heal. Assoc. Am. Water Work. Assoc. Water Environ. Fed.* (2012).

17. Fricker, C. R., Warden, P. S. & Eldred, B. J. Understanding the cause of false negative β-d-glucuronidase reactions in culture media containing fermentable carbohydrate. *Lett. Appl. Microbiol.* **50**, 547–551 (2010).

18. Mulvey, M. R., Grant, J. M., Plewes, K., Roscoe, D. & Boyd, D. A. New Delhi metallo-β-lactamase in *Klebsiella pneumoniae* and *Escherichia coli*, Canada. *Emerg. Infect. Dis.* **17**,

103–106 (2011).

19. Mataseje, L. F. *et al.* Carbapenem-resistant gram-negative bacilli in Canada 2009-10: Results from the Canadian nosocomial infection surveillance program (CNISP). *J. Antimicrob. Chemother.* **67**, 1359–1367 (2012).

20. Brink, A. J. *et al.* Emergence of OXA-48 and OXA-181 carbapenemases among Enterobacteriaceae in South Africa and evidence of in vivo selection of colistin resistance as a consequence of selective decontamination of the gastrointestinal tract. *J. Clin. Microbiol.* **51**, 369–372 (2013).

21. LeChevallier, M. W. Coliform Regrowth in drinking water. A review. *J. / Am. Water Work. Assoc.* **82**, 74–86 (1990).

22. Characklis, W.G., McFeters, G.A. and Marshall, K. . Physiological ecology in biofilm systems, In: Characklis, W.G. and Marshall, K.C. (Ed.), Biofilms. *John Wiley Sons, Inc., New York, N.Y.* 341–394 (1990).

23. Byrne, J. *et al.* Current and future water issues in the Oldman River Basin of Alberta, Canada. *Water Sci. Technol.* **53**, 327–334 (2006).

24. Lamba, M., Sreekrishnan, T. R. & Ahammad, S. Z. Sewage mediated transfer of antibiotic resistance to River Yamuna in Delhi, India. *J. Environ. Chem. Eng.* **8**, (2020).

25. Yin, Q., Yue, D., Peng, Y., Liu, Y. & Xiao, L. Occurrence and distribution of antibiotic-resistant bacteria and transfer of resistance genes in Lake Taihu. *Microbes Environ.* **28**, 479–486 (2013).

26. Jørgensen, S. B. *et al.* A comparison of extended spectrum β-lactamase producing

Escherichia coli from clinical, recreational water and wastewater samples associated in time and location. *PLoS One* **12**, e0186576 (2017).

27. Zhang, M. *et al.* Selective antibiotic resistance genes in multiphase samples during biofilm growth in a simulated drinking water distribution system: Occurrence, correlation and low-pressure ultraviolet removal. *Sci. Total Environ.* **649**, 146–155 (2019).

28. Hooban, B., Joyce, A., Fitzhenry, K., Chique, C. & Morris, D. The role of the natural aquatic environment in the dissemination of extended spectrum beta-lactamase and carbapenemase encoding genes: A scoping review. *Water Research* vol. 180 115880 (2020).

29. Mombini, S., Rezatofighi, S. E., Kiyani, L. & Motamedi, H. Diversity and metallo-β-lactamase-producing genes in *Pseudomonas aeruginosa* strains isolated from filters of household water treatment systems. *J. Environ. Manage.* **231**, 413–418 (2019).

30. Xi, C. *et al.* Prevalence of antibiotic resistance in drinking water treatment and distribution systems. *Appl. Environ. Microbiol.* **75**, 5714–5718 (2009).

31. Ceri, H. *et al.* The Calgary Biofilm Device: New technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J. Clin. Microbiol.* **37**, 1771–1776 (1999).

32. Liu, R. *et al.* Molecular analysis of long-term biofilm formation on PVC and cast iron surfaces in drinking water distribution system. *J. Environ. Sci. (China)* **26**, 865–874 (2014).

33. Becker, K., Heilmann, C. & Peters, G. Coagulase-negative staphylococci. *Clin. Microbiol. Rev.* **27**, 870–926 (2014).

34. Martinez, J. L. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution* vol. 157 2893–2902 (2009).

35. Hartman, B. J. & Tomasz, A. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. *J. Bacteriol.* **158**, 513–516 (1984).

36. Katayama, Y., Ito, T. & Hiramatsu, K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus. Antimicrob. Agents Chemother.* **44**, 1549–1555 (2000).

37. Ito, T. *et al.* Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. *Antimicrobial Agents and Chemotherapy* vol. 53 4961–4967 (2009).

38. Cole, K. *et al.* Draft Genome Sequences of 64 Type Strains of 50 Species and 25 Subspecies of the Genus *Staphylococcus* Rosenbach 1884. *Microbiol. Resour. Announc.* **8**, (2019).

39. Li, B., Brinks, E., Franz, C. M. A. P. & Cho, G. S. Draft genome sequence of *Staphylococcus fleurettii* strain MBTS-1 isolated from Cucumber. *Genome Announc.* **5**, (2017).

40. Naushad, S. *et al.* Comprehensive Phylogenetic Analysis of Bovine Non-aureus Staphylococci Species Based on Whole-Genome Sequencing. *Front. Microbiol.* **7**, 1990 (2016).

41. Tsubakishita, S., Kuwahara-Arai, K., Sasaki, T. & Hiramatsu, K. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. *Antimicrob. Agents*

Chemother. **54**, 4352–4359 (2010).

42. Government of Canada. Short-term drinking water advisories. *Indigenous Services Canada* https://www.sac-isc.gc.ca/eng/1562856509704/1562856530304 (2019).

43. Government of Canada. Ending long-term drinking water advisories. *Indig. Serv.* 1–10 (2019).

44. Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D. & Kamal, M. A. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J. Biol. Sci.* **22**, 90–101 (2015).

45. Adesoji, A. T. & Ogunjobi, A. A. Detection of Extended Spectrum Beta-Lactamases Resistance Genes among Bacteria Isolated from Selected Drinking Water Distribution Channels in Southwestern Nigeria. *Biomed Res. Int.* **2016**, (2016).

46. Schmidt, A. S., Bruun, M. S., Dalsgaard, I. & Larsen, J. L. Incidence, Distribution, and Spread of Tetracycline Resistance Determinants and Integron-Associated Antibiotic Resistance Genes among Motile Aeromonads from a Fish Farming Environment. *Appl. Environ. Microbiol.* **67**, 5675–5682 (2001).

47. Poppe, C. *et al.* Characterization of antimicrobial resistance of *Salmonella* Newport isolated from animals, the environment, and animal food products in Canada. *Can. J. Vet. Res.* **70**, 105–114 (2006).

48. Macauley, J. J., Adams, C. D. & Mormile, M. R. Diversity of tet resistance genes in tetracycline-resistant bacteria isolated from a swine lagoon with low antibiotic impact. *Can. J. Microbiol.* **53**, 1307–1315 (2007).

49. Zhang, X. X., Zhang, T. & Fang, H. H. P. Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology* vol. 82 397–414 (2009).

50. Wu, D., Huang, Z., Yang, K., Graham, D. & Xie, B. Relationships between antibiotics and antibiotic resistance gene levels in municipal solid waste leachates in Shanghai, China. *Environ. Sci. Technol.* **49**, 4122–4128 (2015).

Chapter 3: Characterization of methicillin-resistant gene, *mecA*, harboring *Mammaliicoccus fleurettii* isolated from lake water in a First Nation community, Manitoba, Canada

3.0 Characterization of methicillin-resistant gene, *mecA*, harboring *Mammaliicoccus fleurettii* isolated from lake water in a First Nation community, Manitoba, Canada.

3.1 Abstract

The goal of our study was to examine the diversity of methicillin-resistant gene, *mecA*, harboring *Staphylococcus* spp. from a lake water of First Nation reserves. A total of four methicillin-resistant presumptive *Staphylococcus* spp. were isolated from lake water which showed *mecA* gene positive results in PCR. Using MALDI-TOF and the whole genome sequencing approach by NGS confirmed that all the methicillin-resistant bacteria are *Mammaliicoccus fleurettii* (previously known as *Staphylococcus fleurettii*), a commensal in animals. Phylogenetic analysis of the *mecA* gene from isolated *Mammaliicoccus fleurettii* revealed 99% nucleotide identity with *mecA* allele type. Moreover, a genomic region flanking the *mecA* gene in *Mammaliicoccus fleurettii* in our study showed more than 90% nucleotide identity with other reference genomes of *Mammaliicoccus fleurettii* collected from goat cheese milk, cucumber, and mastitis. Also, a part of the genomic regions flanking *mecA* gene showed nucleotide more than 90% sequence identity with different species of *Staphylococcus* spp. from human and animal sources dispersed over the world. Monitoring of antibiotic resistance genes harboring bacterial species in recreational water is required as it will enhance useful information on the source of antimicrobial resistance genes in water.

3.2 Introduction

Infections from recreational waters are not only restricted to enteric diseases but also extend to different organ targeted infections such as skin infection caused by *Staphylococcus* spp.[1] Though *Escherichia coli* and total coliforms are important indicators for the presence of enteric bacteria in drinking water, assessment of bacteria causing skin infection is more important for the study of

recreational water. Moreover, antibiotic resistance genes or bacteria carrying antimicrobial resistance genes are regarded as environmental pollutants.[2] *Staphylococcus* spp. are normal microbiota of mammals and birds but may behave as opportunistic pathogens. Not only coagulase-positive *Staphylococcus* (CPS) (such as *S. aureus*) but also coagulase-negative *Staphylococcus* (CNS) (such as *S. epidermidis, S. fleurettii, S. hemolyticus,* etc.) are gaining interest due to their increased detection as responsible agents of infections.[1] Recent taxonomic study recategorized several species of CNS into a new family known as Mammaliicoccaceae.[3] Besides, methicillin-resistant *Staphylococcus* (MRS), associated with the expression of the *mecA* gene symbolizes a significant clinical problem. The *mecA* gene encoding penicillin-binding protein, called PBP2a, that has a low affinity to virtually all β-lactam antibiotics is considered one of the most important mechanisms of resistance to β-lactam antibiotics.[4] This gene is on a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*) that can also carry determinants of resistance to other antimicrobials, virulence determinants, and other genes important for bacterial survival under stress conditions.[5] SCC*mec* is composed of two essential elements, the *mec* complex, consisting of *mecA* and its regulators (*mecR1* and *mecI*), and the *ccr* complex, containing cassette chromosome recombinase (*ccr*) genes that ensure the mobility of the cassette.[5,6] Evolutionary study suggested that *mecA* first emerged in *S. fleurettii* (which is now known as *Mammaliicoccus fleurettii*) and then horizontally transferred into different *Staphylococcus* spp. including MRSA.[3,7-9] This type of *mecA* gene evolutionary study is done mostly in *Staphylococcus* spp. of human and animal origins and little is known about the relationship with environmental species. Our previous study showed a high copy number of *mecA* gene in a different point of lake water in a First Nation community, Manitoba.[10] *mecA* gene is prominent in *Staphylococcus* species.[7,9] Thus, the objective of this study was to determine the presence and diversity of *mecA*

positive staphylococcal species in surface water (Lake water) in a First Nation community, Manitoba, and to perform molecular and antimicrobial resistance characterization of recovered isolates by whole genome sequencing approach. Furthermore, *mecA* gene complex and its phylogenic relationship among the collected environmental spp. to different sources (such as human, animal, food, etc.) spp. were also analyzed.

3.3 Materials and methods

3.3.1 Collection of lake water sample

The selected lake water sample was collected from Island lake water of Manitoba which surrounds a First Nation reserve (community D), located 600 km away from Winnipeg, and is reachable via a two-hour flight followed by a half-hour boat ride. Community D is unreachable by road during summer but accessible by ice roads during winter. In June 2019, one water sample was collected from Island lake water where children were swimming. In community D, there are more than 200 homes that use lake water for its recreation purpose as well as source water (S) that is treated through a water treatment plant (WTP). The water sample was collected using the standard method as described by Rice *et al*. (2012): SM 9060A for sample bottle pre-treatment and SM 9060B for sample preservation and storage.[11] From water sample collection to receiving samples to the University of Manitoba, the water sample was maintained in standard condition. For example, during transportation, the water bottles were stored in coolers with enough icepacks or being stored in a refrigerator until transportation to Winnipeg. Also, autoclaved waters that were collected from tap water of the University of Manitoba were used for negative control.

3.3.2 Isolation of methicillin-resistant presumptive *Staphylococcus* spp. (MRS) and phenotypic screening for antibiotic resistance

The water sample was instantly processed for isolation of MRS after receiving it at the laboratory. In brief, 100 mL of water sample and 100 mL of negative control were filtered through sterile polyethersulfone membranes (0.45-µm pore size, 47-nm diameter; Pall Corporation, Mississauga, ON, Canada) followed by placing the filter papers on agar plates having Lysogeny Broth (LB) agar (Bacto LB Agar, Lennox, BD, France) supplemented with 6 µg/mL oxacillin and incubated at 37°C for 48 h. For isolation and phenotypic identification of MRS, colonies that grew on LB were sub-cultured on mannitol salt agar (MSA; Oxoid, Canada) supplemented with 6 µg/mL oxacillin and incubated for 37°C for 48 h. *S. aureus* clinical isolate HA-MRSA 100697 was used as a reference strain. All MSA-Oxacillin-positive strains showing the expected morphological appearance of *Staphylococcus* spp. were subjected to matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Bruker Daltonics Japan, Yokohoma, Japan) analysis. This experiment was conducted under supervision of Dr. George Golding in National Microbiology Lab, Winnipeg, Canada. Mass spectrometry-grade water was used in the preparation of all reagents. A chemical extraction (Ethanol/Formic Acid/Acetonitrile) of each bacterial isolate was performed following the manufacturer's suggested protocol (Bruker Daltonics Japan, Yokohoma, Japan). 1 µl of sample supernatant was spotted onto an MSP 96-well polished steel plate (in triplicate), dried at room temperature, and then overlayed with 1 µl of a saturated solution of alpha-Cyano-4-hydroxycinnamic acid (HCCA, Sigma Aldrich) dissolved in 2.5% trifluoroacetic acid and 50% acetonitrile. Mass spectra were acquired using a Microflex MALDI-TOF mass spectrometer (Bruker Daltonik, Bremen, Germany). Bruker Bacterial Test Standard was used to calibrate the Microflex MS system before analysis; an isolate of

Staphylococcus aureus was also included as an extraction control. Spectra were acquired using flexControl software, version 3.4., with a 60 HZ UV laser. 240 laser shots were accumulated for each mass spectrum. The spectra were analyzed using MALDI Biotyper Compass Explorer 4.1 and compared against the latest spectral database (BDAL DB, 8468 MSPs). Scoring was based on Bruker Daltonik's Biotyper algorithm.

For screening antibiotic susceptibility, MIC's were determined by broth microdilution using the Sensititre system (Sensititre automated microbiology system; Trek Diagnostic Systems Ltd., Westlake, OH, USA) and interpreted according to CLSI breakpoints.[12] The Sensititre panel GPALL1F is a commercially available panel for Gram-positive organisms. It consists of different antimicrobials: Ampicillin, Chloramphenicol, Ciprofloxacin, Clindamycin, Daptomycin, Dtest1, Dtest2, Erythromycin, Gentamicin, Levofloxacin, Linezolid, Moxifloxacin, Nitrofurantoin, Oxacillin +2% NaCl, Penicillin, Quinupristin/dalfopristin, high-level Streptomycin (1000 µg/ml), Tetracycline, Tigecycline, Trimethoprim/sulfamethoxazole, and Vancomycin.

3.3.3 Genotypic analysis

DNA was extracted from all MSA-Oxacillin-positive strains and was further screened for nuclease (*nuc*), *mecA,* and *Staphylococcus* spp. specific *rpoB* genes (**Table 3.1**) by the colony PCR. For DNA template preparation, an isolated single bacterial colony was resuspended in 50 µL of sterile water and boiled at 100ºC for 10 min followed by a short spin. From this lysate containing crude extract of the DNA template, 1µL was used in 10 µL of PCR reaction. The final PCR reaction mixture contains 0.2 mM primers, 200 mM dNTPs, 1.25 U of Taq polymerase, and 10X PCR buffer. The parameter for PCR reaction steps was initial denaturation at 95ºC for 10 min, followed by denaturation at 94ºC for 30 s, annealing temperature 51ºC and 45ºC for 30 s (for *nuc* and *mecA* gene, respectively), extension at 72ºC for 1 min, from denaturation to extension steps repeated for

34 cycles. This was followed by a final extension step at 72ºC for 5 min. For *rpoB*, the parameter for PCR reaction steps were initial denaturation at 95ºC for 10 min, followed by denaturation at 94 °C for 45 s, annealing temperatures 52 °C for 60 s, extension at 72 °C for 90 s, and a final extension at 72 °C for 10 min, from denaturation to extension steps repeated for 30 cycles.[13-15] The amplified PCR products from these reactions were resolved on a 1.3% agarose gel and then visualized in a UV transilluminator.

3.3.4 Identification of the *mecA* gene harboring bacterial spp.

Whole genome sequencing was performed for the isolated *mecA* gene harboring bacteria from lake water sample. The sequencing and library preparation was done under supervision of Dr. Georg Golding in National Microbiology Lab, Winnipeg, Canada. The Genomic DNA was extracted by the EpiCentre MasterPure Complete DNA and RNA purification kit (Illumina, Madison, WI, USA). Sequencing Libraries were prepared using the Nextera XT DNA Sample Prep kit (Illumina, Madison, WI, USA). and 150 bp paired-end sequencing was performed on the Miseq platform (Illumina, San Diego, USA).

After receiving the genomes, further analysis was done by me. Quality check of the sequence was done by FastQC and *de novo* assembly was conducted using SPAdes version 3.14.0 with default parameters.[16] Quality of assembled genomes were checked by QUAST. Antimicrobial resistance genes were analyzed using CARD whereas virulence genes were analyzed using tools available from Center for Genomic Epidemiology.[17] The contigs obtained after *de novo* assembly and quality checking were annotated via genomes Prokka v1.1.[18] Phylogenic relationship of *mecA* genes were analyzed in MEGA X.[19] All the sequences were submitted to NCBI.

Table 3.1: Primers used in this study.

Primer Name	Sequence	Amplicon size (bp)	Genes	Reference
mecA F	AACAGGTGAATTATTAGCACTTGTAAG	174	*mecA*	[13]
mecA R	ATTGCTGTTAATATTTTTTGAGTTGAA			
nuc F	GCGATTGATGGTGATACGGTT	279	*nuc*	[14]
nuc R	AGCCAAGCCTTGACGAACTAAAGC			
Staph rpoB 1418F	CAATTCATGGACCAAGC	899	*rpoB*	[15]
Staph rpoB 3554R	CCGTCCCAAGTCATGAAAC			

3.4 Results

3.4.1 *mecA* gene harboring presumptive MRS detected in lake sample of community D

A total of 20 isolates grew on oxacillin supplemented LB plates. From these oxacillin-resistant isolates, four colonies grew on MSA with oxacillin plates. The colonies were smooth, elevated, circular, and yellow in colour. The four isolates only showed resistance toward oxacillin (resistance breakpoint ≥4µg/mL) and penicillin (resistance breakpoint 0.254µg/mL) in MIC test (**Table 3.2**). All these isolates showed a positive result for methicillin-resistant gene, *mecA*, and *Staphylococcus* spp. specific *rpoB* gene in PCR (**Figure 3.1 (a)** and **Figure 3.1 (c)**). We further tested for *S. aureus* specific *nuc* gene but none of them showed a positive result for *nuc* gene (**Figure 3.1 (b)**). MALDI-TOF test further confirmed that these isolates are non- *S. aureus*.

3.4.2 Genetic features of isolated *mecA*-harboring *Mammaliicoccus fleurettii*

WGS confirmed that the bacteria are *Mammaliicoccus fleurettii* which were previously known as *Staphylococcus fleurettii*.[9] Genome assembly of all *Mammaliicoccus fleurettii* yielded multiple contigs. The genome characteristics of four isolates are described in **Table 3.3**. Genome annotation was done by Prokka v1.1 and it identified more than 2400 protein-coding genes in each of the isolates. Antibiotic resistance genes were predicted using the Comprehensive Antibiotic. Resistance Database (CARD) with “strict” and “perfect” parameters. These parameters exhibited 99.7% sequence identity with *mecA*, 99.83% sequence identity with *mecR1,* and 100% identity with *mecI*. All four isolates of *Mammaliicoccus fleurettii* possess these described genes. We set 90% nucleotide identity as a cut-off value for the detection of ARG by CARD and we did not find any other ARGs above this cut-off value. However, virulence genes were analyzed using VirulenceFinder 2.0 from Center for Genomic Epidemiology (http://www. genomicepidemiology.org/) and no acquired virulence genes were detected in these isolates.

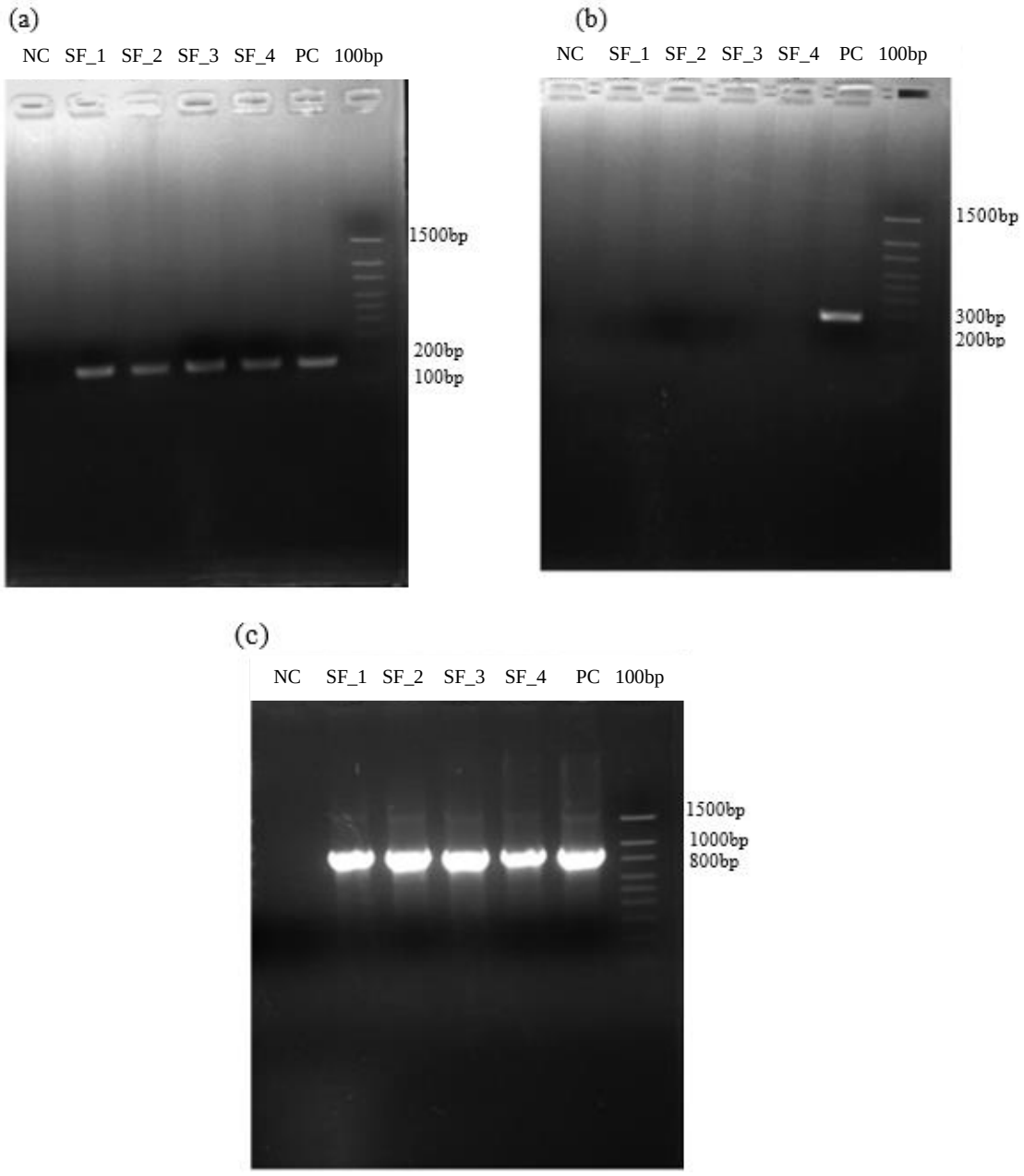

Figure 3.1: Agarose gel electrophoresis of PCR-amplified 174 bp of *mecA* gene (a), 279 bp of *nuc* gene (b), and 899 bp of *rpoB* gene (c). Presumptive MRS isolates (SF_1, SF_2, SF_3 and SF_4) in this study are indicated on the top of the lanes. Here, NC=negative control and PC= positive control which is *S. aureus* clinical isolate HA-MRSA 100697. Molecular sizes are indicated at the right in base pairs (bp).

Table 3.2: MIC (µg/mL) of antimicrobials for *Mammaliicoccus fleurettii* from this study.

Antimicrobials	**SF_1**	**SF_2**	**SF_3**	**SF_4**
Ampicillin	0.5	0.5	0.5	0.5
Chloramphenicol	8.0	8.0	8.0	8.0
Ciprofloxacin	≤1.0	≤1.0	≤1.0	≤1.0
Clindamycin	≤0.5	≤0.5	≤0.5	≤0.5
Daptomycin	≤0.5	≤0.5	≤0.5	≤0.5
Dtest1	≤4.0	≤4.0	≤4.0	≤4.0
Dtest2	≤8.0	≤8.0	≤8.0	≤8.0
Erythromycin	≤0.25	≤0.25	≤0.25	≤0.25
Gentamicin	≤2.0	≤2.0	≤2.0	≤2.0
Levofloxacin	0.5	0.5	0.5	0.5
Linezolid	2.0	2.0	2.0	2.0
Moxifloxacin	0.5	0.5	0.5	0.5
Nitrofurantoin	≤32.0	≤32.0	≤32.0	≤32.0
Oxacillin +2% NaCl	>4.0	>4.0	>4.0	>4.0
Penicillin	0.5	1.0	0.5	0.5
Quinupristin/dalfopristin	≤0.5	≤0.5	≤0.5	≤0.5
High-level Streptomycin (1000 µg/ml)	≤1000	≤1000	≤1000	≤1000
Tetracycline	≤2.0	≤2.0	≤2.0	≤2.0
Tigecycline	≤0.03	0.06	0.06	≤0.03
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5	≤0.5
Vancomycin	1.0	1.0	1.0	1.0

*Blue color=resistant
**White color=sensitive

To know further the allelic type of the *mecA* gene, we performed a phylogenetic tree analysis using MEGA X. The analysis showed that the *mecA* identified in SF_1, SF_2, SF_3, and SF_4 belongs to the *mecA* allele type with 99.95% nucleotide sequence identity with *S. pseudintermedius* KM241(AM90473.1) *mecA* and MRSA N315 (D86934) *mecA* (**Figure 3.2**).[20]

We observed the same *mecA* flanking regions from the genomes of our study (Figure 3.3). Next, we did the comparative genomic analysis of the region flanking the *mecA* gene complex using, draft, or complete genomes of *Mammaliicoccus fleurettii* available through NCBI GenBank (Table 3.4). We compared the *mecA* flanking regions from the genomes of our study (SF_1, SF_2, and SF_4) to the reference genomes. As the genome quality of SF_3 was not good, we did not include this isolate for our further analysis (**Table 3.3**).

Comparison of the genetic structure of the region surrounding the *mecA* gene revealed that a 21,881-bp sequence of *brnQ_1-mvaA-mvaC-mvaS-ugpQ_1-maoC-mecA-mecR1 -mecI-psm-mec-xylR-cstB-DsrE/DsrF/DsrH-cstR-TauE/SafE-xylA-xylB-xylE* shared with the genome of CCUG, NCTC13289, MBTS-1 and SNUC182. An extra piece of a gene, *IS256* transposase-like element observed in CCUG and NCTC13289 strain, and both isolates were collected from goat cheese milk (Figure 3.4). Also, this specific region was compared with MRSA 315 (Figure 3.5) and other methicillin-resistant *Staphylococus* spp., isolated from different sources, which showed more than 99% nucleotide identity (**Table 3.5**).

Table 3.3: Genomic characteristics of *Mammaliicoccus fleurettii* isolated in this study.

	SF_1	**SF_2**	**SF_3***	**SF_4**
Contigs	54	52	887	48
Largest contig (bp)	217942	436487	42799	352823
Total length (bp)	2477406	2480156	2329073	2478073
GC (%)	31.65	31.66	32.01	31.66
N50	113393	113420	5479	117447
N75	59627	58365	2751	66624
L50	9	7	121	7
L75	16	15	270	14
CDSs	2445	2443	2201	2444
rRNA (5S, 16S, 23S)	2, 2, 1	2, 2, 2	2, 2, 1	2, 2, 2
tRNA	48	48	48	49
Pseudo genes (total)	28	29	28	28
CDSs	2445	2443	2201	2444

*SF_3 has 887 contigs with N50=5479. As the genome quality of SF_3 is not good, we withdraw this genome for further analysis.

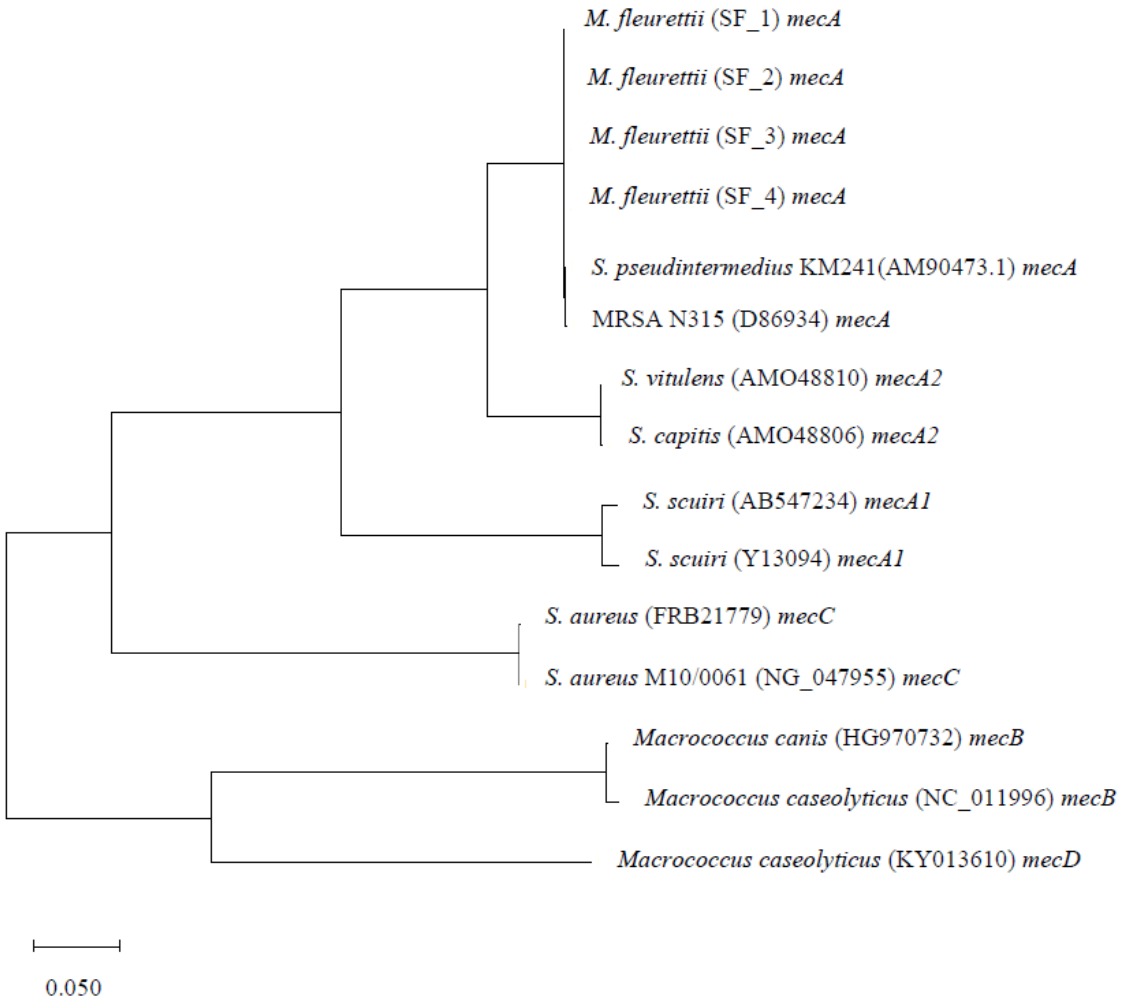

Figure 3.2: Phylogenetic tree of the *mecA* gene of *Mammaliicoccus fleurettii* in this study was inferred using the Neighbor-Joining method.[21] The optimal tree with the sum of branch length = 1.373 is shown. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site.[22] This analysis involved 15 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 2042 positions in the final dataset. Evolutionary analyses were conducted in MEGA X.[19]

Table 3.4: Genome assembly and annotation results of eight *M. fleurettii* strains found in NCBI.

Strain	**Biosample source**	**Size (Mbp)**	**GC%**	**Ref**
FDAARGOS_682	Jugular catheter, USA	2.53	36.0	[23]
NCTC13829	Goat cheese, Germany	2.68	31.60	[38]
DSM 13212	Goat milk cheese, UK	2.47	35.90	[39]
MBTS-1	Cucumber, Germany	2.58	31.70	[40]
SNUC 182	Mastitis, Canada	2.87	31.90	[27]
SNUC 248	Mastitis, Canada	2.53	31.60	[27]
ssch2	Periprosthetic tissue, Italy	2.51	36.0	Unpublished
ssch3	Fistula, Italy	2.51	36.0	Unpublished

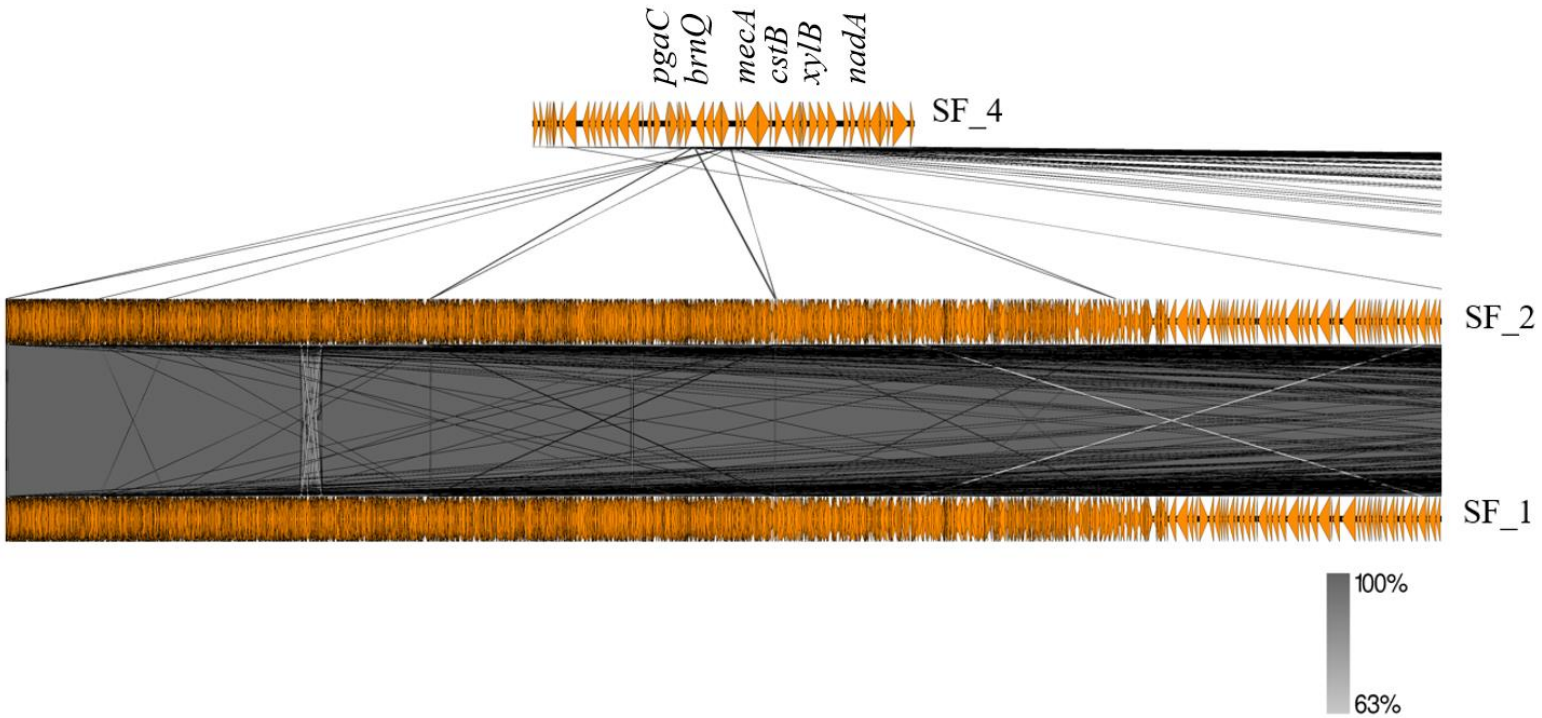

Figure 3.3: Genomic organization of the *mecA* gene locus of *Mammaliicoccus fleurettii* (SF_4, SF_2, and SF_1) in this study and comparison of the locus-containing *mecA* gene complex among the isolates. Arrows indicate the translation orientation of the coding genes. Figure was generated with EasyFig (http://mjsull.github.io/Easyfig/).

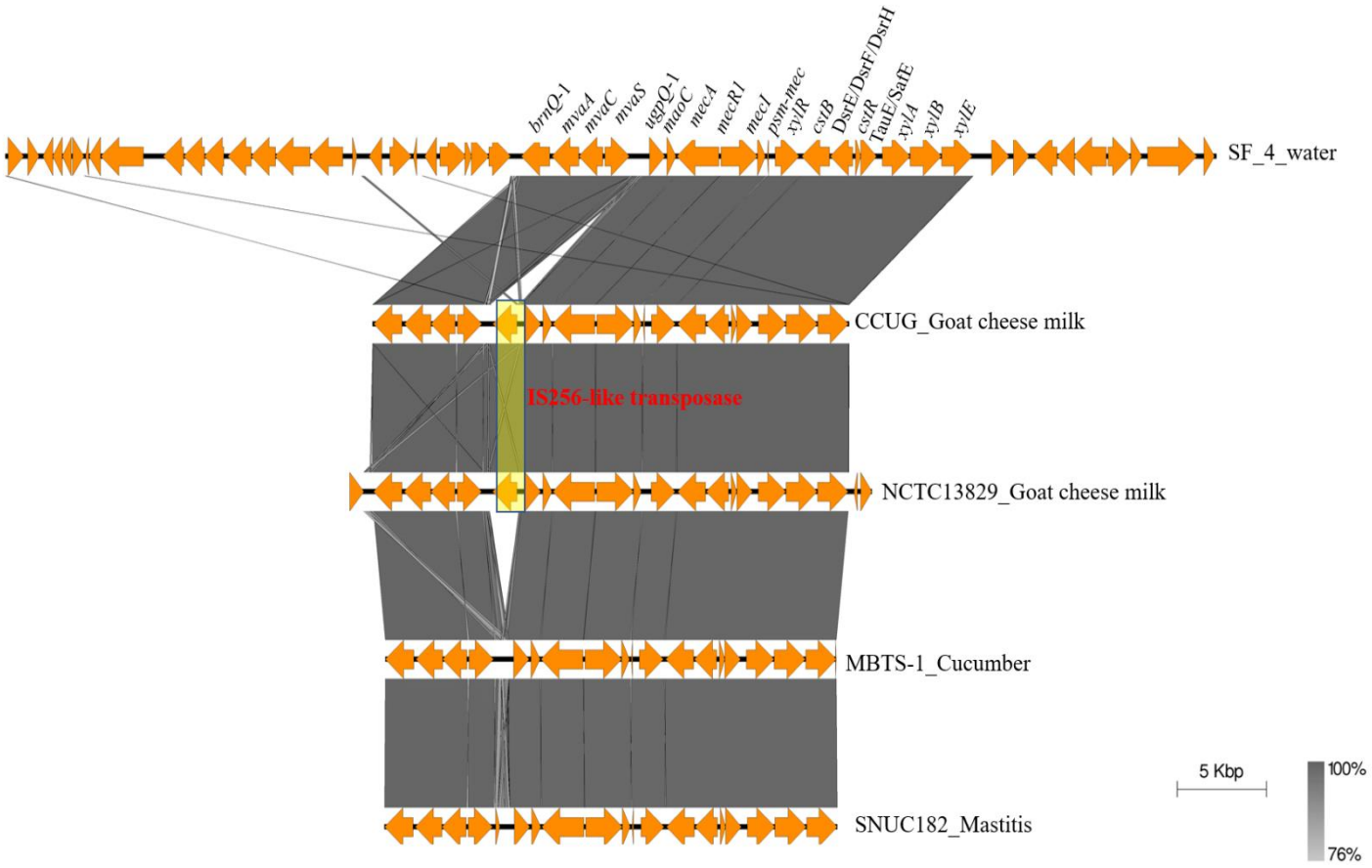

Figure 3.4: Genomic organization of the *mecA* gene locus of *Mammaliicoccus fleurettii* (SF_4) in this study and comparison of the locus-containing *mecA* gene complex among *M. fleurettii* CCUG, *M. fleurettii* NCTC13829, *M. fleurettii* MBTS-1 and *M. fleurettii* SNUC182. Arrows indicate the translation orientation of the coding genes. The yellow region indicating an extra genetic element, *IS256*-transposase like element, present in CCUG and NCTC13829. Figure was generated with EasyFig (http://mjsull.github.io/Easyfig/).

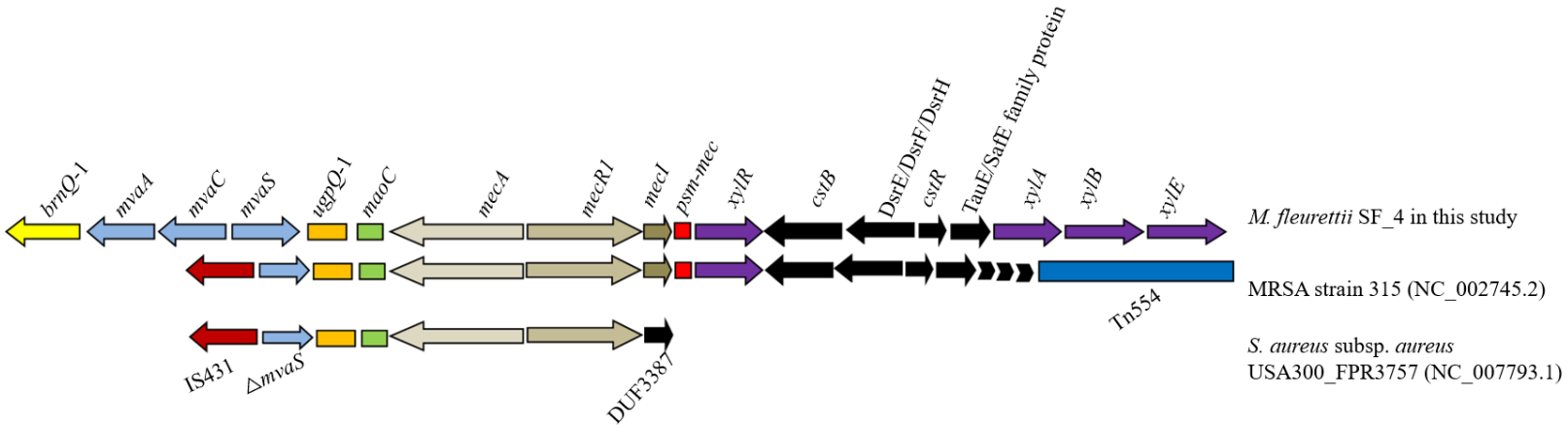

Figure 3.5: Genomic comparison of the locus-containing *mecA* gene complex among *Mammaliicoccus fleurettii* in this study (upper), MRSA strain N315 (middle) and *S. aureus* USA300 strain FPR3757 (bottom). Arrows indicate the translation orientation of the coding genes.

Table 3.5: Nucleotide sequence identity of locus flanking *mecA* gene complex in *Mammaliicoccus fleurettii* and various *Staphylococcus* spp. found in GenBank.

Strain ID	**Biosample source**	**Country**	**Nucleotide identity (%)**	**Accession number**
MRSA strains N315	Human	Japan	99.80	D86938934.2
***S. pseudintermedius* strains KM241**	Human	Switzerland	99.84	AM904731.1
MRSA strain C10682	Human	Canada	99.80	FJ390057.1
***S. aureus* strain OC3**	Human	Russia	99.82	AB983237.1
***S. lentus* strain H29**	Horse	China	99.86	CP059679.1

3.5 Discussion

Methicillin, a semi-synthetic penicillin-resistant to penicillinases, was pioneered in 1960, and, unfortunately, methicillin-resistant strains emerged in 1961.[28-30] Coagulase-positive methicillin-resistant *Staphylococcus aureus* (MRSA) remains to be identified as a major public health concern because of the therapeutic challenges associated with this pathogen.[31] During the late 1980s and early 1990s, the National Nosocomial Infections Surveillance System revealed that coagulase-negative *Staphylococcus* spp. (CNS) were associated with nosocomial infections.[7,32] One of the CNS member is *Staphylococcus fleurettii*, a commensal organism for different animals and occasionally associated with bovine mastitis, has been recently isolated from human (**Table 3.5**). A recent study was done to re-evaluate the taxonomy of Staphylococcaceae by employing a core genome phylogeny complemented with overall genome-related indices. Phylogenomic analyses offered strong support for assigning several species of the Staphylococcaceae family in the novel genus *Mammaliicoccus* including *S. fleurettii*.[3] Thus, all the published bacterial genome in NCBI previously known as *S. fleurettii* is now renamed as *Mammaliicoccus fleurettii. In* this study, the *mecA* gene harboring four isolates of *M. fleurettii* were isolated from water samples which were collected from a particular location of Island lake water where children from community D used to swim during summer 2019. In our previous study, a high copy number of *mecA* was observed in Island lake water that led us to investigate which *Staphylococcus* spp. carrying this gene.[10] This study also investigated to gain a better understanding of probable risks for human health via exposure to this organism by molecular characterization of this CNS.

MICs for antibiotic resistance were measured in the presence of different antibiotics but only penicillin resistance was observed in *M. fleurettii* of this study (**Table 3.2**). Whereas the study conducted in Spain showed that *M. fleurettii* strains isolated from water were resistant to penicillin,

cefoxitin, erythromycin, clindamycin, and fusidic acid.[33] The primary characterization for the isolates in this study was analyzed by MALDI-TOF which confirmed that the isolates were non-*S. aureus* and finally the species were confirmed by the whole-genome sequencing approach.

All the four isolates in this study carrying *mec* gene complex, including methicillin-resistance gene *mecA* with its regulators, *mecR1,* and *mecI*.[20] *M. fleurettii* naturally contains *mecA* in its chromosome and evolutionary study proved that *mec* gene of SCC*mec* of MRS, including MRSA, originated from *M. fleurettii*.[41] The *mec* gene complex has been categorized into five distinct classes, classes A to E, based on insertion sequences (IS) and regulatory elements upstream and downstream of the *mec* gene.[6,34,35] Phylogenetic analysis revealed all the *M. fleurettii* isolates are *mecA* gene type (Figure 3.1). There are three allotypes, *mecA*, *mecA1*, and *mecA2*, under *mecA* gene type and our isolated *M. fleurettii* showed 99.98% nucleotide identity with *mecA* allotype (**Figure 3.1**).[20]

There are eight whole-genome sequences of *M. fleurettii* from different sources are available in NCBI (Table 3.4) but no genome sequence of *M. fleurettii* from a water source has been found in NCBI. In our study, genetic structure of the region surrounding *mecA* gene revealed that a 21,881-bp sequence of *brnQ-1-mvaA-mvaC-mvaS-ugpQ-1-maoC-mecA-mecR1-mecI-psm-xylR-cstB-DsrE/DsrF/DsrH-cstR-TauE/SafE-xylA-xylB-xylE* which was compared with the available genomic sequences of *M. fleurettii*. This region showed similar genomic arrangements among SNUC182 (sample source: mastitis), MBTS-1 (sample source: cucumber), and NCTC13829 (sample source: goat cheese) except one extra gene, *IS256*-transposase like element, in NCTC13829. The human-sourced *M. fleurettii* genomes (FDAARGOS_682, ssch2, and ssch3) do not contain this region. We observed that *M. fleurettii* had an intact *mvaS* gene followed by *mvaAC* genes representing the mevalonate pathway.[36] Besides, *ugpQ* as well as *maoC* genes are present

in between the *mvaS* and *mecA* genes on the chromosome of *M. fleurettii*. In upstream of *mecA-mecR1-mecI* complex in *M. fleurettii* in this study showed the presence of genes encoding a phenol-soluble modulin mec (*psm-mec*),[37] ROK family transcriptional regulator (xylose repressor, *xylR*), persulfide dioxygenase-sulfurtransferase (*cstB*), dihydroneopterin aldolase, and persulfide-sensing transcriptional repressor (*cstR*). In this study, *M. fleurettii* also contains a xyl operon with *xylABE* encoding xylose isomerase, xylulokinase, and the xylose transporter, respectively, which might be regulated by *xylR* (**Figure 3.2**).[38]

Genome analysis around *mecA* gene in *M. fleurettii* was also compared with MRSA strain N315 which has SCC*mec* gene complex with truncated *mvaS* by *IS431* (**Figure 3.3**). This SCC*mec* element had also *ugpQ* and *maoC* genes as described before in *M. fleurettii*. Upstream of *mecA-mecR1-mecI* N315 SCC*mec* had similar genes as *M. fleurettii* as described before except *xylABE* which was replaced by Tn554. Also, clinical isolate *S. aureus* subsp. *aureus* USA 300_FPR3757 contain a similar locus of *ugpQ-maoC- mecA-mecR1-mecI* with truncated *mvaS* at upstream of the region.

The locus *ugpQ-1-maoC-mecA-mecR1-mecI-psm-mec-xylR-cstB-DsrE/DsrF/DsrH-cstR-TauE/SafE* of *M. fleurettii* in this study shared 99% nucleotide identity with the corresponding locus of type II SCC*mec* carried by MRSA strain N315. This association was found not only in MRSA strain N315 but also in strains containing different classes of SCC*mec* type had been distributed worldwide. According to GenBank database, this association was found in, for example, SCC*mec* type IIIA of *S. aureus* from Russia, methicillin-resistant *S. lentus* strain H29 from China, SCC*mec* type II of MRSA strains N315 from Japan, methicillin-resistant *S. pseudintermedius* strains KM241 from Switzerland; and SCC*mec* type VIII of MRSA strain C10682 from Canada. (**Table 3.5**)

Though the *M. fleurettii* isolated from our study is only resistant towards penicillin and did not show any virulence genes, the *mecA* gene complex might be able to transfer to other virulent organisms as shown in previous epidemiological studies. Moreover, Seyfried et al. (1985) showed the correlation between staphylococcal counts with eye and skin illness among swimmers and predicted the morbidity rates associated with total counts of staphylococci.[39] Though our study was done with a small number of water samples examined in a specific period, the information gained from this study leads to the conclusion that monitoring antibiotic-resistant bacteria at recreational water is necessary.

3.6 Conclusion

Methicillin-resistant *mecA* gene harboring *M. fleurettii* were detected in this study in recreational water in Island lake water, including diverse genetic lineages of *M. fleurettii* with other *Staphylococcus* species of potential human and animal origins with a high content of antimicrobial resistance genes. Although high abundance of ARGs is more likely to be associated with high frequency of mobilization of resistance genes, a single potential donor cell can transfer resistance gene(s) by interacting with a suitable receiver. Thus, rigorous monitoring of the antimicrobial resistance bacteria, and their molecular characteristics in the environment as a potential reservoir and vehicle for transmission is important for both public and veterinary health concerns.

3.7 Acknowledgment

We thank the First Nation community for the research partnership. We are thankful to Dr. George Golding from National Microbiology Lab, Canada for conducting MIC, MALDI-TOF, and NGS in his lab. This research is supported by the Canadian Institutes of Health Research under its Project Grant program.

3.8 References

1. Becker, K., Heilmann, C. & Peters, G. Coagulase-negative staphylococci. *Clin. Microbiol. Rev.* **27**, 870–926 (2014).
2. Martinez, J. L. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environmental Pollution* vol. 157 2893–2902 (2009).
3. Hartman, B. J. & Tomasz, A. Low-affinity penicillin-binding protein associated with β-lactam resistance in *Staphylococcus aureus*. *J. Bacteriol.* **158**, 513–516 (1984).
4. Madhaiyan, M., Wirth, J. S. & Saravanan, V. S. Phylogenomic analyses of the staphylococcaceae family suggest the reclassification of five species within the genus *Staphylococcus* as heterotypic synonyms, the promotion of five subspecies to novel species, the taxonomic reassignment of five *Staphylococcus* species to *Mammaliicoccus* gen. Nov., and the formal assignment of *Nosocomiicoccus* to the family staphylococcaceae. *Int. J. Syst. Evol. Microbiol.* **70**, 5926–5936 (2020).
5. Katayama, Y., Ito, T. & Hiramatsu, K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus. Antimicrob. Agents Chemother.* **44**, 1549–1555 (2000).
6. Ito, T. *et al.* Classification of staphylococcal cassette chromosome mec (SCCmec): Guidelines for reporting novel SCCmec elements. *Antimicrobial Agents and Chemotherapy* vol. 53 4961–4967 (2009).
7. Rolo, J. *et al.* Evolutionary origin of the staphylococcal cassette chromosome mec (SCCmec). *Antimicrob. Agents Chemother.* **61**, (2017).
8. Gómez, P. *et al.* Characterization of staphylococci in urban wastewater treatment plants in Spain, with detection of methicillin resistant *Staphylococcus aureus* ST398. *Environ.*

Pollut. **212**, 71–76 (2016).

9. Tsubakishita, S., Kuwahara-Arai, K., Sasaki, T. & Hiramatsu, K. Origin and molecular evolution of the determinant of methicillin resistance in staphylococci. *Antimicrob. Agents Chemother.* **54**, 4352–4359 (2010).

10. Fernando, D. M. *et al.* Detection of antibiotic resistance genes in source and drinking water samples from a first nations community in Canada. *Appl. Environ. Microbiol.* **82**, 4767–4775 (2016).

11. Rice EW, Baird RB, Eaton AB, C. L. Standard methods for the examination of water and wastewater, 22nd ed. *Am. Public Heal. Assoc. Am. Water Work. Assoc. Water Environ. Fed.* (2012).

12. Weinstein, M. P. *et al. M100 Performance Standards for Antimicrobial Susceptibility Testing A CLSI supplement for global application. Performance Standards for Antimicrobial Susceptibility Testing Performance Standards for Antimicrobial Susceptibility Testing*. (2020).

13. Martineau, F. *et al.* Multiplex PCR assays for the detection of clinically relevant antibiotic resistance genes in staphylococci isolated from patients infected after cardiac surgery. *J. Antimicrob. Chemother.* **46**, 527–533 (2000).

14. Brakstad, O. G., Aasbakk, K. & Maeland, J. A. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *J. Clin. Microbiol.* **30**, 1654–1660 (1992).

15. Mellmann, A. *et al.* Sequencing and staphylococci identification. *Emerg. Infect. Dis.* **12**, 333–336 (2006).

16. Bankevich, A. *et al.* SPAdes: A new genome assembly algorithm and its applications to

single-cell sequencing. *J. Comput. Biol.* **19**, 455–477 (2012).

17. McArthur, A. G. *et al.* The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother.* **57**, 3348–3357 (2013).

18. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* **30**, 2068–2069 (2014).

19. Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* **35**, 1547–1549 (2018).

20. Lakhundi, S. & Zhang, K. Methicillin-Resistant *Staphylococcus aureus*: Molecular Characterization, Evolution, and Epidemiology. *Clinical microbiology reviews* vol. 31 (2018).

21. Saitou, N. & Nei, M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**, 406–425 (1987).

22. Tamura, K., Nei, M. & Kumar, S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 11030–11035 (2004).

23. Sichtig, H. *et al.* FDA-ARGOS is a database with public quality-controlled reference genomes for diagnostic use and regulatory science. *Nat. Commun.* **10**, (2019).

24. Vernozy-Rozand, C. *et al. Staphylococcus fleurettii* sp. nov., isolated from goat's milk cheeses. *Int. J. Syst. Evol. Microbiol.* **50**, 1521–1527 (2000).

25. Cole, K. *et al.* Draft Genome Sequences of 64 Type Strains of 50 Species and 25 Subspecies of the Genus *Staphylococcus* Rosenbach 1884. *Microbiol. Resour. Announc.* **8**, (2019).

26. Li, B., Brinks, E., Franz, C. M. A. P. & Cho, G. S. Draft genome sequence of

Staphylococcus fleurettii strain MBTS-1 isolated from Cucumber. *Genome Announc.* **5**, (2017).

27. Naushad, S. *et al.* Comprehensive Phylogenetic Analysis of Bovine Non-aureus Staphylococci Species Based on Whole-Genome Sequencing. *Front. Microbiol.* **7**, 1990 (2016).
28. Barber, M. Methicillin-resistant staphylococci. *J. Clin. Pathol.* **14**, 385–393 (1961).
29. Jevons, M. P. *"Celbenin" - resistant Staphylococci*. *British Medical Journal* vol. 1 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1952888/ (1961).
30. Chambers, H. F. *Methicillin-Resistant Staphylococci*. *Clinical Microbiology Reviews* vol. 1 (1988).
31. Chambers, H. F. & DeLeo, F. R. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nature Reviews Microbiology* vol. 7 629–641 (2009).
32. Jarvis, W. R. & Martone, W. J. Predominant pathogens in hospital infections. *J. Antimicrob. Chemother.* **29**, 19–24 (1992).
33. Gómez, P. *et al.* Diversity of species and antimicrobial resistance determinants of staphylococci in superficial waters in Spain. *FEMS Microbiol. Ecol.* **93**, 208 (2017).
34. Shore, A. C. *et al.* Detection of staphylococcal cassette chromosome mec type XI carrying highly divergent mecA, mecI, mecR1, blaZ, and ccr genes in human clinical isolates of clonal complex 130 methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **55**, 3765–3773 (2011).
35. Katayama, Y., Ito, T. & Hiramatsu, K. Genetic organization of the chromosome region surrounding mecA in clinical staphylococcal strains: Role of IS431-mediated mecI deletion in expression of resistance in *mecA*-carrying, low-level methicillin-resistant

Staphylococcus haemolyticus. *Antimicrob. Agents Chemother.* **45**, 1955–1963 (2001).

36. Wilding, E. I. *et al.* Identification, evolution, and essentiality of the mevalonate pathway for isopentenyl diphosphate biosynthesis in gram-positive cocci. *J. Bacteriol.* **182**, 4319–4327 (2000).

37. Queck, S. Y. *et al.* Mobile Genetic Element-Encoded Cytolysin Connects Virulence to Methicillin Resistance in MRSA. *PLoS Pathog.* **5**, e1000533 (2009).

38. Takeda, Y., Takase, K., Yamato, I. & Abe, K. Sequencing and characterization of the xyl operon of a gram-positive bacterium, Tetragenococcus halophila. *Appl. Environ. Microbiol.* **64**, 2513–2519 (1998).

39. Seyfried, P. L., Tobin, R. S., Brown, N. E. & Ness, P. F. A prospective study of swimming-related illness II. Morbidity and the microbiological quality of water. *Am. J. Public Health* **75**, 1071–1075 (1985).

Chapter 4: Discussion

4.0 Discussion

4.1 Summary of findings

Safe water is important for life. It is important to maintain multiple checkpoints in drinking water systems for microbials to ensure safe drinking water. This includes steps such as source water protection, checking different operational steps of water treatment, and ensuring the quality of treated drinking water during water distribution.[1] A lack of access to safe drinking water in homes on First Nation reserves is not uncommon in Canada, and this is in violation of the basic Human Rights to safe, running drinking water As of March 9, 2021, 58 long-term DWAs remained in First Nation reserves in place because of the unsafe water quality issues across Canada. In Manitoba, there are 3 long-term boil water advisories active in First Nation communities.[2,3]

In our previous studies, *E. coli* and total coliforms which are indicators of fecal contamination were detected in drinking water distribution systems of different First Nation reserves in Manitoba, Canada.[4–6] Moreover, different types of antibiotic resistance genes (ARGs) were observed in drinking water distribution systems and source (lake) water including a high copy number of methicillin-resistant gene in lake water indicating the plausible presence of notorious *Staphylococcus* spp..[5,6] Our earlier findings suggest that residents of some homes on First Nation reserves are exposed to pathogenic bacteria as well as ARGs through drinking water. Residents of one community were also potentially exposed to methicillin-resistant bacteria as evident from the detection of methicillin-resistant gene in lake water.[6] The previous studies tested the water quality one or two times in each community, however, water quality varies throughout the year. This current study expanded on monitoring multiple times throughout the year, and on the extent of microbial parameters analysed for.

In Chapter 2, we examined the microbiological quality of drinking water in two First Nation communities (community B and community D). The study screened for free residual chlorine concentrations, the number of enteric bacteria (total coliforms, *E. coli*), and the presence of beta-lactamase and carbapenemase genes in the drinking water of these reserves.

In community B and community D, no *E. coli*/ total coliforms were observed in water samples of WTP and pipeline water although most of the pipeline water samples from community D and some of pipeline water samples from community B had free residual chlorine concentrations that were below the recommended concentrations of 0.2 mg/L. ARGs were not observed in the samples collected from WTP and piped homes in either communities, except for two pipeline homes from community D that showed positive results in different sampling months and both had <0.2 mg/L free residual chlorine concentration (**Figure 2.6**).

In community B, most of the water samples collected from cistern-based homes were positive for *E. coli*/coliforms as well as ARGs but there was variation between home to home. Besides, water from concrete cisterns showed higher bacterial counts than water from polyethylene cisterns (**Figure 2.3**, **Table 2.4**). The reasons for this might be (a) concrete cisterns were much older than polyethylene cisterns, (b) some concrete cisterns were reported to be cracked, (c) the surface of the concrete is more suitable for biofilm formation than the polyethylene surface. Also, the *E. coli* counts were higher during warmer months than the colder month (**Table 2.4**). In community D, fecal bacteria and ARGs were also observed in cistern-based homes but in less frequency than community B (**Figure 2.3**, **Figure 2.4**). In both communities, the free residual chlorine concentration in most of the cisterns was below <0.2 mg/L.

This observation suggests that residents living in homes with cisterns are more susceptible to being exposed to fecal bacteria and ARGs than the residents living in homes with direct pipelines to WTP.

In Chapter 3, our study investigated the *mecA* gene harboring *Staphylococcus* spp. isolated from Island lake water, Manitoba, and further characterized the bacteria at the molecular level. In our previous study, Fernando et al. (2016) showed the presence of a high copy number of *mecA* gene in Island lake water that led us to investigate which *Staphylococcus* spp. carrying this gene.[6] Island lake water is used both as a source of water (which is treated by WTP) for drinking purpose and recreational purposes in the community D First Nation reserve in Manitoba. During summer (August) 2019, we collected a water sample from a location of the lake where children used to swim.

The study confirmed the presence of four isolates of a methicillin-resistant gene, *mecA*, carrying *Mammaliicoccus fleurettii (*previously known as *Staphylococcus fleurettii*) by whole genome sequencing approach. All the four isolates in this study carrying *mec* gene complex, *mecA* regulators, *mecR1* and *mecI*. Phylogenetic analysis revealed all the *M. fleurettii* isolates are *mecA* gene type with 99.98% nucleotide identity with *mecA* allotype. We further compared the genetic structure of the region surrounding the *mecA* gene with other *M. fleurettii* available in GenBank and the results showed similar genomic arrangements among SNUC182 (sample source: mastitis), MBTS-1 (sample source: cucumber), and NCTC13829 (sample source: goat cheese) except one extra gene, *IS256*-transposase like element, in NCTC13829. The same regions of the *mecA* gene complex also compared with other pathogenic methicillin-resistant *Staphylococcus* spp which showed 99% nucleotide identity with the corresponding locus indicating the plausible transfer of *mecA* gene complex to other virulent organisms.

Though our study was performed with a small number of water samples examined in a specific period, the information gained from this study leads to the conclusion that the monitoring of methicillin-resistant bacteria at recreational water is necessary for public health concern.

In summary, the observations from Chapter 2 and Chapter 3 show microbial and ARG contamination in surface water (lake water) and treated drinking water in various water distribution points in two First Nation reserves.

4.2 Significance of the study

Both communities in this study are not under any kind of water advisories although we found *E. coli* and other coliforms in the water distribution systems of these communities. WTPs from both communities are functioning well regarding ensuring good microbiological quality of drinking water which is the likely reason for the lack of boil water advisories in these communities. Homes with piped water supply overall have better quality water than homes with cistern water. Our study shows that the use of concrete cisterns on First Nation reserves is not providing access to safe drinking water to homes. The poor microbiological quality of drinking water in cisterns, especially concrete cisterns in community B, is a health concern as these cisterns showed a high quantity of *E. coli* and other coliforms. Moreover, *E. coli* counts were higher during warmer months than colder months (**Table 2.4**). Cisterns more recently cleaned (less than 6 months ago) showed a lower count of bacteria, which suggest that enhanced resources directed to First Nations reserves for cleaning cisterns is essential. Most of the cisterns from community B showed ARGs positive (**Figure 2.5**). Whereas in community D, where chlorine levels in water distribution systems were mostly below 0.2 mg/L, ARGs are detected in both cistern and piped water (**Figure 2.2 (B), Figure 2.6**)

Overall, our study suggests that there needs to be greater attention in Canada to solve drinking water crisis that families on First Nations reserves are experiencing.

4.3 Future study

This study provided insights into the seasonal variation as well as water storage systems on the microbiological quality of drinking water from First Nation communities in Manitoba. In the future, this study can be modified in the following manner to get a better sense of factors that influence the water quality in these communities.

It would be helpful to examine whether the time that the cistern was cleaned last (e.g., days, weeks, or months, or years ago) is associated with bacterial count and/ARG results in drinking water. This information might help First Nations reserves with evidence of what financial resources are needed to maintain clean, running drinking water to home.

Future studies should focus on the identification of bacteria-harboring β-lactamases and carbapenemases to determine the association of bacterial species harboring these resistance genes. For example, bla_{SHV} gene is not only common in *E. coli* but also other species of Enterobacteriaceae.[7] Moreover, in community D, ARGs were observed in piped water samples although the water samples were free from total coliforms. It indicates that other than *E. coli* /coliform, different bacterial species might carry these ARGs. For example, *Bacillus thuringiensis* have been found from treated water and municipal tap water carrying bla_{TEM} and bla_{SHV} genes.[8]

The study can be expanded to determine the presence of several other resistance genes. For instance, *tetA, tetB, tetC, tetD,* and *tetE* are frequently detected in different water environments including drinking water, surface water, swine lagoon, fish ponds, etc.[9–11] Moreover, ARGs of aminoglycoside, macrolide–lincosamide–streptogramin, chloramphenicol, and vancomycin are

frequently being detected in aquacultures throughout the world.[12] Thus, for getting the complete picture of the frequency of ARGs present in water samples in our study, the data of other groups of ARGs need to be included in this study.

Finally, we were not able to determine what kind of selection pressure(s) result in the selection of ARGs in water. For instance, it has been previously shown that the selective pressure tetracycline in municipal waste, which was measured by high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS), was associated with the abundance of *tetQ* and *tetM* genes.[13] In our future study, the selective pressure of antibiotics can be determined by using HPLC-MS/MS, and then the association between ARGs and their corresponding antibiotic could be determined.

4.4 References

1. World Health Organization. *Guidelines for drinking-water quality, fourth edition*. (2011).

2. Government of Canada. Short-term drinking water advisories. *Indigenous Services Canada* https://www.sac-isc.gc.ca/eng/1562856509704/1562856530304 (2019).

3. Government of Canada. Ending long-term drinking water advisories. *Indig. Serv.* 1–10 (2019).

4. Farenhorst, A. *et al.* Bacteria in drinking water sources of a First Nation reserve in Canada. *Sci. Total Environ.* **575**, 813–819 (2017).

5. Mi, R. *et al.* Detection of fecal bacteria and antibiotic resistance genes in drinking water collected from three First Nations communities in Manitoba, Canada. *FEMS Microbiol. Lett.* **366**, (2019).

6. Fernando, D. M. *et al.* Detection of antibiotic resistance genes in source and drinking water samples from a first nations community in Canada. *Appl. Environ. Microbiol.* **82**, 4767–4775 (2016).

7. Shaikh, S., Fatima, J., Shakil, S., Rizvi, S. M. D. & Kamal, M. A. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. *Saudi J. Biol. Sci.* **22**, 90–101 (2015).

8. Adesoji, A. T. & Ogunjobi, A. A. Detection of Extended Spectrum Beta-Lactamases Resistance Genes among Bacteria Isolated from Selected Drinking Water Distribution Channels in Southwestern Nigeria. *Biomed Res. Int.* **2016**, (2016).

9. Schmidt, A. S., Bruun, M. S., Dalsgaard, I. & Larsen, J. L. Incidence, Distribution, and

Spread of Tetracycline Resistance Determinants and Integron-Associated Antibiotic Resistance Genes among Motile Aeromonads from a Fish Farming Environment. *Appl. Environ. Microbiol.* **67**, 5675–5682 (2001).

10. Poppe, C. *et al.* Characterization of antimicrobial resistance of Salmonella Newport isolated from animals, the environment, and animal food products in Canada. *Can. J. Vet. Res.* **70**, 105–114 (2006).

11. Macauley, J. J., Adams, C. D. & Mormile, M. R. Diversity of tet resistance genes in tetracycline-resistant bacteria isolated from a swine lagoon with low antibiotic impact. *Can. J. Microbiol.* **53**, 1307–1315 (2007).

12. Zhang, X. X., Zhang, T. & Fang, H. H. P. Antibiotic resistance genes in water environment. *Applied Microbiology and Biotechnology* vol. 82 397–414 (2009).

13. Wu, D., Huang, Z., Yang, K., Graham, D. & Xie, B. Relationships between antibiotics and antibiotic resistance gene levels in municipal solid waste leachates in Shanghai, China. *Environ. Sci. Technol.* **49**, 4122–4128 (2015).

5.0 Supplementary information

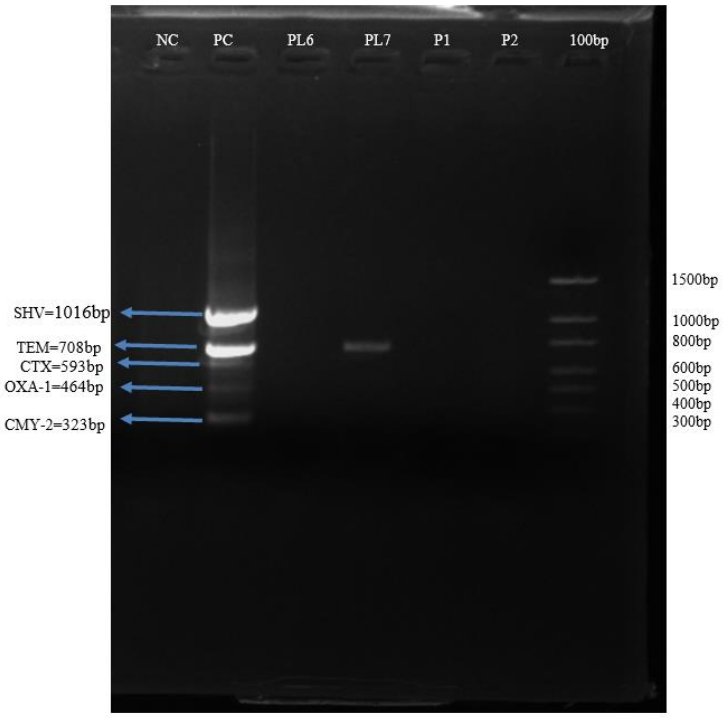

Figure S1: Agarose gel electrophoresis result of multiplex PCR-amplified β-lactamase genes of samples collected from community D, October 2018. Sample ID are indicated on the top of the lanes. Here, NC=negative control; PC= positive control *Klebsiella pneumoniae* N09-00080 carrying five different β-lactamase genes; PL= Polyethylene cistern water; P=Piped water. PL7 showed positive result for bla_{TEM} gene. Molecular sizes are indicated at the right in base pairs (bp).

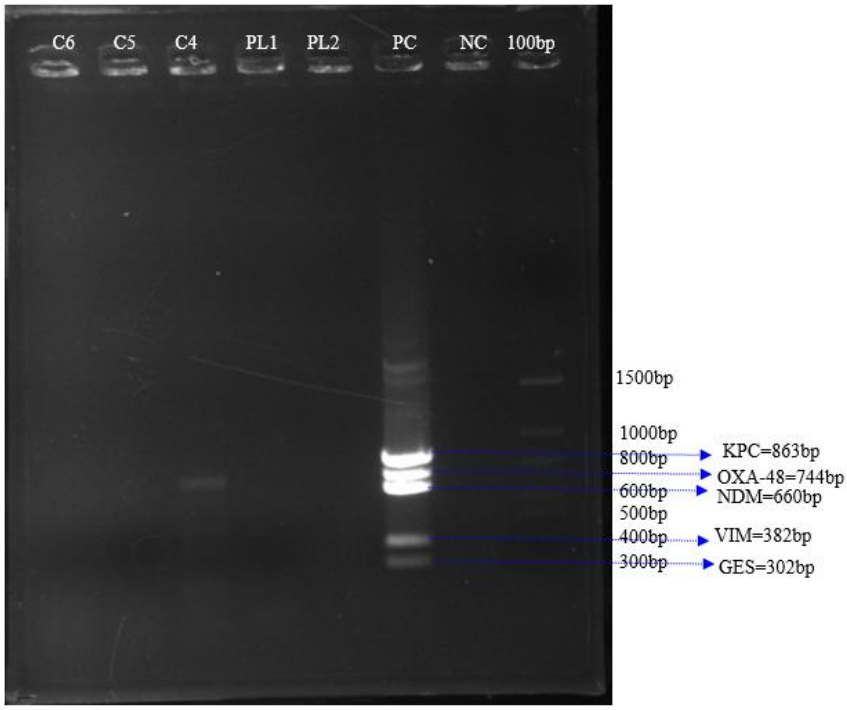

Figure S2: Agarose gel electrophoresis result of multiplex PCR-amplified carbapenemase genes of samples collected from community B, August 2018. Sample ID are indicated on the top of the lanes. Here, NC=negative control; PC= positive controls carrying five different carbapenemase genes; PL= Polyethylene cistern water; C=Concrete cistern water. The gene *bla*$_{IMP}$ was determined by a separate single-point PCR reaction. In this figure, C4 showed positive result for *bla*$_{OXA-48}$ gene. Molecular sizes are indicated at the right in base pairs (bp).