

**Surveillance of antimicrobial resistant pathogens in Canadian retail meat
using a One-Health lens: Linkages to the human sector through the farm-to-
fork continuum**

by

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Abstract

Antimicrobial resistance (AMR) remains a pressing public health issue and has been emphasized in Canadian and global public health priorities. AMR pathogens have arisen and spread across clinical, animal, and environmental sectors, which together can be studied using a One-Health perspective. Using this framework, the farm-to-fork continuum links agri-food and human clinical sectors, where AMR pathogens may spread to humans through the exposure and consumption of retail meat. Beyond foodborne pathogens, retail meat has been noted as a source of AMR pathogens often associated with the nosocomial environment; however, linkages to human clinical infections typically rely on traditional typing methods.

This study reports a low prevalence of *Clostridioides difficile* (0.9%, 3/318), methicillin-resistant *Staphylococcus aureus* (MRSA) (0.9%, 3/318), and varying prevalences of carbapenemase-producing (CP) organisms (*Acinetobacter* spp.: 4.1%, 13/318; *Pseudomonas* spp.: 4.1%, 13/317; *Aeromonas* spp.: 59.8%, 189/316) from retail meat samples collected through the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). When available, whole genome sequencing was used to evaluate genomic linkages between retail meat and human clinical infections collected through the Canadian Nosocomial Infection Surveillance Program (CNISP) or the Canadian Ward Study (CANWARD). Comparisons were performed with *C. difficile*, MRSA, and CP-*Acinetobacter* spp., where retail meat isolates shared sequence types with human clinical isolates, whereas core genome multilocus sequence typing and single-nucleotide variant analysis showed varying degrees of relatedness.

Further genomic investigation identified species-associated, chromosomal carbapenemases across CP-organisms where *cphA*-type, *blaOXA*-type, and *blaPFM*-type/*blaPOM*-1 were identified from *Aeromonas* spp., *Acinetobacter* spp., and *Pseudomonas* spp., respectively. Two *Pseudomonas paracarnis* tested positive for carbapenemase production *in vitro*, but no carbapenemases were identified *in silico*, indicating potential novel carbapenemases in these isolates. All genera harboured additional resistance genes beyond carbapenemases, including the presence of *mcr* in 10/311 *Aeromonas* spp.

Together, this study shows that retail meat is a source of exposure to AMR pathogens along the farm-to-fork continuum, harbouring organisms that have previously been involved in human clinical infections in Canada, and a reservoir of species-associated carbapenemases and other AMR

genes of high importance. Future work is required to demonstrate the direct transmission of AMR pathogens from retail meat to humans.

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List of abbreviations

ACB	<i>Acinetobacter calcoaceticus</i> - <i>Acinetobacter baumannii</i> complex	CMRSA	Canadian MRSA
AMR	antimicrobial resistance	CNISP	Canadian Nosocomial Infection Surveillance Program
AMRNet	Antimicrobial Resistance Network	CP	carbapenemase-producing
AMU	antimicrobial use	CPE	carbapenemase-producing Enterobacterales
ANI	average nucleotide identity	CPO	carbapenemase-producing organism
ARG	antimicrobial resistance gene	CR	carbapenem-resistant
bp	base pair	CT	complex type
BPW	buffered peptone water	DNA	deoxyribonucleic acid
BSI	bloodstream infection	GAP- AMR	Global Action Plan on Antimicrobial Resistance
CA	community-associated	GLASS	Global Antimicrobial Resistance and Use Surveillance System
CARSS	Canadian Antimicrobial Resistance Surveillance System	HA	healthcare-associated
CMRSA	community-associated methicillin-resistant <i>Staphylococcus aureus</i>	KPC	<i>Klebsiella pneumoniae</i> carbapenemase
CC	clonal complex	LA	livestock-associated
CDI	<i>Clostridioides difficile</i>	MALDI- TOF MS	matrix-assisted laser desorption/ionization time- of-flight mass
CDMN	<i>Clostridioides difficile</i> moxalactam norfloxacin	mCIM	Modified carbapenem- inactivation method
cgMLST	core genome multilocus sequence type	MDR	multidrug-resistance
CIM	carbapenem-inactivation method	MGE	mobile genetic elements
CIPARS	Canadian Integrated Program for Antimicrobial Resistance Surveillance	MIA	medically important antimicrobial
CLSI	Clinical and Laboratory Standards Institute	MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>

MLST	multilocus sequence typing
NAP	North American pulsotype
NDM	New Delhi metallo- β - lactamase
NML	National Microbiology Laboratory
OX	Oxford
OXA-48	Oxacillinase-48
PA	Pasteur
PCAP	Pan-Canadian Action Plan
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PFM	<i>Pseudomonas fluorescens</i> metallo- β -lactamase
PHAC	Public Health Agency of Canada
POM	<i>Pseudomonas otitidis</i> metallo- β -lactamase
RT	ribotype
SNV	single nucleotide variant
ST	sequence type
TFA	trifluoroacetic acid
TSA	tryptic soy agar
WGS	whole-genome sequencing

Statement of author contributions

Chapter 1: Introduction

Writing (original draft, review, and editing) was performed by Paula Pidsadny (PP). Writing (review and editing) was performed by Dr. George Golding (GG).

Chapter 2: Materials and methods

Writing (original draft, review, and editing) was performed by PP. Writing (review and editing) was performed by GG. Sections of this chapter (2.1, 2.2.1, 2.4.1, 2.4.2, 2.5, 2.7.1) have been published previously but have been modified through the inclusion of additional detail for this thesis. Sections 2.6.1 and 2.7.2 reflect the original publication with minor grammatical changes. The publication details and author contributions are described below with Chapter 3. Supplementary data from the publication has not been included with this thesis.

Chapters 3-6: Results

The following is applicable to Chapters 3-6:

Data collection, methodology, data analysis, figures and tables, and writing (original draft, review, and editing) was performed by PP. Conceptualization, funding acquisition, project administration, resources, supervision, validation, and writing (review and editing) was performed by GG. Conceptualization, supervision, and writing (review and editing) was performed by Dr. George Zhanel (GZ). Conceptualization, supervision, and writing (review and editing) was performed by Dr. Denice Bay (DB). Conceptualization, funding acquisition, investigation, methodology, resources, and writing (review and editing) was performed by Dr. Richard Reid-Smith (RRS). Data curation, funding acquisition, investigation, methodology, resources, and writing (review and editing) was performed by Dr. Audrey Charlebois (AC). Methodology of pathogen isolation was developed by Derek Tan (DT). A subset of pathogen isolations were performed by DT. A subset of sample preparations for proteomic species identification was performed by James Katipunan (JK), DT, Neil Reyes, and Marco Scerbo (MS). A subset of sample preparations for whole-genome sequencing was performed by MS. Retail meat rinsates were collected and prepared by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS). Mass Spectrometry and Proteomics Core at

the NML provided proteomic identification of unknown isolates. NML Robotics Support Laboratory performed DNA extractions. NML Genomics Core performed Illumina sequencing.

Chapter 3: Surveillance of *Clostridioides difficile* in Canadian retail meat and genomic linkages to community-associated human clinical infections in Canada

This chapter has been published in the Canadian Journal of Microbiology (<https://doi.org/10.1139/cjm-2024-0193>) with minimal modifications from the original publication. Author contributions are as follows: retail meat isolate data curation, formal analysis, investigation, bioinformatic methodology, and writing (original draft, review and editing) was performed by PP. CNISP data curation, CNISP methodology, and writing (review and editing) was performed equally by Tim Du, Romeo Hizon (RH), and Sean Ahmed. Retail meat rinsate pathogen isolation methodology and writing (review and editing) was performed by DT. Conceptualization, supervision, and writing (review and editing) was performed by GZ. Conceptualization, supervision, and writing (review and editing) was performed by DB. Conceptualization, funding acquisition, investigation, methodology, resources, and writing (review and editing) was performed by RRS. Data curation, funding acquisition, investigation, methodology, resources, and writing (review and editing) was performed by AC. Conceptualization, funding acquisition, project administration, resources, supervision, validation, and writing (review and editing) was performed by GG.

Additionally, human clinical isolates for genomic comparisons were provided by the Canadian Nosocomial Infection Surveillance Program (CNISP) and processed by members of the Antimicrobial Resistance & Nosocomial Infections (ARNI) lab at the NML. Audez Bangit performed PCR ribotyping of retail meat isolates. RH performed antimicrobial susceptibility testing (Etest) of retail meat isolates.

Chapter 4: Surveillance of methicillin-resistant *Staphylococcus aureus* in Canadian retail meat and linkages to human clinical infections in Canada

In addition to the contributions pertaining to all results chapters, human clinical isolates for genomic comparisons were provided by CNISP and processed by members of ARNI at the NML. This chapter has been written with the intent to publish its contents in a scientific journal in the future.

Chapter 5: Genomic characterization of carbapenemase-producing-*Pseudomonas* spp. and *Acinetobacter* spp. in Canadian retail meat and linkages to human clinical infections in Canada

In addition to the contributions pertaining to all results chapters, JK performed the majority (>90%) of phenotypic carbapenemase tests in this section. PP performed the remainder of phenotypic carbapenemase tests. Human clinical isolates for genomic comparisons were provided by CNISP and the Canadian Ward Study (CANWARD) and processed by members of ARNI at the NML.

This chapter has been written with the intent to publish its contents in a scientific journal in the future.

Chapter 6: Genomic characterization of carbapenemase-producing *Aeromonas* spp. isolated from Canadian retail meat

In addition to the contributions pertaining to all results chapterse, JK performed the majority (>90%) of phenotypic carbapenemase tests in this section. PP performed the remainder of phenotypic carbapenemase tests.

This chapter has been written with the intent to publish its contents in a scientific journal in the future.

Chapter 7: Final summary and discussion of thesis findings, limitations, future directions, and conclusions

Writing (original draft, review, and editing) was performed by PP. Writing (review and editing) was performed by GG.

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The publication has been modified to provide additional detail and fit the thesis style in sections 2.1, 2.2.1, 2.4.1, 2.4.2, 2.5, and 2.7.1 of this thesis. The publication is presented as-is with minimal grammatical modification in sections 2.6.1, 2.7.2, and Chapter 3 of this thesis. Supplementary data from the publication has not been included in this thesis.

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Chapter 1: Introduction

1.1 Overview of antimicrobial resistance

Antimicrobial resistance (AMR) is an increasingly overwhelming and ongoing public health issue both in Canada and globally. In contrast to recent public health crises like the COVID-19 pandemic, AMR has been described as a silent pandemic, where antimicrobials have been rendered ineffective against microorganisms due to an array of resistance mechanisms¹. Once-treatable infections are becoming resistant, with the burden of AMR felt in the healthcare system through increased risks associated with medical procedures and treatment, as well as economic impacts across different sectors^{2,3}. While AMR is a phenomenon observed in bacteria, viruses, fungi, and parasites, this study investigates AMR bacteria in retail meat; therefore, this review will focus on AMR in bacteria.

AMR is a naturally occurring phenomenon where bacteria can persist in the presence of antimicrobial compounds that would otherwise be used to treat infections and control their spread. Bacteria can have innate mechanisms of AMR through an absence of antimicrobial targets or through the presence of an intrinsic (chromosomal) resistance mechanism that serves a normal metabolic function⁴. They can also become resistant to antimicrobials through random genetic events that offer a fitness advantage in the presence of selective pressures⁴. These random genetic events include random chromosomal mutations or the acquisition of specialized resistance genes through horizontal transmission from other organisms (transduction, transformation, conjugation, transposition), both of which can be passed down to progeny once acquired⁴. Through selective pressure from antimicrobial compounds, bacteria that harbour one or more of these resistance mechanisms can persist in higher concentrations of antimicrobials, thus ‘selecting’ for an AMR population over one which is antimicrobial-susceptible⁵. Selective pressures exist naturally in the environment, as environmental organisms can produce antimicrobial compounds⁶; therefore, it is no surprise that AMR determinants can also be found in other environmental bacteria. Selective pressures can also be found in the environment as contaminants of human activity where mining⁷, landfills⁸, and pesticide use⁹ have been linked with the presence of AMR organisms. These activities can contribute to AMR, and human-affected environments have been shown to have distinct AMR profiles in comparison to pristine environments¹⁰.

Despite intrinsic resistance mechanisms in some organisms, the spread and propagation of

AMR is a human-driven phenomenon, with associations observed between antimicrobial use (AMU) and AMR¹¹. Other sociopolitical and socioeconomic factors have also been associated with AMR, including population density, national regulations, and global influence and accountability¹¹. It has been suggested that these socioeconomic and sociopolitical factors can have important effects on AMR rates, and that antimicrobial use is only one of several AMR drivers^{11,12}. The spread of AMR is therefore a complex and multifaceted issue, and approaches beyond limiting AMU such as surveillance, infection prevention and control, antimicrobial stewardship, and research and innovation are necessary to address this public health crisis¹³.

While AMR has been a concern since the introduction of antimicrobials in the late 1930s⁶, it remains a pressing public health issue that left unaddressed, will continue to worsen in coming years. In 2019, it was estimated that AMR was associated with 4.95 million deaths globally, while 1.27 million deaths could be attributed to AMR annually¹⁴. More recent estimates have determined there were 4.71 million AMR-associated deaths and 1.14 million AMR-attributable deaths in 2021¹⁵. In Canada, the burden of AMR can be felt through patient mortality and financial impacts. There were an estimated 14,000 deaths linked to first-line-resistant AMR infections (resistant to the first antimicrobial typically chosen for treatment) in 2018, with 5,400 deaths being AMR-attributable, and deaths from first-line-resistant infections accounted for 1 in 19 deaths in Canada in 2018¹³. The costs associated with hospitalization and loss in production due to disability and premature mortality were estimated to be between \$143.8 and \$271.95 million dollars in an analysis of just two pathogens: methicillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridioides difficile*¹⁶.

Recent global forecasts of AMR rates predict an increase to 8.22 million AMR-associated deaths and 1.91 million AMR-attributable deaths in the year 2050 without increased efforts in addressing this public health crisis¹⁵. Thus, AMR requires an urgent and multifaceted response strategy which can include measures such as antimicrobial stewardship, surveillance, novel treatments, regulations, and shared priorities on both national and global scales.

1.2 Addressing AMR: Global and Canadian priorities

To address AMR on a global level, the World-Health Organization established a Global Action Plan on AMR (GAP-AMR) in 2015. This action plan has five objectives related to AMR: awareness and understanding, surveillance and research, infection prevention and control,

antimicrobial stewardship in humans and animals, and investment into medicine and diagnostics¹⁷. Additionally, the WHO released a document in 2017 that prioritized bacterial AMR pathogens that urgently required research and development of novel antimicrobials, with the aim of guiding both public and private research and development¹⁸. This list was recently updated in 2024, where the new document incorporates improvements to surveillance systems and their surveillance data, reflects a better understanding of global AMR, and addresses limitations from the original list. This document also provides more specific aims and scope, which can be used to lead efforts to address their priority list of AMR pathogens¹⁹.

In a similar vein, the Public Health Agency of Canada (PHAC) published the Pan-Canadian Action Plan on Antimicrobial Resistance (PCAP) in 2023, a document outlining Canada's five-year commitment to address AMR through five pillars of action: research and innovation, surveillance, stewardship, infection prevention and control, and leadership. Within each pillar of action are desired outcomes and actionable steps, with strategies that can be used to address AMR²⁰.

The PCAP pillar of AMR surveillance aims to establish and expand upon One-Health surveillance infrastructure in Canada for the capture and timely use of both AMR and AMU data across sectors. The second aim of this pillar is to understand AMR and AMU across different sectors and scales in order to implement and measure intervention outcomes²⁰. Fortunately, these aims build upon existing surveillance systems, where Canada continues to perform surveillance activities on AMR and AMU. These surveillance systems aid our understanding of the historical and current context, and as suggested with PCAP, can be leveraged for future applications.

1.3 Surveillance of AMR in Canada

1.3.1 Surveillance systems

Surveillance is essential in understanding the burden and dynamics of AMR in Canada, with several systems existing to capture different AMR pathogens, demographics, and sectors. In general, the coverage and scope of these systems requires collaboration between partners. The following surveillance systems have linkages to different federal agencies; however, AMR surveillance can also occur within provincial, territorial, and regional institutions. The surveillance systems summarized in the sections below are key to understanding AMR in Canada, especially in the context of common nosocomial pathogens, but are not an exhaustive list of Canadian

surveillance systems that might capture AMR pathogens.

1.3.1.1 CNISP: The Canadian Nosocomial Infection Surveillance Program

The Canadian Nosocomial Infection Surveillance Program (CNISP) captures AMR data from a sentinel hospital system across Canada, integrating both patient and microbiological data to monitor important AMR infections in Canada. Several pathogens and infection types are captured through CNISP, including *Candida auris*, carbapenemase-producing (CP) organisms (CPOs), *C. difficile* infections (CDI), MRSA bloodstream infections (BSI), and vancomycin-resistant *Enterococcus* BSI. Additionally, CNISP collects data on AMU and hospital antibiogram data²¹. Data is reported directly to hospitals, and more broadly, CNISP disseminates surveillance data in the form of publications, including a publicly available annual report²² and interactive public-facing dashboards²¹. Surveillance data from CNISP has informed evidence-based policy, infection prevention and control guidelines, and provides data to the Canadian Antimicrobial Resistance Surveillance System (CARSS) and the Global Antimicrobial Resistance and Use Surveillance System (GLASS), which updates national and international stakeholders in AMR²³.

1.3.1.2 CIPARS: The Canadian Integrated Program for Antimicrobial Resistance Surveillance

The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) is a system that both captures AMU data and performs surveillance of AMR enteric bacteria in animal and human reservoirs. Using active and passive surveillance methods, CIPARS focuses their efforts on humans, animals, and food, collaborating with stakeholders across sectors to capture AMU and AMR data across Canada. Data collected through CIPARS is integrated and used to evaluate trends in AMR and AMU, identify linkages, detect novel resistance, and inform evidence-based policies²⁴.

1.3.1.3 AMRNet: The Antimicrobial Resistance Network

The Antimicrobial Resistance Network (AMRNet) is a surveillance system developed by the National Microbiology Laboratory (NML) in a collaborative effort between PHAC and provincial and territorial organizations. Taking an integrative approach to surveillance, AMRNet aims to capture data from both humans and animals, focusing on the collection of antimicrobial susceptibility testing data from fungal and bacterial pathogens across Canadian human and

veterinary labs. AMRNet is a relatively young surveillance system, with routine data collection in select provinces commencing in 2022. AMRNet aims to build upon existing surveillance systems such as CIPARS, but focuses their efforts on addressing previously unfilled gaps such as smaller hospitals, long-term care, and community settings. Future aims of this network includes the integration of data from other Canadian surveillance systems as part of a One-Health approach to AMR surveillance in Canada^{25,26}.

1.3.1.4 CANWARD: The Canadian Ward Surveillance Study

CANWARD is an ongoing surveillance study focused on AMR pathogens collected from sentinel hospital wards across Canada. The study is a collaborative effort between the Canadian Antimicrobial Resistance Alliance and Health Canada, evaluating both pathogen epidemiology and AMR in isolates obtained from human infection sites and various hospital wards²⁷. CANWARD isolate collection targets wound, urinary, respiratory, and BSIs, as opposed to targeting specific pathogens. Several notable AMR pathogens have been recorded through this study, including MRSA, VRE, extended-spectrum β -producing *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, among others, with the focus of antimicrobial susceptibility testing²⁸. CANWARD collects and reports longitudinal data, allowing for global comparison with other countries²⁷.

1.3.1.5 CARSS: The Canadian Antimicrobial Resistance Surveillance System

CARSS integrates data across surveillance systems to report AMR and AMU from humans, animals, and agri-food sectors. Several surveillance systems discussed previously contribute to CARSS: AMRNet, CIPARS, and CNISP, among other surveillance systems not discussed²⁹. In addition to providing an annual report, they also provide an interactive dashboard to disseminate information³⁰. By integrating data across from different surveillance systems, CARSS provides a comprehensive report of AMR and AMU across different Canadian sectors.

1.3.2 Human surveillance of top priority AMR pathogens in Canada

AMR surveillance systems in Canada encompass a range of resistant phenotypes and pathogens which are in line with national and global priorities. In 2015, PHAC released a list of priority AMR pathogens to inform surveillance efforts, research, and interventions in Canada. Using public and internal surveillance data, pathogens were scored using 10 different criteria.

Results from this assessment ranked extended-spectrum β -lactamase producing and carbapenem-resistant (CR) *Enterobacteriaceae*, *C. difficile*, and MRSA as the highest priority³¹. This assessment is in line with global priorities, where the WHO Bacterial Priority Pathogen List has ranked carbapenem and third-generation cephalosporin-resistant *Enterobacteriaceae*, as well CR-*Acinetobacter baumannii* as critical priority for the discovery, research and development of new antibiotics¹⁹. These priority lists aid in informing public health decision making, including targeted surveillance efforts to assess trends surrounding a given pathogen. A key aspect of AMR surveillance are the methods and tools used to monitor, identify, and characterize a given pathogen. Below, current methods of bacterial identification, characterization, and typing for *C. difficile*, MRSA, and CPOs are reviewed.

1.3.2.1 Bacterial identification, typing, and characterization

Several laboratory methods can be used for the surveillance of AMR pathogens, many of which are tailored to specific pathogens and AMR mechanisms of interest. Standards in surveillance methods may vary by country or even by institute, depending on local expertise and resources. In general, essential steps of AMR surveillance are identification, typing, and characterization.

1.3.2.1.1 Bacterial identification with MALDI-TOF MS

Bacterial identification is the initial step in bacterial AMR surveillance, and several approaches can be used which may vary by laboratory or target organism. In general, these approaches can be biochemical, molecular, or proteomic³². This review focuses on matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), a common proteomic method of bacterial identification in clinical laboratories.

MALDI-TOF MS is a proteomic method of bacterial identification. In general, this method involves desorption and ionization of a bacterial sample that is crystallized within a matrix followed by the acceleration and separation of protonated ions by their mass-to-charge ratio. Using the time of flight, a peptide mass fingerprint is generated, which can then be compared to a database of known peptide mass fingerprint spectra to identify the bacterial isolate. Bacterial identifications are based primarily on ribosomal proteins due to their abundance³³. MALDI-TOF MS is a desirable method for its accuracy, ability to identify a broad number of species, speed of identification, and low cost³⁴. In a clinical setting, it can facilitate rapid identification of pathogens

to determine subsequent treatment methods³³. Conversely, this method may not be suitable when distinguishing closely related or rare species as a potential lack of reference spectra for a given organism may lead to a misidentification³⁵. Researchers and clinicians may therefore perform additional testing for reliable identification depending on their suspected isolate and application.

1.3.2.1.2 Bacterial typing with multilocus sequence typing

Multilocus sequence typing (MLST) is a molecular typing method that has been widely adopted. Traditionally, this method involves the PCR amplification of 450-500 bp fragments of seven to eight housekeeping genes, followed by DNA sequencing of PCR amplicons. Based on the sequence of amplicons, DNA fragments are assigned an allele number, with a given combination of alleles corresponding to a sequence type (ST)^{36,37}. MLST has proven to be an attractive method for researchers due to its highly portable nature and ease of comparison across laboratories, with a common curated database (PubMLST) serving as a central repository for STs and their associated alleles³⁸⁻⁴⁰. This method has a moderate resolution, which while not as discriminatory as whole-genome-based methods, is sufficient for many applications⁴¹. STs can be further grouped into clonal complexes (CC), typically referring to a group of closely related STs⁴². This typing strategy has been important in identifying and tracking global clones of importance and has allowed for the study of global strains with insights into bacterial evolution^{39,43}.

Despite these advantages, MLST is not without its drawbacks, with an important factor being the cost associated with DNA sequencing³⁹. However, with the increased accessibility of whole-genome sequencing (WGS), MLST can easily be implemented *in silico* into a genomic workflow, therefore this method will likely continue to be used by researchers.

1.3.2.1.3 Whole-genome sequencing for bacterial AMR surveillance

WGS is a powerful tool that can be used for bacterial typing and characterization, offering the highest level of discriminatory power and information to researchers³⁶. WGS can be employed in several ways for bacterial surveillance, including through genomic typing methods, for bacterial phylogenies, and to gain additional insight into AMR genes (ARGs), virulence factors, and mobile genetic elements (MGE), which are essential components of bacterial AMR surveillance. Below, common WGS-based methods which are used in bacterial AMR surveillance are discussed.

1.3.2.1.3.1 Core genome multilocus sequence typing

As discussed previously, MLST, based on seven to eight housekeeping genes, is a common typing method that can be achieved via whole-genome sequences. Core genome MLST (cgMLST) uses the rationale of MLST, but increases the discriminatory power of this method through the inclusion of a large number of genes in a given scheme. In this method, a curated set of core genes for a given species are defined in a cgMLST scheme, and complex types are assigned in a similar manner as MLST, where a combination of alleles across the entire scheme corresponds to the complex type. cgMLST can further be used to infer phylogenetic relationships through the generation of minimum spanning trees or neighbour-joining trees³⁶.

Unlike MLST, there is no central platform for hosting cgMLST schemes, with multiple schemes existing across platforms for select organisms. For example, Enterobase (<https://enterobase.warwick.ac.uk/>) and Ridom SeqSphere+ (<https://www.cgmlst.org/ncs>) both host separate schemes for *Clostridioides*⁴⁴ and *C. difficile*⁴⁵, respectively. With several platform-specific schemes used, complex types do not translate across schemes, limiting the portability of this method³⁶. Despite this limitation, cgMLST has proven to be a useful tool for outbreak investigation and the identification of pathogen transmission⁴⁶.

1.3.2.1.3.2 Single nucleotide variant analysis

In single nucleotide variant (SNV) analysis, nucleotide variants between bacterial isolates are identified in order to delineate samples in an analysis⁴⁷. Both reference-free and reference-based methods exist, each with different advantages and considerations. In reference-based analyses, reads are mapped against a chosen reference genome to determine variants in query sequences. When using a reference, there should be careful consideration for the reference genome used, with the potential for different reference sequences to influence phylogenetic outcomes³⁸. In a reference free SNV analysis, K-mers, or short strings of nucleotides of a specified length, are compared across genomes³⁸. Using SNV data to generate a distance matrix, further computation can be performed to calculate a phylogeny³⁸. Importantly, with the discriminatory power of SNV analysis comes challenges in interpreting results and phylogenetic relationships with a notable challenge being the determination of appropriate SNV thresholds to conclude genetic relatedness. Standardized SNV thresholds for a given species remains a limitation of this method, however relatedness and transmission can be strengthened with the implementation of epidemiological data

to draw conclusions³⁸.

1.3.2.1.3.3 Antimicrobial resistance and mobile genetic element detection

A major advantage of WGS is the detail of genomic information that can be gained beyond the typing methods discussed previously. Key information into organism resistance, virulence, and insights into MGEs can be gained using WGS-based tools. This *in silico* characterization complements typing methods in AMR surveillance efforts and provides insights into clones of interest. In general, two different approaches are used for *in silico* AMR prediction: the detection of DNA mutations that give rise to a resistant phenotype, and the detection of ARGs using a reference database. There are several AMR detection tools that have different functionalities, such as AMRFinderPlus⁴⁸, which uses BLAST-based tools to search nucleotide or amino acid sequences against the Reference Gene Database. This tool also has additional functionalities, including the use of hidden markov models and the identification of resistant point mutations, depending on the input and parameters desired. Staramr⁴⁹ is another BLAST-based AMR identification tool, with additional MLST and plasmid-prediction functionalities. Staramr relies on two curated databases in order to make AMR predictions: ResFinder⁵⁰ for ARGs, and PointFinder⁵¹ for point mutations, although PointFinder functionality is only available for specific organisms. In contrast to AMRFinderPlus, Staramr does not accept amino acid queries⁴⁹. It should be noted that this is not an exhaustive list of all ARG identification tools and databases⁵², and factors such as updated and comprehensive databases may influence ARG predictions by a given tool.

In addition to *in silico* ARG prediction, MGEs can add another dimension to bacterial AMR surveillance. MGEs such as plasmids, insertion sequences, and transposons have been associated with ARGs, facilitating their transfer from one host to the next⁵³. While AMR prediction can be relatively straight-forward, MGE prediction can be challenging due to the presence of repetitive regions, therefore hybrid assemblies that include both short and long-read sequencing can be used to address this issue⁵⁴.

One comprehensive tool that can be used to detect plasmids and other MGEs is MOB-suite, a plasmid prediction and typing tool which has three different functions that enable plasmid typing and characterization through the use of mash distances and reference databases, plasmid reconstruction, and plasmid clustering⁵⁵. MGE prediction tools such as MOB-suite can add another dimension to bacterial AMR surveillance, providing context and insight into the potential mobility

of ARGs of interest.

1.3.2.2 Introduction of priority AMR pathogens

As discussed previously, *C. difficile*, MRSA and CPOs are among the top priority AMR pathogens in Canada and globally. The methods discussed above are general and apply to some or all of these priority AMR pathogens, however the surveillance of each of these pathogens also includes organism-specific methods. Below, the top priority AMR pathogens *C. difficile*, MRSA, and CPOs are described, along with organism-specific methods of typing and characterization which are useful in the surveillance of these AMR pathogens.

1.3.2.2.1.1 *C. difficile* overview

C. difficile is a Gram-positive, spore-forming anaerobe, commonly associated with hospital-acquired diarrhea and known as the causative agent of pseudomembranous colitis^{56,57}. Most CDI are associated with antibiotic use, however they are also associated with other risk factors, including acid-suppressant medications, age, hospitalization, and immunosuppression⁵⁸. In the instance of antibiotic use, dormant spores can germinate due to the reduced microbial competition, leading to CDI⁵⁸. Globally, CDI is most prevalent in North America, with highest incidences being reported from Poland and the US^{59,60}. In Canada, *C. difficile* incidence is declining, however it remains a common pathogen associated with both hospital and community acquisition⁶¹.

C. difficile are known to colonize the gastrointestinal tracts of humans and other mammals⁵⁶, with some strains equipped to cause infections. Toxins produced by *C. difficile* are the primary contributors to disease symptoms: toxin A (TcdA), toxin B (TcdB), and *C. difficile* binary toxin (CDT). TcdA/B are known to damage the intestinal epithelium resulting in a loss of barrier function, and the binary toxin enhances their effects by increasing the adherence of this pathogen^{62,63}. *C. difficile* can harbour all, none, or some of these toxins, but toxigenic strains are considered to have TcdB, or more commonly, both TcdA and TcdB⁶⁴. Additionally, *C. difficile* both toxigenic and non-toxigenic can be carried by asymptomatic individuals⁶⁵, adding complexity to understanding the epidemiology of this pathogen.

C. difficile is also associated with AMR and has been reported most commonly as resistant to second generation fluoroquinolones and clindamycin, and rarely resistant to vancomycin,

fidaxomicin, and metronidazole, among other antimicrobials⁶⁶. Among the challenges in understanding this pathogen are the genetic determinants of resistance, where *C. difficile* can harbour a variety of resistance mechanism beyond acquired ARGs. For example, resistance to fluoroquinolones is typically attributed to mutations to the housekeeping gene *gyrA*, whereas resistance to metronidazole can be plasmid-mediated or attributed to mutations in FeoB1 and IscR, reducing the activation of metronidazole⁶³. Resistance to the first-line antimicrobial vancomycin can be attributed to the presence of *van* genes, plasmid-mediated resistance decreasing membrane permeability, gene mutations, or other more general mechanisms of resistance⁶⁷. Despite a lack of association with a specific ARGs, *C. difficile* continues to be an important AMR organism with diverse and complex genetic determinants of AMR.

1.3.2.2.1.2 Molecular epidemiology of *C. difficile* in Canada

Recent reports from CNISP have shown *C. difficile* remains the most common AMR infection captured through this surveillance system, with 3,846 cases captured over a two-month surveillance period in 2022²². Of the subset of 399 isolates tested, resistance to clindamycin (23.6%), moxifloxacin (7.3%), and rifampin (1.0%) was observed. Over the five-year study period (2018-2022), there was a non-significant 7% decrease in CDI. Interestingly, while CDI is associated with acquisition in hospitals or healthcare environments, healthcare-acquired CDI (HA-CDI) has decreased in most regions, while infections determined to be community-acquired CDI (CA-CDI) remained stable over the five-year study period²².

1.3.2.2.1.3 Methods used in *C. difficile* surveillance and characterization

C. difficile surveillance can include general typing methods discussed previously, such as MLST, which is based on the seven housekeeping genes *adk*, *atpA*, *dxr*, *glyA*, *recA*, *soda*, and *tpi* in *C. difficile*⁶⁸. In addition to this and other WGS-based methods, researchers commonly use two traditional techniques: pulsed-field gel electrophoresis (PFGE) and PCR ribotyping.

1.3.2.2.1.3.1 Pulsed-field gel electrophoresis

PFGE was once considered the gold standard in bacterial typing⁶⁹. In this method, the bacterial genome is digested with restriction endonucleases, resulting in long DNA fragments which are separated using an alternating pulsed field. This separation generates a banding pattern that corresponds to a given pulsotype^{37,69}. As a bacterial typing method, PFGE has several

advantages including its low cost and wide availability. However drawbacks of this method includes challenges in pathogen-specific standardization and inter-laboratory comparisons, as well as the time and skill required in order to execute this method^{37,38}.

Despite these limitations, PFGE has been used for bacterial typing of several pathogens including *C. difficile*⁶⁹. In Canada and elsewhere in North America, *C. difficile* have been typed according to their PFGE types, commonly known as North American pulsotypes (NAP), which has been proven useful in identifying outbreaks and transmission of *C. difficile*⁷⁰. PFGE and the use of NAP types is commonly used in North America, whereas PCR ribotyping has been predominant in Europe, which may present challenges in international comparisons³⁹. Furthermore, while PFGE was once the predominant method used for *C. difficile* typing in Canada and the US, it has been replaced by molecular and genomic methods³⁹.

1.3.2.2.1.3.2 PCR ribotyping

PCR ribotyping is a typing method that involves the PCR amplification of the 16S-23S intergenic spacing region, and gel electrophoresis is used to produce the resulting banding pattern that corresponds to a given ribotype³⁹. PCR ribotyping has several advantages, including its speed, cost, and ease of comparisons between laboratories due to standardized nomenclature⁷¹. These advantages come with drawbacks, in particular with limitations in discriminatory power and challenges in data interpretation³⁹. It should be noted that while PCR ribotyping has been the predominant typing method of *C. difficile* in Europe, this method has been adopted by researchers in North America^{61,72}.

1.3.2.2.2 Methicillin-resistant *Staphylococcus aureus* (MRSA)

1.3.2.2.2.1 MRSA overview

S. aureus is a Gram-positive organism that can be found as part of the normal human microbiome and as an opportunistic pathogen than can cause serious infections such as endocarditis, bacteremia, osteomyelitis, and skin and soft tissue infections⁷³. *S. aureus* is regarded as an important AMR pathogen due to the acquisition of the *mecA* gene, a penicillin-binding protein 2a that confers resistance to methicillin and nearly all other β -lactam antimicrobials, known as MRSA⁷³. *mecA* is located on the staphylococcal cassette chromosome (SCC*mec*), which houses site-specific recombinases allowing for the transmission of this genetic element⁷³. While SCC*mec*

is transmissible, it should be noted that the spread of MRSA is mostly due to the clonal transmission of strains and not solely due to the transmission of this gene, with important known MRSA epidemic strains in circulation^{73,74}. This is unsurprising, as MRSA is a highly transmissible pathogen, where contact with any contaminated object that comes in contact with skin could lead to its transmission, thus increasing the risk of infection⁷³.

1.3.2.2.2.2 Molecular epidemiology of MRSA in Canada

In Canada, MRSA infections are captured through CNISP surveillance, however it is important to note that only BSIs are recorded. Recent reports from CNISP have shown a 2.9% decrease in the rate of infection from 2018-2022, with 820 infections recorded in 2022. Stratified by infection type, differences in infection rates can be observed for healthcare associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA), with steady rates of HA-MRSA, but a 12% increase in CA-MRSA over the study period. Between the two case types, clindamycin resistance was more commonly associated with HA-MRSA, however no additional difference in AMR phenotypes were observed between HA-MRSA and CA-MRSA²².

1.3.2.2.2.3 Methods used in MRSA surveillance and characterization

While MRSA in Canada are now characterized by ST and *spa* type (described below), they were previously characterized by their PFGE epidemic CMRSA1-10 types (Canadian MRSA)⁷⁵. The transition away from PFGE and CMRSA epidemic types can present challenges when comparing past and present epidemic types, however there are associations between CMRSA types and current typing methods like MLST³⁷ and *spa* typing⁷⁶.

1.3.2.2.2.3.1 *spa* typing

spa typing is a common method used in MRSA characterization, which is based on the sequence of protein A. Similar to MLST, where conserved housekeeping genes are used for typing, the *spa* gene is conserved in *S. aureus*, however with this method, typing relies on a single locus utilizing variable-number tandem repeats in the repeat region of protein A. This region contains a variable number of repeats that range from 21-27 bp in length, with a given repeat corresponding to an arbitrary value. The number and organization of repeats then correspond to a *spa* type, which can be used for epidemiological purposes³⁷.

spa typing is an attractive method for several reasons, including its efficiency and cost

effectiveness. Across laboratories, standard nomenclature is used, with a central database hosted by ridom.spaserver.de⁷⁷ allowing for inter-laboratory comparison and reproducibility³⁷. Similar to MLST, *spa* typing, once performed solely via Sanger sequencing, can be performed through WGS and has remained a relevant MRSA typing tool in the genomics era⁷⁸. While this method has a role in epidemiological investigation⁷⁹, the potential for false transmission links has been highlighted by the use of more discriminatory WGS-based methods⁸⁰.

1.3.2.2.3 Carbapenemase-producing organisms (CPOs)

1.3.2.2.3.1 CPO overview

CPOs encompass any organism that produces a carbapenemase, but is most commonly associated with members of the order Enterobacterales in addition to other Gram-negative organisms including *P. aeruginosa* and *A. baumannii*^{81,82}. CPOs present a serious threat to human medicine, as carbapenems are a last-resort antimicrobial used to treat complicated Gram-negative infections and as empiric treatment to serious infections such as extended-spectrum β -lactamase-producing organisms^{81,83}. CR-Enterobacterales, which includes select CPOs, have been deemed the most critical pathogens for the development of novel antibiotics by the World Health Organization¹⁸. Currently, CR infections require the use of less desirable antimicrobials, such as polymyxins or fosfomycin, or the use of combination therapy with or without antimicrobials⁸⁴.

CPOs can be distinguished from CR-organisms as they present a unique and urgent threat. Carbapenemases are β -lactamases that confer resistance to nearly all β -lactam antimicrobials through hydrolysis, resulting in a high minimum inhibitory concentration⁸⁴. When first identified, carbapenemases were known as species-associated genes that were located on the chromosome and spread through clonal transmission. The emergence of plasmid-mediated carbapenemases added a novel dimension to this resistance, where interspecies transmission is now a concern⁸⁵. Clinically important carbapenemases now include both chromosomal species-associated carbapenemases, such as NmcA and SME, however the most notorious and prolific carbapenemases are highly mobile interspecies genes such as *K. pneumoniae* carbapenemase (KPC), which is found on self-conjugative plasmids⁸¹.

Carbapenemases can be broadly categorized into β -lactamase Ambler classes A, B, and D, which are based on their amino acid sequences⁸⁶, as well as into Bush functional classes based on their active sites⁸⁷. Carbapenemases in classes A and D contain a serine in their active site, while

class B carbapenemases are known as metalloenzymes and contain a zinc in their active site⁸⁵. All classes contain both chromosomal and plasmid-located carbapenemases⁸⁵.

1.3.2.2.3.2 Molecular epidemiology of CPOs in Canada

While this study broadens its focus to all CPOs, Canadian surveillance of carbapenem resistance reports primarily on CP-Enterobacterales (CPEs). Canada remains a country with a low rate of CPE infections, however in recent years, these numbers have increased by 133% from 2018-2022 in Canadian hospitals. Common carbapenemases detected in Canada were KPC, New Delhi metallo- β -lactamase (NDM), and Oxacillinase-48 (OXA-48) with *E. coli* being the most common CPE detected in this study²². While previous reports of extensively-drug-resistant CPE from 2019-2020 identified international travel as a risk factor for CPE infection, CNISP data from 2020-2022 show that 85.3% of cases were acquired domestically^{22,88}.

1.3.2.2.3.3 Methods of carbapenemase detection

There are two general approaches to carbapenemase detection: molecular methods and phenotypic methods. While molecular PCR-based methods can be useful, they are limited to their target genes, therefore there is opportunity for rare or novel carbapenemases to be missed with this method⁸⁹. Several phenotypic approaches to carbapenemase detection exist, including inhibitory methods, hydrolysis-based methods, and lateral flow immunoassays⁹⁰. Below, an inhibitory method, the modified carbapenemase inactivation method (mCIM), and a hydrolysis-based method, Carba NP, and their variants are discussed.

1.3.2.2.3.3.1 The modified carbapenemase inactivation method and its variants

mCIM is a simple method of carbapenemase detection that is recommended for carbapenemase testing of Enterobacterales by the Clinical and Laboratory Standards Institute (CLSI)⁹¹. This is an improved method of the previously recommended carbapenem inactivation method (CIM), with the same rationale behind the two methods. In this method, a meropenem disk is incubated in an aqueous solution with the organism of interest. If the organism is carbapenemase-positive, the carbapenemase produced will hydrolyze the meropenem present in the disk. Following an incubation period, the disk is placed on a lawn of susceptible *E. coli*. A carbapenemase-positive phenotype is reflected by a reduced zone of inhibition resulting from the hydrolyzed meropenem⁹². This is a simple and cost effective method, is easily interpretable, and

is effective for detecting all carbapenemase types, however the time to results is longer than other methods⁹³.

While mCIM is a common method used for carbapenemase detection in Enterobacterales and *P. aeruginosa*, it is not recommended for *A. baumannii*. To address this limitation and improve upon mCIM, variant methods have been suggested such as CIMTris, which uses Tris-HCl and has a reduced incubation time⁹⁴, or the simplified carbapenem inactivation method (sCIM), which involves smearing the culture directly on the meropenem disk⁹⁵, among others.

1.3.2.2.3.3.2 The Carba NP method

The Carba NP method takes a different approach to carbapenemase detection using the hydrolysis of imipenem to identify CPOs. In this method, a red to orange/yellow colour change is used to indicate the presence of a carbapenemase⁹⁶. This is another common method, however Carba NP fails to accurately capture OXA-48 carbapenemases. Additionally, limitations of this method include the time and equipment required to prepare reagents yet it remains fast and cost-effective, with same-day results available in contrast to mCIM's required overnight incubation⁹⁷.

1.4 One-Health

One-Health is a concept that can be applied to several fields of study, however increasingly, the One-Health framework has become essential for the study and management of AMR. One-Health has been defined in several ways, but at its core, it emphasizes the relationships between the health of humans, animals, and our environment, calling for a collaborative effort between sectors to address health issues such as AMR⁹⁸. One-Health can be integrated into all aspects of AMR action plans, from research and surveillance, to antimicrobial stewardship and policy. One-Health is central to PCAP, emphasizing the need for collaboration to address AMR in Canada²⁰.

A One-Health approach to AMR research and surveillance allows for sector-specific study, as well as the identification of linkages between sectors, suggesting the potential transmission of ARGs or pathogens between two reservoirs. Using this approach can reveal how practices in one sector may influence AMR in another, such as the use of important antimicrobials in both human and animal sectors⁹⁹.

1.4.1 Livestock as important contributors to AMR

Livestock have been identified as important contributors to the spread of AMR. AMR

organisms have been isolated from livestock and their environments^{100,101}, which has largely been attributed to the use of antimicrobials in livestock¹¹. Animals are important consumers of antimicrobials in Canada, where 82% of all antimicrobials sold were intended for use in production animals, compared to only 17% being sold for use in humans in 2020. Adjusting for biomass, this represents a use in animals that is 1.8 times more than use in humans¹⁰². Similar to humans, antimicrobials may be administered to animals for therapeutic or prophylactic use; however, historically antimicrobials had an important role as growth promoters of livestock⁹⁹.

Growth promotion is the practice of administering sub-therapeutic doses of antimicrobials to increase the productivity of animals¹⁰³. The effects of growth promotion have clearly been illustrated in other countries, with one such example being the use of the glycopeptide avoparcin in Denmark. Avoparcin was previously used as a growth promoter in nearly all feed of broiler chickens and pigs. At the same time, there were high levels of vancomycin-resistant *Enterococcus faecium* from livestock. A ban prior to a voluntary withdrawal of avoparcin use by farmers was followed by a drop in resistant *E. faecium* isolates from broiler chickens and pigs, illustrating the cause and effect of growth promotion. Furthermore, this example demonstrated how excessive antimicrobial use in livestock may select for clinically important AMR organisms such as vancomycin-resistant *E. faecium*¹⁰⁴.

Antimicrobial growth promotion has been long banned elsewhere, such as a total ban on antibiotics as growth promoters in 2006 in Europe¹⁰⁵. Other countries, including Canada, were later to legislate the use of antimicrobials as growth promoters. In Canada, regulations were implemented in 2018 surrounding antimicrobial use in livestock. These regulations targeted the use of medically important antimicrobials (MIA), which are antimicrobials that are of human clinical importance¹⁰⁶. In December 2018, growth promotion claims were to be removed from drug labels, while responsible use statements were to be added for select MIAs. Additionally, MIA use in livestock required veterinary prescription¹⁰⁶. Despite these efforts, growth promotion persists, with CIPARS reporting antimicrobial growth promotion at four sentinel surveillance farms in 2020¹⁰².

Understanding AMR and AMR-driving practices in the animal sector is important for investigating potential linkages to the human sector through the One-Health continuum, as AMR in animals can have important implications for human health, especially in instances where

humans interface with animals, such as with agri-food products.

1.4.2 The farm to fork continuum

The farm-to-fork continuum is a critical human-animal interface within the One-Health continuum where pathogens from animals can spread to humans through exposure to contaminated food products. Foodborne pathogens such as *Salmonella* clearly demonstrate the importance of the farm-to-fork continuum, as human *Salmonella* infections can be transmitted via the fecal-oral route, either directly through exposure to animals, or indirectly by eating contaminated foods¹⁰⁷.

While it is unclear exactly how AMR pathogens from retail meat affect humans directly¹⁰², correlations between animal and human AMR have been described in Canada with AMR *Salmonella*. A previous CIPARS study investigating *Salmonella enterica* serovar Heidelberg in Quebec found a link between ceftiofur resistance found in retail chicken, and the incidence of these infections in humans. Researchers found that resistance levels in chickens was related to the use of this antimicrobial in hatcheries, demonstrating the importance of studying the farm-to-fork continuum¹⁰⁸.

This is a valuable Canadian study that illustrates a clear linkage between AMU in animals, and AMR in humans. This study, however, is rare, as such clear correlations are challenging to capture without robust sampling. Furthermore, the pathogen in question, *Salmonella*, is a known foodborne pathogen associated with poultry¹⁰⁹. Illustrating a farm-to-fork linkage with other pathogens, especially those that may not readily result in foodborne illness, proves to be more challenging and nuanced. While there is evidence of other pathogens in agri-food products, their linkages to the human sector are less obvious.

1.4.3 Prevalence of AMR pathogens in retail meat

The priority AMR pathogens *C. difficile*, MRSA, and CPOs have been isolated from retail meat both locally in Canada and elsewhere across the globe from a variety of meat commodities. The prevalence of AMR pathogens in retail meat differ for a given pathogen, and can reflect sampling and isolation methods, product type, and geographical region among other factors. It is challenging to summarize a global prevalence in retail meat across studies and time, although some systematic reviews have attempted to do so^{110,111}. The following is a review of the current literature investigating priority AMR pathogen prevalence in retail meat, with a focus on retail pork and

beef, when available.

1.4.3.1 Prevalence of *C. difficile* in retail meat

In Canada, there have been several studies focused on *C. difficile* isolated from retail pork and beef, in addition to other commodities, with reported prevalences ranging from 1.6% to 20%^{112–116}. Additionally, AMR phenotypes have been investigated and reported, with a study of ground meat from Ontario and Quebec detecting resistance to clindamycin and levofloxacin¹¹⁴ and another detecting 1/10 isolates from 644 retail meat samples being resistant to moxifloxacin¹¹². The global prevalence of *C. difficile* in retail meat has been estimated to be 5.6% in raw meat, compared to 6.3% estimated across all food types in a systematic review and meta-analysis¹¹¹.

1.4.3.2 Prevalence of MRSA in retail meat

Canadian studies evaluating the prevalence of MRSA in retail meat are less common than *C. difficile*, yet varying MRSA prevalences have been reported. Two studies of retail chicken in Canada have reported similar prevalences of 1.2% and 1.3%^{117,118}. One of these studies also investigated the prevalence in retail pork and beef, finding MRSA in 9.6% and 5.6% of samples, respectively¹¹⁸. Another study of retail pork from four Canadian provinces found a prevalence of 7.7%, or 5.8% after adjusting for provincial-level clustering¹¹⁹. Globally, the prevalence of MRSA in raw meat was estimated to be 3.2%, with a prevalence of 4.1% in pork and 2.5% in beef¹¹⁰.

1.4.3.3 CPOs in retail meat and related reservoirs

In contrast with the other pathogens of interest, CPOs are not commonly reported from retail meat both globally and in Canada. One study from China reported the isolation of *bla*NDM-positive *E. coli* (n=30), and *K. pneumoniae* (n=4) screened from 520 meat samples¹²⁰. This study appears to be an anomaly, as other studies have reported lower rates of CPO recovery. Another study targeting *Proteus mirabilis* from meat (114 chicken, 141 pork) purchased in China only identified one isolate that harboured *bla*NDM from retail chicken, although other bacterial species were not investigated¹²¹. Cloacal swabs from whole chickens purchased in Peru (n=61) revealed one *E. coli* that harboured *bla*KPC, although only a subset of the isolated *E. coli* were sequenced¹²². In Germany, slaughterhouses, meat plants, fresh food, and the environment were tested for CPEs. PCR results showed 9% of the 100 fresh food isolates tested positive for CPE, which was the lowest source of CPE across reservoirs. All CPE-positive isolates from food harboured *bla*OXA-

48: 1/10 beef samples, 4/37 pork samples, and 4/53 poultry samples¹²³. A *bla*VIM-positive *Enterobacter hormachei* was reported from beef patty in Egypt¹²⁴, and a *bla*VIM-positive *E. coli* was isolated from 178 minced pork samples in Belgium¹²⁵. In the US, a *Routella ornithinolytica* that co-harboured *bla*NDM and *bla*KPC was isolated from retail meat samples¹²⁶. These reports are rare, and many studies only report single CPO isolates recovered from retail meat collections.

In Canada, there are currently no published studies reporting carbapenemases in retail meat, however they have been detected in Canadian food products. Specifically, the presence of *bla*IMI and *bla*NDM has been reported from select seafood products¹²⁷. Another study of imported seafood in Canada identified *bla*OXA-48 from 3.3% of tested samples that originated in China and Korea¹²⁸. Last, one study found *Vibrio cholerae* that harboured a novel VCC carbapenemase from imported retail shrimp¹²⁹. These reports highlight food as a reservoir of clinically relevant carbapenemases. It remains unknown, however, if this potential foodborne exposure is important for the spread of these organisms to humans.

While CPOs have not been detected in Canadian retail meat, they have been found in environmental reservoirs related to livestock production in Canada. Notably, environmental CPOs have been isolated from cattle feedlots in Alberta, where *Pseudomonas* spp. and *Acinetobacter* spp. harboured OXA-type carbapenemases, and *Stenotrophomonas maltophilia* harboured species-associated L1 carbapenemases¹³⁰. Clearly, there is an environmental presence of certain CPOs; however, there is a lack of evidence that demonstrates a linkage to humans through the farm-to-fork continuum.

1.5 Thesis rationale, aims, and objectives

As reviewed above, three priority AMR pathogens *C. difficile*, MRSA, and CPOs have been detected at varying rates in Canada and have also been identified as community-associated infections. These pathogens are acquired from unknown community sources that remain to be investigated. The isolation of these pathogens from retail meat both in Canada and other countries indicates that retail meat may be a source of exposure, although previous investigations are limited to traditional typing methods. The transmission of AMR between agri-food and human sectors is accepted through the One-Health lens, with Canadian efforts like PCAP aiming to improve health and food safety along the farm-to-fork continuum²⁰. This linkage, however, has traditionally been investigated through the study of foodborne pathogens¹³¹ rather than those associated with the

nosocomial environment.

Investigations into AMR pathogens in Canadian retail meat have been described previously, but an updated perspective is required in the face of increasing AMR. To gain a current perspective, this study aimed to provide updated *C. difficile* and MRSA prevalences in retail meat in Canada, and to investigate the potential novel presence of CPOs. As demonstrated with ceftiofur-resistant *Salmonella* in Quebec, AMR pathogens in retail meat can be correlated with AMR infection incidence in humans¹⁰⁸ and current linkages to the human sector in Canada exist primarily through traditional typing methods which quantify isolate relationships at lower levels of resolution. Thus, the second aim of this study was to investigate relationships between AMR pathogens from retail meat and humans at a high level of resolution using WGS which could provide insight into the potential farm-to-fork transmission of these pathogens.

These aims were addressed by leveraging two Canadian surveillance programs, CIPARS and CNISP, for retail meat sampling and human clinical isolate collections, respectively. When the CNISP isolate collection was limited, isolates collected through CANWARD were used for genomic comparisons. Retrospective surveillance was performed through selective culture-based methods prior to further characterization with phenotypic or molecular methods. All isolates of interest were sequenced using WGS for *in silico* typing and/or characterization. Genomic relationships to human clinical isolates collected through CNISP were investigated using PCR ribotyping, sequence typing, *spa* typing, cgMLST, and SNV analysis depending on the organism. In several instances, no human clinical linkages could be investigated due to the species of pathogens isolated. The implications of these pathogens in retail meat are discussed. The following research exemplifies a One-Health approach to AMR surveillance and demonstrates genomic similarities between priority AMR pathogens isolated across the Canadian farm-to-fork continuum. Results from this study can contribute to risk modelling of AMR transmission, and can be used to identify novel reservoirs, pathogens of interest, or resistance types. Additionally, this may provide insights into gaps in current infection prevention and control measures across the farm-to-fork continuum and be used to influence interventions to address AMR.

Chapter 2: Materials and methods

2.1 CIPARS sampling and sample preparation

2.1.1 Retail meat collection

Retail meat was purchased in Alberta (West), British Columbia (West), Ontario (Central), and Quebec (Central) from 2016 – 2018 through CIPARS. CIPARS sampled both retail chains and independent stores or butchers, and preferentially selected ground beef over other beef products, and pork chops over other pork products. Immediately after purchase, fresh meat commodities were placed in coolers between 1- 6 °C for transport to the NML (Saint Hyacinthe) for retail meat processing and the transport temperature was recorded. Relevant information on each retail meat sample can be found in Appendix A Table A1.

2.1.2 Retail meat processing

Retail meat commodities were processed at the NML (Saint Hyacinthe). Retail meat packages were cleaned with 70% ethyl alcohol before sample processing. To process ground meat products, 25g of sample was added to 255 ml of buffered peptone water (BPW) and mixed in a homogenizer at 200 rpm for 1 minute. To process whole sectioned meat products, samples were placed in a bag depending on the section size, and BPW was added (255 ml for 18 x 30 cm bags and 450 ml for 37 x 38 cm bags). A rotating plate was used to mix bags at 30 rpm for 15 minutes. Afterwards, all samples were incubated at 35°C ±1 °C for 18-24 hours before 600 uL of the rinsate was stored with 600 ml of brucella broth with 15% glycerol at 80 °C±10°C. Frozen rinsates were later shipped on dry ice to the NML (Winnipeg) for pathogen isolation.

2.2 Pathogen isolation

2.2.1 Isolation of *C. difficile*

C. difficile was isolated as described previously with modification using both direct and selective enrichment methods¹¹². All incubations were performed anaerobically at 37 °C unless specified. A positive *C. difficile* control strain, a negative *S. aureus* control ATCC® 29213, and a blank control (blank plate for the direct method and blank broth for the selective enrichment method) was run alongside all isolation steps. Direct isolation was performed by spreading 100 uL of BPW rinsate on to *C. difficile* moxalactam norfloxacin (CDMN) agar and incubated for an initial 48 hours. Selective enrichment was performed by inoculating 100 uL of retail meat rinsate in 9 mL

of CDMN broth with taurocholic acid and incubating broths for 5-7 days. Following the incubation period, an ethanol shock was performed, where 2 ml of the broth culture was incubated at room temperature in ambient air with 2 ml of anhydrous ethanol. After a 1 hour incubation period broths were centrifuged at 3500 – 4500 x g for 10 minutes to pellet spores and the supernatant was removed. Pellets were spread directly or resuspended in a small volume of phosphate-buffered saline before being spread on to brucella blood agar and incubated for 48 hours. Following the incubation period of both direct and selective enrichment methods, plates were inspected for colony morphology and fluorescence using long-wave ultraviolet light (365 nm). Both plate types were incubated and inspected for an additional 3-5 days. Colonies of interest were isolated on to brucella blood agar and confirmed to be *C. difficile* using MALDI-TOF MS (described below).

2.2.2 Isolation of MRSA

MRSA isolation used two different solid media: chromID® MRSA (bioMérieux, France) and mannitol salt agar supplemented with 8 mg/L of cefoxitin. MRSA isolation was performed by streaking a 10 uL loopful of retail meat rinsate onto each selective solid media. Additionally, 100 uL of rinsate was inoculated into mannitol salt broth supplemented with 6 mg/L cefoxitin and incubated for 48 hours. After the incubation period, a 10 uL loopful of broth was streaked onto the two solid medias described above. All plate incubations were 48 hours in length at 35 ± 2 °C.

MRSA isolations were run alongside a MRSA-positive control (MRSA 21S-0391 *mecA*⁺), a methicillin-sensitive negative control strain (*S. aureus* ATCC® 29213), and a blank control. Colonies of interest were selected according to colour and resemblance to the positive control: blue/green colonies were selected from chromID® MRSA and pale yellow colonies were selected from mannitol salt agar plates. Colonies of interest were isolated onto tryptic soy agar (TSA) with 5% sheep's blood for MALDI-TOF MS identification.

2.2.3 Isolation of carbapenem-resistant organisms for Chapters 5 and 6

CR-organisms were isolated using chromID® CARBA and chromID® CARBA SMART (bioMérieux, France). The chromID® CARBA SMART is a bi-plate, consisting of half CARBA, and half OXA-48 chromogenic agar. CPO isolation was performed by streaking a 10 uL loopful of retail meat rinsate onto a chromID® CARBA whole plate, and on to each half of a chromID® CARBA SMART bi-plate. Additionally, 100 uL of rinsate was inoculated in to brain-heart infusion broth supplemented with 5 mg/L ertapenem broth. The broth was incubated for 48 hours prior to

streaking a 10 uL loopful of the incubated broths on the solid media as described above. All plate incubations were 48 hours in length at 35 ± 2 °C.

CPO isolations were run alongside two positive controls (*E. coli*, N22-01155, NDM⁺, OXA-48⁺; *Enterobacter cloacae* complex N22-01172, NDM⁺ (chromID® CARBA agar only)), a negative control (*E. coli* ATCC® 25922), and a blank control. N22-01155 colonies were purple on both agar types, and N22-01172 was green on chromID® CARBA agar. There was no selection criteria for colony isolation, although colonies were selected with the aim of isolating one of each colour and morphology from a given plate. Colonies of interest were isolated onto TSA with 5% sheep's blood for MALDI-TOF MS identification.

2.3 Pathogen identification with MALDI-TOF MS

Two different methods were used for MALDI-TOF MS identification. A quick-spotting method, and a formic acid extraction method (described below). Common across the two methods was the matrix overlay consisting of α -cyano-4-hydroxycinnamic acid saturated in a solution of 2.5% trifluoroacetic acid (TFA) and 50% acetonitrile. In each method, target plates included two positive *S. aureus* control spots, a matrix blank to check for contamination, and three replicate spots of Bacterial Test Standard (Bruker Daltonik GmbH, Germany) prepared in a solution of 2.5% TFA and 50% acetonitrile.

Bacterial identification was performed using the Bruker MicroFlex system using MBT Compass Explorer Libraries revH v11, revK v12, and v13 by the Mass Spectrometry and Proteomics Core at the NML according to manufacturer recommendations (Bruker Daltonik GmbH, Germany). A genus-level identification was recorded if the two replicate spots were in concordance. Samples without reliable genus-level identifications were recorded as non-identifiable. Non-identifiable isolates from MRSA and *C. difficile* isolations were discarded, whereas those from the CPO isolations were subject to phenotypic carbapenemase testing (described below).

2.3.1 Quick spotting method

In the quick spotting method, the unknown sample was smeared and emulsified into 2 uL of 70% formic acid added to the target spot. The spot was allowed to air-dry and 1 uL of matrix was overlaid over dry target spots¹³². This was the choice method used for sample identification

due to the speed of this protocol.

2.3.2 Formic acid extraction method

The formic acid extraction method was performed according to recommendations from the manufacturer. In this method, test cultures were extracted before their supernatant was added to target plates. Approximately half a 1 uL loopful of test culture was added to 300 uL of H₂O, followed by the addition of 900 uL of anhydrous ethanol. Samples were mixed through inversion, and solids were pelleted via centrifugation for two minutes at 16,000 RPM. The supernatant was decanted and pellets were allowed to air dry for 10 minutes to remove any remaining ethanol. Afterwards, 20 uL of 70% formic acid was mixed with the pellet and incubated at room-temperature for 5 minutes before gently mixing in 20 uL of acetonitrile and performing a final centrifugation for two minutes at 16,000 RPM. The final extraction mixture was frozen for up to a week before identification, or identified immediately by adding 1 uL of supernatant on to the target plate, allowing spots to dry, and overlaying 1 uL of matrix.

2.4 *In vitro* pathogen characterization

2.4.1 PCR ribotyping of *C. difficile*

PCR ribotyping was performed as described previously⁷¹. In brief, DNA was extracted from *C. difficile* culture grown on solid media by resuspending 1 uL of test cultures in 100 uL of Instagene matrix (Bio-Rad, United States) and heated at 99 °C for 10 minutes. Cells were pelleted, and the lysate was removed for further use. PCR amplification of the 16S-23S intergenic spacer region was performed and PCR products were analyzed using capillary gel electrophoresis. PCR primers, reaction composition, and cycling conditions can be found in Appendix B Table B1.1-3. Ribotypes (RTs) were assigned from a database of RTs validated in an international multicentre study⁷¹.

2.4.2 Antimicrobial susceptibility testing of *C. difficile* via Etest

Antimicrobial susceptibility testing was performed via Etest for the following antimicrobials: clindamycin, metronidazole, moxifloxacin, rifampin, tigecycline, and vancomycin. Etest was performed on Brucella agar with hemin and vitamin K + 5% laked sheep's blood following the manufacturer's recommendations (bioMérieux, France), using a 1.0 McFarland standard. Etest breakpoints were interpreted according to manufacturer recommendations and

CLSI (Table 1)⁹¹.

2.4.3 Phenotypic carbapenemase detection

Phenotypic carbapenemase detection was performed with two different methods. mCIM was used to screen any Enterobacterales isolated, whereas CIMTris was used to screen isolates identified as *Aeromonas* spp. and *Pseudomonas* spp. Samples without a reliable MALDI-TOF MS identification were screened using both mCIM and CIMTris. As there is no recommended phenotypic carbapenemase test for non-aeruginosa *Pseudomonas* spp. and *Aeromonas* spp. in CLSI⁹¹, a small validation was performed comparing mCIM, CIMTris⁹⁴, and a modified CIMTris II assay¹³³. The results from this validation are shown in Appendix B Table B2, where CIMTris was chosen as the best method for carbapenemase testing in *Aeromonas* spp. and *Pseudomonas* spp.

Due to a broad CPO colony selection criteria coupled with the genus-level resolution of MALDI-TOF MS identification, only a selection of isolates underwent further phenotypic testing. After MALDI-TOF MS identification, only Gram-negative organisms were selected for further characterization. Additionally, isolates identified as *Stenotrophomonas* spp. were not included in this study. Accounting for both isolates of chromID® CARBA and chromID® OXA-48 origin, 3978 CR-isolates were screened from 317/318 retail meat rinsates. The number of isolates selected for further testing ranged from 1 – 30, with exception of one rinsate where no isolates were recovered (Figure 1). Additionally, three retail meat rinsates 145, 146, and 148, were only screened on CHROMID® OXA-48 agar, due to limited access to these samples. Appendix C Table C1 shows the genera of isolates screened.

2.4.3.1 mCIM

mCIM was performed according to the methods outline in the CLIS M100⁹¹. 1 uL of fresh overnight test culture was emulsified in 2 mL tryptic soy broth. A 10 ug meropenem disk was added into the culture tube, and incubated for 4 hours at 35°C ± 2°C. Following the incubation period, a 0.5 McFarland suspension of *E. coli* ATCC 25922 was prepared in saline, which was used to streak a lawn on Mueller Hinton agar and the 10 ug meropenem disk was removed from the culture suspension and plated. The zone of inhibition was measured and recorded between 18 – 24 hours of incubation at 35°C ± 2°C. Isolates with zones of inhibition measuring 6-15 mm in diameter or 16-18 mm with pinpoint colonies were considered carbapenemase-positive. Zones of

Table 1. *C. difficile* minimum inhibitory concentration breakpoints used for Etest.

Antimicrobial	Susceptible (ug/mL)	Intermediate (ug/mL)	Resistant (ug/mL)
Clindamycin	≤ 2	4	≥ 8
Metronidazole	≤ 8	16	≥ 32
Moxifloxacin	≤ 2	4	≥ 8
Rifampin	≤ 1	2	≥ 4
Tigecycline	≤ 4	8	≥ 16
Vancomycin	≤ 8	8-16	≥ 32

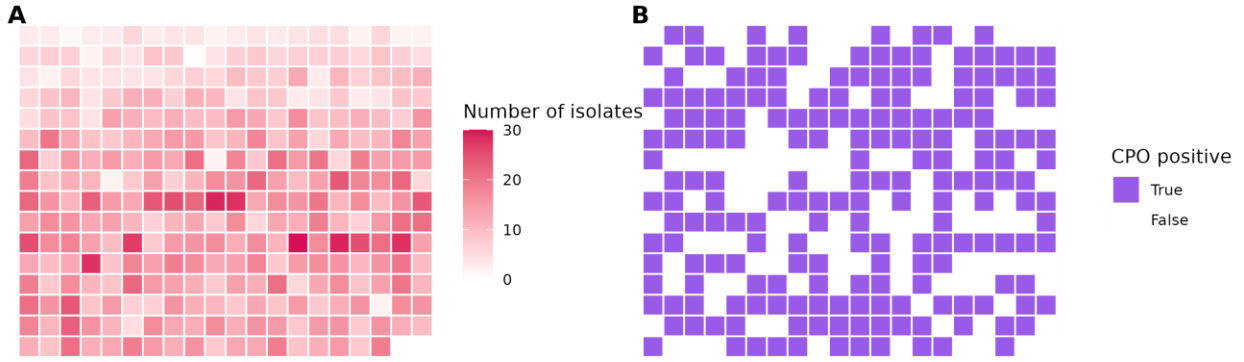


Figure 1. Overview of carbapenem resistance from retail meat rinsates. Tiles are ordered from 1-318, left to right. Both heatmaps exclude any *Acinetobacter* spp. recovered since they were not tested *in vitro*. (A) Number of CR isolates tested for phenotypic carbapenemase production per retail meat rinsate. (B) Overview of retail meat rinsates that harboured at least one carbapenem-producing isolate confirmed by phenotypic testing.

inhibition measuring 16-18 mm with no pinpoint colonies was considered indeterminate, and ≥ 19 mm was considered carbapenemase-negative. Only carbapenemase-positive isolates were used in subsequent analyses.

K. pneumoniae ATCC® BAA-1706™ was used as a negative control, and *K. pneumoniae* ATCC® BAA-1705™ was used as a positive control for each batch of samples. Additionally, each batch included a saline blank and a quality control meropenem disk plated directly onto the lawn of susceptible *E. coli*.

2.4.3.2 CIMTris

CIMTris was performed as described in Uechi et al⁹⁴. In brief, 10 uL of culture was emulsified in 400 uL 0.5 M Tris-HCl (pH 7.6). A 10 ug meropenem disk was added into the solution and incubated at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 2 hours. Following the incubation period, a 0.5 McFarland suspension of *E. coli* ATCC® 25922 was prepared in saline, which was used to streak a lawn on Mueller Hinton agar. Following the 2 hour incubation, the 10 ug meropenem disk was removed from the culture suspension and placed on the lawn of *E. coli*, where it was incubated for 18-20 hours at $35^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Zones of inhibition were read according to the methods outlined in section mCIM. Positive and negative controls were prepared in the same manner as mCIM.

2.5 Genomic methods

2.5.1 DNA extraction

C. difficile grown from fresh 2-day culture on solid media was inoculated into brain-heart infusion broth and incubated anaerobically at 37°C for 4-6 hours and pelleted prior to DNA extraction. All other organisms were extracted directly from fresh overnight culture grown on solid media. DNA extractions were performed by the Robotics Support Laboratory at the NML using the LuminUltra RNA1k-480 (LuminUltra, Canada) modified for bacterial DNA extractions using an in-house 3% cetyltrimethylammonium bromide solution buffer or the Omega-Mag Bind Universal DNA Kit (Omega Bio-tek, Georgia).

2.5.2 Illumina sequencing

All WGS was performed by the Genomics Core at the NML. Sequencing libraries were prepared with the Nextera XT library preparation kit (Illumina, USA) and sequenced on Illumina NextSeq instruments (Illumina, USA). The Mid Output kit was used with the NextSeq 500, the

P1-300 kit was used with the NextSeq 1000 and 2000, and the P2-300 kit was used with the NextSeq 1000.

2.6 Selection criteria for human clinical isolates included in genomic comparisons

2.6.1 Inclusion criteria for the genomic comparisons of *C. difficile* in Chapter 3

Select CDI isolates from 44-45 participating CNISP hospitals within Alberta (West), British Columbia (West), Ontario (Central), and Quebec (Central) from 2016-2018 were sequenced and used for comparison to retail meat isolates, all of which corresponded to the same years and provinces of isolation as meat samples in this study. More specifically, the selection criteria included isolates that were community-associated or of indeterminate origin as defined through CNISP¹³⁴. Briefly, CA-CDI is defined as CDI symptoms occurring less than 3 days (or <72 hours) after admission, with no history of hospitalization or any other healthcare exposure within the previous 12 weeks. For cases defined as indeterminate, the symptom onset was more than 4 weeks but less than 12 weeks after the patient was discharged from any healthcare facility or after the patient had any other healthcare exposure¹³⁴. Human clinical CA-CDI and indeterminate CDI isolates included RT027/ST1 (n=33; associated with NAP1), RT002/ST8 (n=32; associated with NAP6), or RT015/ST10 (n=26; associated with NAP12).

2.6.2 Inclusion criteria for the genomic comparisons of MRSA in Chapter 4

MRSA of corresponding *spa* and STs collected through CNISP were selected for genomic comparison to retail meat isolates. The CNISP collection of MRSA whole-genome sequences began in 2017 at the time of this study, therefore isolates dating beyond this point were not included in genomic analyses. Specific inclusion criteria per *spa*/ST vary per comparison and are described in Chapter 4. In the instance where there was no corresponding *spa*/ST available through CNISP, publicly available genomic data of *S. aureus* of human origin were used and are listed in Appendix D Table D1.

2.6.3 Inclusion criteria for the genomic comparisons of *Acinetobacter* spp. in Chapter 5

Available sequenced isolates collected through CNISP and CANWARD were included if they were identified as members of the *Acinetobacter calcoaceticus*-*A. baumannii* (ACB) complex and belonged to the same ST. There are two MLST schemes available for *Acinetobacter* spp.: the Pasteur scheme and the Oxford scheme. CNISP isolates were searched according to their Pasteur

(PA) ST whereas CANWARD isolates were searched according to their PA and Oxford (OX) schemes. The selection criteria was not limited by year, region, or site of isolation. At the time of this study, the availability of *Acinetobacter* spp. sequenced from CANWARD dates back to isolates collected in 2017. Sequences for CP-*Acinetobacter* spp. collected through CNISP were available from 2010-onward.

2.7 Bioinformatic analysis

2.7.1 Illumina read and assembly quality

Illumina reads were filtered using fastp v0.23.2¹³⁵ using a qualified phred quality of ≥ 20 . Read quality was assessed before and after trimming with FastQC v0.72¹³⁶. Contamination was assessed with MetaPhlAn2 v2.6.0.0¹³⁷ and initial species identity was confirmed with RefSeq Masher v0.1.2¹³⁸. Reads were assembled using Shovill v1.0.4¹³⁹ and assembly quality was assessed with Quast v5.0.2¹⁴⁰. Read and assembly quality metrics for all organisms investigated can be found in Appendix E.

2.7.2 Analysis of *C. difficile* for Chapter 3

Retail meat isolates were subject to an *in silico* toxin panel using BLASTn v2.10.1¹⁴¹. STs were identified by searching PubMLST⁴⁰ using mlst v2.22.0¹⁴². cgMLST analysis was performed using Ridom SeqSphere+ v9.0.10¹⁴³. Minimum spanning trees were calculated with the parameter “pairwise ignore missing values”, and clusters were identified using a threshold of ≤ 6 allele differences⁴⁵. SNVPhyl v1.1¹⁴⁴ was run on cgMLST clusters containing meat isolates using an internal reference and a SNV density filtering threshold of 2 in 20. In the instance where the meat isolate did not cluster via cgMLST, SNVPhyl was run using isolates of the nearest cgMLST cluster. The R package ape v5.7.1¹⁴⁵ was used to remove references from newick files and maximum likelihood SNV trees were presented with ggtree v3.11.0¹⁴⁶.

2.7.3 Analysis of MRSA for Chapter 4

In silico spa typing was performed using spaTyper v0.3.3¹⁴⁷. starAMR v0.10.0⁴⁹ was used to identify MRSA STs, and ARGs were identified with a threshold of 90% blastn identity. cgMLST analysis was performed using Ridom SeqSphere+ v9.0.10¹⁴³ and distance calculations were based on 1861 columns, pairwise ignoring missing values. Clusters were identified using the default threshold of 24 allelic differences. SNV analyses on trimmed reads were performed using the

SNVPhyl v1.1¹⁴⁴ paired-end pipeline using an internal reference sequence. SNV density filtering was adjusted to 2 in 20 and all other parameters were left default. Maximum likelihood trees from SNVPhyl were processed using ape v5.7-1¹⁴⁵ was used to remove the internal reference from newick files, and the resulting trees were visualized with ggtree v3.11.0¹⁴⁶.

2.7.4 Analysis of *Pseudomonas* spp. and *Acinetobacter* spp. for Chapter 5

2.7.4.1 Additional isolate quality and species identification

Beyond initial assembly quality described previously, an additional quality assessment was performed on retail meat *Acinetobacter* spp. and *Pseudomonas* spp. assemblies using BUSCO v5.8.0¹⁴⁸ employing the Pseudomonadales dataset, as well as CheckM2 v1.0.2¹⁴⁹ to check for genome completeness. Species were identified with the Genome Taxonomy Database Toolkit v2.4.2¹⁵⁰ using the “classify” workflow.

2.7.4.2 Resistance genes and mobile genetic element identification

AMRFinderPlus v4.0.3⁴⁸ using database v2024-10-22.1 with default parameters was used to detect ARG and metal tolerance genes. Gene variants were identified using the top reference sequence identified from AMRFinderPlus. Genes related to heat stress were omitted but other non-antibiotic resistance was included in the final results. MGEs were detected with MOB-suite MOB-recon v3.1.9⁵⁵. ARGs were determined to be plasmid-located if they were located on a contig predicted to be a plasmid by MOB-suite.

2.7.4.3 Phylogenetic comparisons of retail meat and human clinical *Acinetobacter* spp.

Both Pasteur and Oxford MLST schemes were used to identify appropriate isolates for genomic comparisons to *Acinetobacter* spp., which were identified with mlst v2.23.0¹⁴² (updated 2024-10-25). cgMLST analysis was performed using Ridom SeqSphere+ v10.5.3 on *A. baumannii* only¹⁴³ and distance calculations were based on 2234 columns, calculated pairwise ignoring missing values. Clusters were identified using the default threshold of 9 allelic differences. SNV analyses on trimmed reads from all retail meat isolates, from CNISP, and from CANWARD were performed using the SNVPhyl v1.1¹⁴⁴ paired-end pipeline using an internal reference sequence. SNV density filtering was adjusted to 2 in 20 and all other parameters were left default. Maximum likelihood trees from SNVPhyl were used in the phylogenetic analyses, where ape v5.7-1¹⁴⁵ was used to remove the internal reference from newick files and the resulting maximum likelihood tree

was visualized with ggtree v3.11.0¹⁴⁶.

2.7.5 Analysis of *Aeromonas* spp. for Chapter 6

2.7.5.1 Additional quality and species identification

In addition to the quality assessment described above, genome assembly completeness was assessed with BUSCO v5.8.0¹⁴⁸ using the dataset for Gammaproteobacteria as well as CheckM2 v1.0.2¹⁴⁸. Species were identified with Genome Taxonomy Database Toolkit v2.3.2¹⁵⁰ using the “classify” workflow.

2.7.5.2 Resistance gene and mobile genetic element identification

Resistance genes and MGEs were identified according to the methods described in Section 2.7.4.2.

2.7.5.3 Phylogenetic tree construction and visualization

To identify duplicate isolates and investigate phylogenetic relationships, a phylogenetic tree was constructed from concatenated MLST alleles. Reference sequences corresponding to each species were included in the initial tree to assess tree quality (*A. allosaccharophila*, GCF_016026615.1; *A. bestiarum*, GCF_028751155.1; *A. hydrophila*, GCF_000014805.1, *A. veronii*, GCF_008693705.1; *A. salmonicida*, GCF_028355655.1). To retrieve allele locations in query isolates, local BLASTn v2.13.0+¹⁴¹ was used to search the PubMLST⁴⁰ database of *Aeromonas* spp. alleles (*gltA*, *groL*, *gyrB*, *metG*, *ppsA*, *recA*) (downloaded 2024-10-26). BEDTools v2.31.1¹⁵¹ was used to extract allele sequences based on sequence positions identified with BLASTn. Individual MAFFT v7.467¹⁵² alignments were performed for each gene, followed by gap and end gap removal of each alignment with trimAl v1.5.rev0¹⁵³ which removed seven total positions across all six alignments. SeqKit v2.8.0¹⁵⁴ was used to concatenate the alignments together, and isolates were analyzed further as a concatenation of the seven housekeeping alleles, which measured 3078 bp in length. Variant sites were identified from the alignment using SNP-sites v2.5.1¹⁵⁵, which identified 769 variant sites across the concatenated alignment. A maximum likelihood tree was generated from the variant sites with iqtree v2.3.6¹⁵⁶ using the model “GTR+ASC” which is recommended for variant-only trees, with 1000 replicates for bootstrap branch support¹⁵⁷.

All phylogenetic tree manipulation and visualization was performed in R⁵⁴. phylotools

v1.5-1⁵⁵ was used to root the maximum likelihood tree at the midpoint, and cluster identification was performed with castor v1.8.2⁵⁶ using a branch length cutoff of 0.0001 to identify duplicate isolates from the same retail meat rinsate. The ARG profile of each isolate was also considered in the identification of duplicate isolates, with isolates from the same rinsate and ST (determined through phylogenetic clustering) included in the final phylogeny if the ARG profile differed. Unique isolates were extracted from the phylogenetic tree using ape v5.7-1³⁸. These unique isolates were then used to construct the final phylogenetic tree of unique *Aeromonas* spp. Finally, ggtree v3.11.0³⁹ was used for both phylogenetic visualizations, where two separate visualizations were generated from the analysis: an initial tree containing duplicates, and a secondary tree containing only unique isolates.

2.8 Statistics

To compare outcomes from pork and beef, Fisher's Exact Test was performed using the R package stats¹⁵⁸.

Chapter 3: Surveillance of *Clostridioides difficile* in Canadian retail meat and genomic linkages to community-associated human clinical infections in Canada

3.1 Introduction

C. difficile is a Gram-positive spore-forming anaerobe that remains an important nosocomial pathogen, being among the most common causes of hospital-acquired diarrhea in developed countries⁵⁷. In Canada, there were an estimated 16 070 cases of CDI in 2019, and a reported 2.2% attributable mortality rate in 2021^{16,134}. Despite being commonly associated with nosocomial acquisition, one quarter of *C. difficile* cases admitted to hospitals in Canada have been identified as CA-CDI⁶¹. This type of infection represents a significant proportion of overall CDI in other countries, with 14%–26% of CDI being reported as CA-CDI in Europe^{159–161}, and 61% of CDI in an American multicentre cohort study¹⁶². Overall, while various rates have been reported, CA-CDI is an important source of infection both in Canada and at an international level, raising questions regarding potential reservoirs and sources of exposure outside of the hospital setting.

CDI can be studied within the framework of One-Health, as *C. difficile* has been isolated from diverse sources, including the natural environment, animals and farm environments, and retail food products, such as seafood, vegetables, and meat^{111,163,164}. There has been speculation regarding the foodborne nature of this pathogen, with retail meat being identified as a potential source of exposure^{164,165}. Investigations into the prevalence of *C. difficile* in Canadian retail meat have reported various results, with select studies aiming to establish linkages to human isolates using traditional typing methods^{112–116}. However, the direct transmission to humans from retail meat has not yet been elucidated. This study therefore aimed to first evaluate the prevalence of *C. difficile* in Canadian retail pork and beef and isolates from meat were then compared to human clinical CA-CDI isolates collected through CNISP to evaluate potential genomic linkages within the One-Health continuum in Canada.

3.2 Results

3.2.1 Prevalence and characterization of *C. difficile* in retail meat

In this study, toxigenic *C. difficile* was isolated from 3/219 (1.4%) retail pork and 0/99 (0%) beef samples. Two of the isolates from pork, 063-A-02 (ST1/RT027) and 180-A-01 (ST8/RT002), were purchased in Western Canada in 2016 and 2018, respectively. The third isolate

from pork 169-A-04 (ST10/RT015) was purchased in Central Canada in 2018. All retail pork isolates were susceptible to the tested antimicrobials, apart from 180-A-01, which showed intermediate resistance to clindamycin, and are compared to corresponding human clinical strain types that displayed variable susceptibility rates to clindamycin (31.3%–73.1%), moxifloxacin (27.3%–93.8%), and rifampin (93.3%–100%) (Table 2).

3.2.2 Comparison of retail meat and human clinical community-associated *C. difficile* isolates

To investigate any genomic relatedness within the context of One-Health, cgMLST complex types of the three pork isolates were compared to human clinical CA-CDI isolates with the same ST/RT collected through CNISP (Figures 2A–4A). cgMLST complex types of the three pork isolates 063-A-02, 169-A-04, and 180-A-01 were 5177, 5179, and 7945, respectively. Minimum spanning trees showed that 063-A-02 and 169-A-04 were located in clusters of 8 (Figure 2A) and 19 isolates (Figure 3A), respectively. Isolate 180-A-01, however, did not cluster with any of the available human clinical CA-CDI isolates and was a unique complex type in its cgMLST analysis (Figure 4A).

SNV analysis was performed to further delineate the genomic relatedness of pork and human clinical CA-CDI isolates. Of the three analyses, 169-A-04 showed the closest genomic relationship to the available human clinical ST10/RT015 CA-CDI isolates, with pairwise SNV distances ranging from 2 to 11 SNVs. Within its maximum likelihood tree, 169-A-04 clustered in a clade of 14 total isolates. This clade contained four human clinical CA-CDI isolates with a year (2018) and region (Central) of collection that corresponded to the pork isolate (Figure 3B). Two human clinical CA-CDI isolates shared a pairwise SNV distance of 2 with 169-A-04 and were collected in Central and Western Canada in 2017.

Pork isolate 063-A-02 showed some genomic similarities when compared to human clinical ST1/RT027 CA-CDI isolates, with pairwise SNV distances ranging from 11 to 29 SNVs. Two human clinical CA-CDI isolates collected in the same year (2016) and region (West) as the pork isolate were included in this comparison, one of which shared a pairwise SNV distance of 11 SNVs to the pork isolate. While this isolate shared the fewest SNV differences with 063-A-02, the two isolates did not cluster closely in the maximum likelihood phylogeny (Figure 2B).

Since the final pork isolate 180-A-01 was not located in a cgMLST cluster, SNV distances

Table 2. Antimicrobial susceptibilities (%) of *C. difficile* isolated from retail meat and human clinical infections.

Ribotype	Source	Metronidazole	Clindamycin	Rifampin	Moxifloxacin	Tigecycline	Vancomycin
RT002	Human (n=32)	100	31.3	100	93.8	100	100
	Pork (n=1)	100	0	100	100	100	100
RT015	Human (n=26)	100	73.1	100	92.3	100	100
	Pork (n=1)	100	100	100	100	100	100
RT027	Human (n=33)	100	60.6	93.9	27.3	100	100
	Pork (n=1)	100	100	100	100	100	100

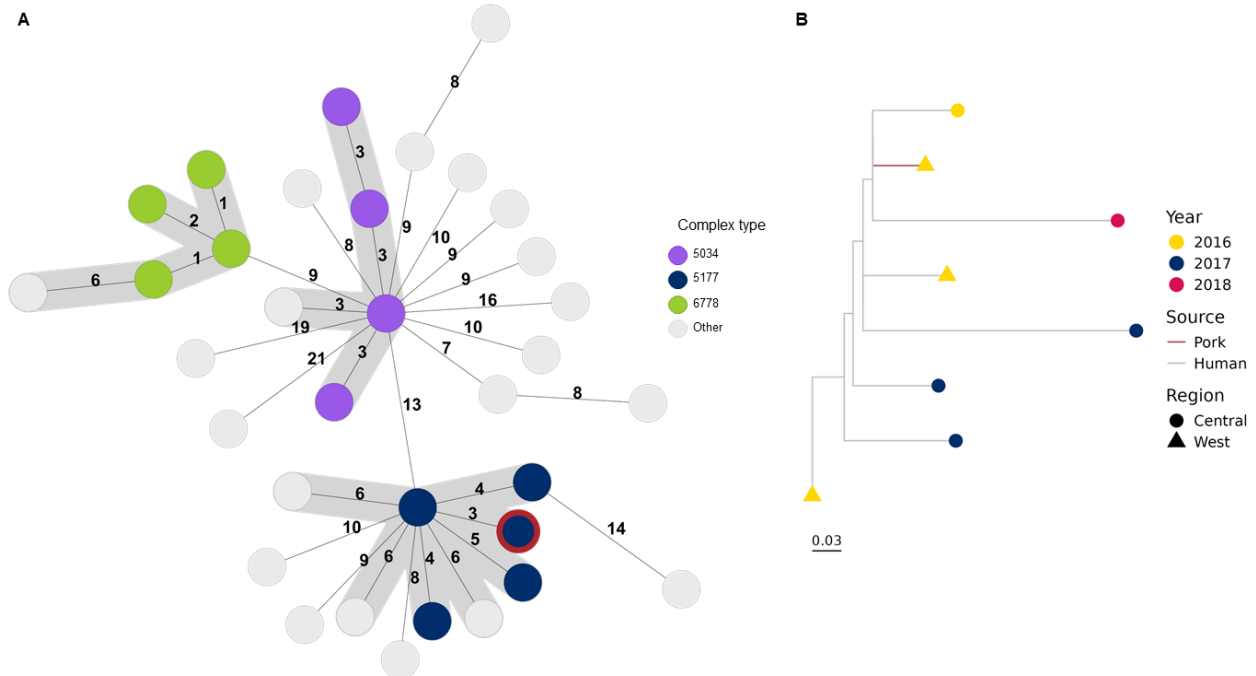


Figure 2. Phylogenetic analyses comparing 063-A-02 to human clinical ST1/RT027 CA-CDI. (A) Minimum spanning tree using 2147 cgMLST alleles, calculated pairwise ignoring missing values, and presented on a logarithmic scale. *C. difficile* from retail meat is outlined in red. Isolates with a unique complex type are grouped as “Other”. (B) Unrooted maximum likelihood tree of SNV analysis of the cgMLST cluster containing complex type 5177. 94.33% of the core genome was used in the analysis and 98 variant sites were used to generate the phylogeny.

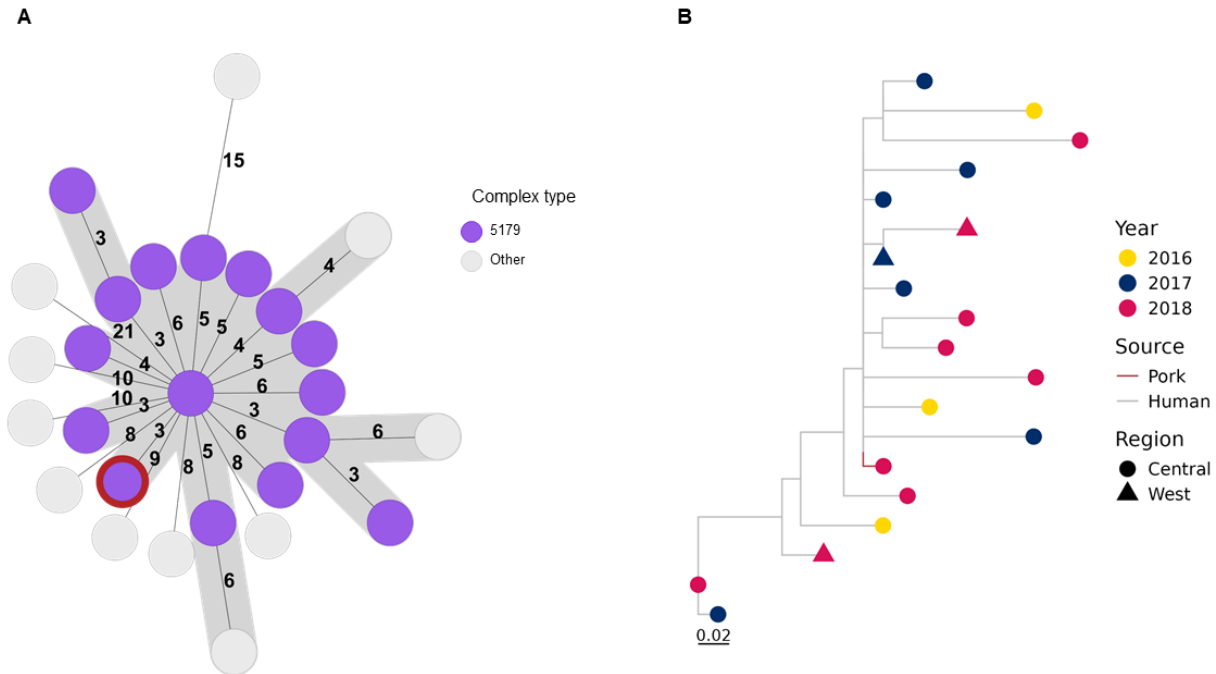


Figure 3. Phylogenetic analyses comparing 169-A-04 to human clinical ST10/RT015 CA-CDI. (A) Minimum spanning tree using 2147 cgMLST alleles, calculated pairwise ignoring missing values, and presented on a logarithmic scale. Grey highlight indicates clusters with ≤ 6 allele differences. *C. difficile* from retail meat is outlined in red. Isolates with a unique or unidentified complex type are grouped as “Other”. (B) Unrooted maximum likelihood tree of SNV analysis of the cgMLST cluster containing complex type 5179. 90.64% of the core genome was used in the analysis and 80 variant sites were used to generate the phylogeny.

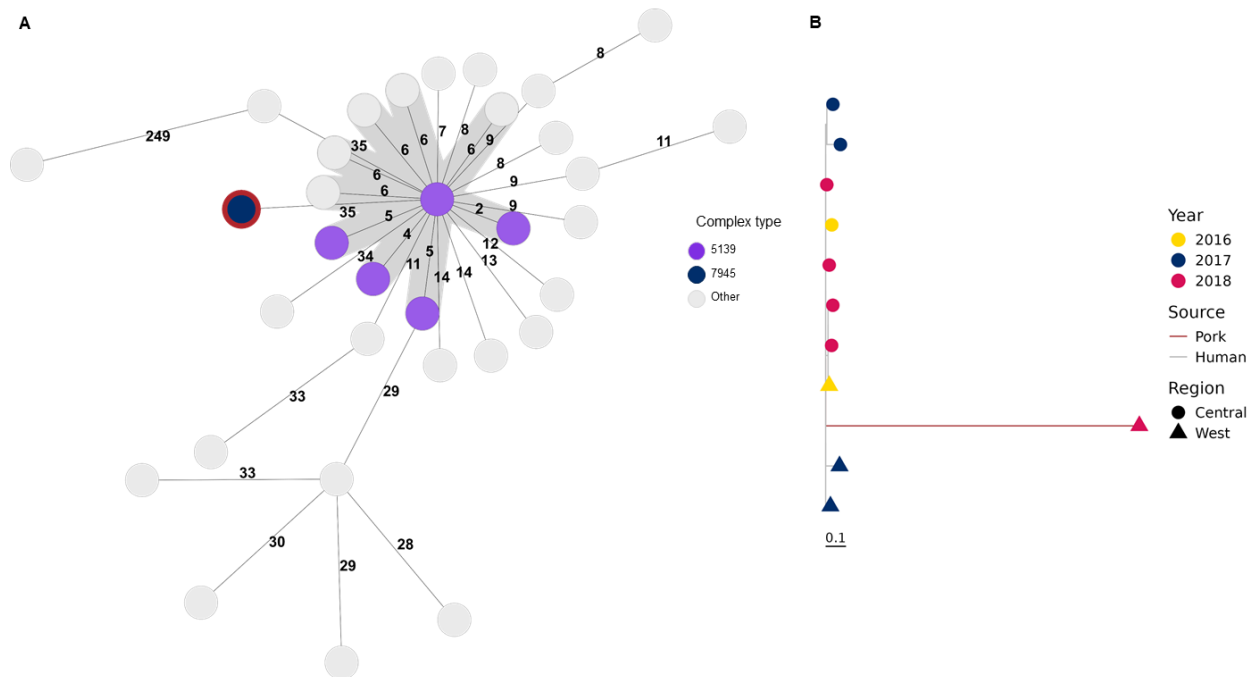


Figure 4. Phylogenetic analyses comparing 180-A-01 to human clinical ST8/RT002 CA-CDI isolates. (A) Minimum spanning tree using 2147 cgMLST alleles, calculated pairwise ignoring missing values, and presented on a logarithmic scale. Grey highlight indicates a cluster with ≤ 6 allele differences. *C. difficile* from retail meat is outlined in red. Clinical isolates with a unique complex type are grouped as “Other”. (B) Unrooted maximum likelihood tree of SNV analysis including isolates with ≤ 6 cgMLST allele differences with the retail pork isolate. 90.00% of the core genome was used in the analysis and 173 variant sites were used to generate the phylogeny.

to human clinical ST8/RT002 CA-CDI were evaluated using isolates of the nearest cgMLST cluster for comparison (highlighted in Figure 4A). This diversity from the cluster was reflected in Figure 4B, where 180-A-01 was located on the longest branch in the tree. Furthermore, it had pairwise SNV distances ranging from 123 to 133 SNVs to human clinical CA-CDI isolates included in the maximum likelihood tree. Conversely, human clinical CA-CDI isolates in this analysis shared pairwise distances ranging from 4 to 22 SNVs. Last, while select human clinical CA-CDI isolates shared the same year (2018) or region (West) of collection, there were no human clinical ST8/RT002 CA-CDI isolates that shared both the year and region of collection with the isolate from pork.

3.3 Discussion

This study identified a low prevalence of toxigenic *C. difficile* in Canadian retail pork (3/219, 1.4%) and beef (0/99, 0%), which is in concordance with a previous Canadian surveillance study, where 1.2% of retail pork, 0% of beef, and 3.4% of veal meat samples tested positive¹¹², and another Canadian study of retail pork, where a prevalence of 1.8% was identified¹¹³. Common across all three studies was the identification of RT027, a predominant RT from 2015 to 2019 amongst Canadian CDI patients admitted in hospital which was associated with increased morbidity and mortality⁶¹.

Beyond similarities with the results of these two previous studies, rates of *C. difficile* in retail meat have been higher in other Canadian studies, with prevalences ranging from 6.3% to 20%^{114–116}. While methods of *C. difficile* isolation are similar across these three studies, there are notable differences in culture inoculation methods, with select studies performing broth enrichment and ethanol shock directly on meat samples^{113–115} and others, including this study, using a meat rinse for culture inoculation^{112,116}. Additionally, discrepant results between studies could be a reflection of product type, with other studies focusing only on ground meat products^{114–116}. Conversely, the work presented here investigates both ground and non-ground products, with 97/99 beef products and 4/219 pork products being ground meat. Furthermore, the context surrounding other Canadian studies may be different, with select former studies purchasing meat products in 2005–2008^{113–116}, whereas retail meat from this study were purchased in 2016–2018. The differences in prevalence observed could be a result of different agricultural, abattoir, and meat packaging practices, and their potential impacts on *C. difficile* reservoirs during this time

period. Last, *C. difficile* from this study was isolated from frozen, rather than fresh rinsates; therefore, the prevalence identified may be reflective of a loss of spore or cell viability that may have occurred while frozen.

Elsewhere, various *C. difficile* prevalences have been reported in retail pork and beef, ranging from none detected in retail pork in Spain¹⁶⁶ to 41.3% in retail pork and 42.4% in retail beef purchased in a single US city¹⁶⁷. A meta-analysis of *C. difficile* prevalence in retail meat concluded a global prevalence of 5.6% in raw meat, and an overall prevalence of 6.3% across all foods¹¹¹. On a local and global scale, varied prevalences of *C. difficile* have been reported, which are likely influenced by several factors beyond the actual prevalence in retail meat. Standardized sampling and isolation methods could reduce variation across studies.

Antimicrobial resistance has been detected in *C. difficile* in both meat products and human CA-CDI^{134,168}; however, the small number of retail meat isolates in this study limits the potential for any comparisons to be drawn between meat and human isolates. While all *C. difficile* STs and RTs identified from pork have previously been observed in human clinical CA-CDI isolates in Canada⁶¹, sources of exposure were not previously investigated. Directly attributing CA-CDI to foodborne exposure is challenging as a detailed epidemiological investigation would be required. It has been suggested, however, that pork is among the highest risk foods for *C. difficile* exposure¹⁶⁴. In the context of foodborne exposure, the livestock-associated RT078/126 is often of interest as it has been described to have zoonotic potential¹⁶⁹. This RT was not identified in this study, nor in the previous recent Canadian study¹¹², but has been identified in 0.8%–3.2% of Canadian human clinical infections identified through CNISP between 2015 and 2019⁶¹. Directly identifying the transmission of CA-CDI remains elusive to researchers, likely in part because *C. difficile* has not been linked to a foodborne outbreak, and other authors have suggested that food is likely only one of several sources of *C. difficile* exposure outside the hospital setting¹⁶⁵. This remains a limitation of this study, and no directionality can be identified between retail meat and clinical infections.

The observed relationships between pork and clinical isolates should be interpreted with caution, as this study does not incorporate detailed epidemiological data. On the basis of cgMLST, 063-A-02 (ST1/RT027) and 169-A-04 (ST10/RT015) were both closely related to select human clinical CA-CDI isolates when applying a ≤ 6 allele difference threshold. At a higher resolution,

only 169-A-04 showed close relationships with human clinical CA-CDI when a ≤ 2 SNV threshold was applied¹⁷⁰. However, epidemiological information on these isolates is limited and while there may be potential for transmission from retail meat due to the relatedness of this clone, conclusions regarding a direct transmission event cannot be made. Additionally, it should be noted that this study is retrospective in nature, consisting of convenience retail meat samples and human clinical isolates available through their respective national surveillance programs; therefore, this study is not intended as a reflection of current CA-CDI in Canada, but rather an investigation of previously unexplored genomic relationships. In summary, a *C. difficile* prevalence of 0.9% was recorded in retail pork and beef purchased in Western and Central Canada. This study evaluated three *C. difficile* at a high resolution by using WGS, with one of three pork isolates being considered genetically related to human clinical CA-CDI isolates and another isolate genetically similar through cgMLST and SNV analysis. Albeit low, retail meat may therefore be a source of exposure for CA-CDI in Canada although further investigation is required to determine if direct transmission from retail meat could result in human infection.

Chapter 4: Surveillance of methicillin-resistant *Staphylococcus aureus* in Canadian retail meat and linkages to human clinical infections in Canada

4.1 Introduction

MRSA is an important nosocomial pathogen that can cause infections such as soft skin tissue infections, bacteremia, and sepsis¹⁷¹. The severity of this pathogen can be seen in Canada, where 820 BSIs were collected through CNISP surveillance with an all-cause mortality rate of 19.8% in 2022²². Despite its linkages to healthcare and nosocomial acquisition, MRSA infections have been increasingly associated with community-acquisition, with a 12% increase in CA-MRSA from 2018-2022, while HA-MRSA BSIs remained stable over the same period²².

CA-MRSA can be transmitted through direct or close contact with healthy or infected individuals, along with contaminated surfaces and environments. While CA-MRSA can cause a variety of infections, it is most commonly associated with skin and soft-tissue infections in humans, including in Canada, where wound and intravenous infections were the most common infection types observed, followed by BSI and respiratory infections^{172,173}. Beyond human-to-human transmission, MRSA has been studied as a One-Health pathogen, with select clones known as livestock-associated MRSA (LA-MRSA)¹⁷². While colonization and infection by LA-MRSA is commonly attributed to close proximity to livestock either occupationally or geographically, infections with no known exposure to livestock have increasingly been reported^{174,175}. Thus, it is challenging to understand the significance of MRSA outside of the nosocomial environment and its reservoirs for transmission within the One-Health continuum.

One potential reservoir for MRSA is retail meat, with a previous Canadian study finding MRSA in 31/402 (7.7%) of retail pork samples, suggesting that contamination is not rare¹¹⁹. Retail meat may serve as a vector for transmission between agri-food and human sectors via the farm-to-fork continuum, however no Canadian studies have established this linkage beyond traditional typing methods. This study therefore investigates retail meat as a potential reservoir for MRSA transmission, aiming to determine the prevalence of MRSA in Canadian retail meat and investigate potential genomic linkages to human clinical isolates in Canada.

4.2 Results

4.2.1 Prevalence and characterization of MRSA from Canadian retail meat

A low prevalence of MRSA was detected in both retail pork (0.9%, 2/219) and beef (1.0%, 1/99) for a combined prevalence of 0.9% (3/318) in all retail meat samples. The two pork isolates, 035-M-07 (t899/ST9) and 099-M-10 (t034/ST398) were purchased in 2016 from Western and Central Canada, respectively. The third isolate 232-M-02 (t008/ST8) was collected from beef purchased in Central Canada in 2018. cgMLST complex types (CT) for 035-M-07, 099-M-10, and 232-M-02 were CT181, CT39815, and CT39840, respectively (Table 3).

4.2.2 Investigation of potential genomic linkages between retail meat and human clinical MRSA

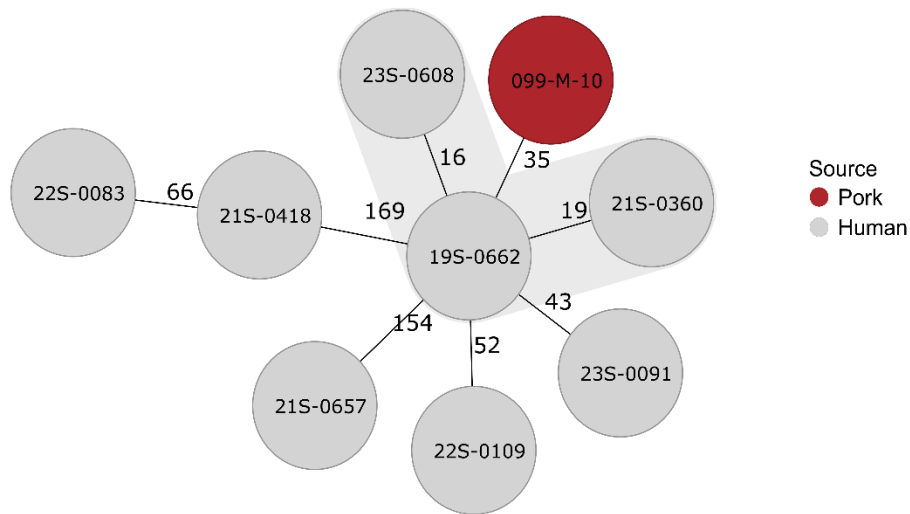
There were a limited number of genomes available for genomic comparison of the t034/ST398 pork isolate (099-M-10) with CNISP human clinical isolates of the same *spa* and ST; therefore human clinical isolates from any year, both CA-MRSA and HA-MRSA as defined through CNISP²² were included. All 9 isolates included in the cgMLST analysis had unique complex types, with 099-M-10 being identified as CT39815. Furthermore, 099-M-10 did not cluster with any other human clinical isolate, showing 35 allelic differences to its nearest neighbor (Figure 5A). SNV analysis further distinguished genomic differences between 099-M-10 and the human clinical isolates where pairwise SNV distances between pork and clinical isolates was 69-93 SNVs (Figure 5B).

The pork isolate is located in a cluster with five other isolates, three of which were HA-MRSA and two of which were CA-MRSA. As observed with cgMLST, 099-M-10 is genetically distinct from the human clinical isolates included in this comparison. Last, 099-M-10 was predicted to harbour ARGs *ant(9)-Ia*, *blaZ*, *erm(A)*, *lnu(A)*, *mec(A)*, *tet(K)*, and *tet(M)*. This profile was shared with 4/8 human clinical isolates, all of which were located in the same phylogenetic cluster (Figure 5B). Genomic comparisons to t008/ST8 from retail beef (232-M-02) with human clinical isolates of the same *spa* and ST were limited to 2017-2019 CA-MRSA collected through CNISP. Through cgMLST, 232-M-02 was identified as CT39840, a unique complex type in this comparison. This isolate showed a large allelic distance of 257 to its nearest neighbor and was therefore not located within any cgMLST cluster (Figure 6A). This is also reflected in the SNV analysis, where 232-M-02 does not cluster with any human clinical isolates, with pairwise SNV

Table 3. Epidemiological and typing data of MRSA recovered from retail meat.

Isolate Name	Year	Region	<i>spa</i> type	ST	cgMLST complex type
035-M-07	2016	Western	t899	9	181
099-M-10	2016	Central	t034	398	39815
232-M-02	2018	Central	t008	8	39840

A



B

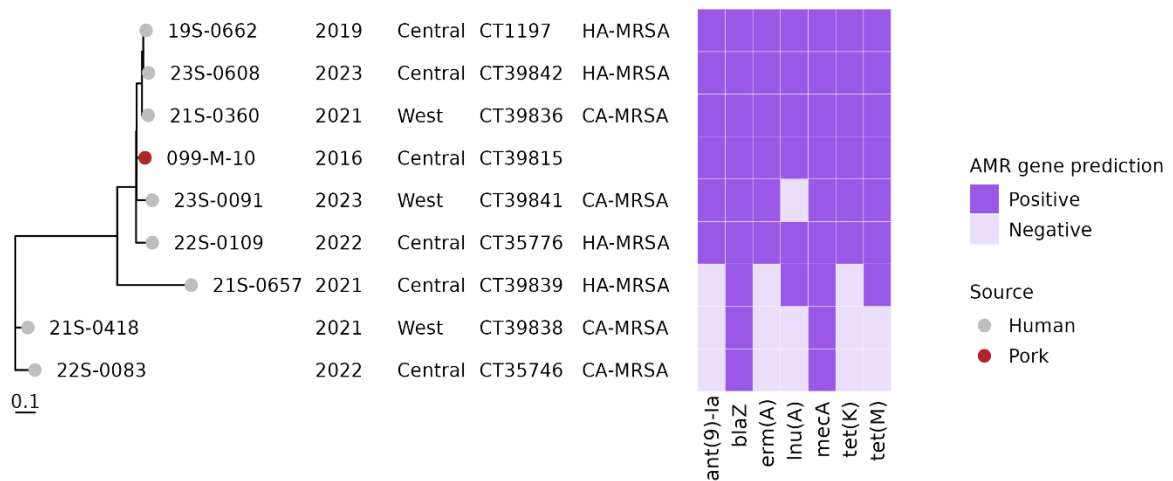


Figure 5. Phylogenetic analyses of 099-M-10 and human clinical t034/ST398 isolates. (A) cgMLST minimum spanning tree presented on a logarithmic scale. Grey highlight indicates clusters containing ≤ 24 allelic differences. (B) Unrooted maximum likelihood tree resulting from the SNV analysis. 90.52% of the core genome was used in the analysis and 811 variant sites were used to generate the phylogeny.

distances ranging from 411-468 SNVs to other isolates, and a low core genome (78.64%) (Figure 6B). Last, it had a unique ARG profile, harbouring *ant(6)-Ia*, *aph(3')-III*, *blaZ*, *mecA*, *mph(C)*, *msr(A)*, and *tet(K)*.

The final pork isolate 035-M-07 was identified as t899/ST9 which has not been identified from human clinical isolates though CNISP at the time of this study, therefore publicly available human clinical isolates were utilized for genomic comparisons. Due to the limited available ST9 sequences of confirmed human origin, *S. aureus* sequences from any year or country were used, and both MRSA and MSSA were accepted (Appendix C Table C1). Furthermore, since the majority of publicly available isolates only had published assembly records, a SNV analysis was not performed on these isolates, although cgMLST analysis indicates that a comparison at the variant level would not be informative due the large number of allelic differences (178) between 035-M-07 and its nearest neighbour. Unsurprisingly, 035-M-07 was a unique complex type in this analysis, identified as CT181 (Figure 7A). Of the 18 ARGs identified across all sequences, 035-M-07 harboured the fewest, with only *blaZ* and *mecA* being detected (Figure 7B). Taken together 035-M-07 appears to be genetically distinct from published human clinical isolates of the same *spa* and ST, with a limited resistome relative to published clinical isolates.

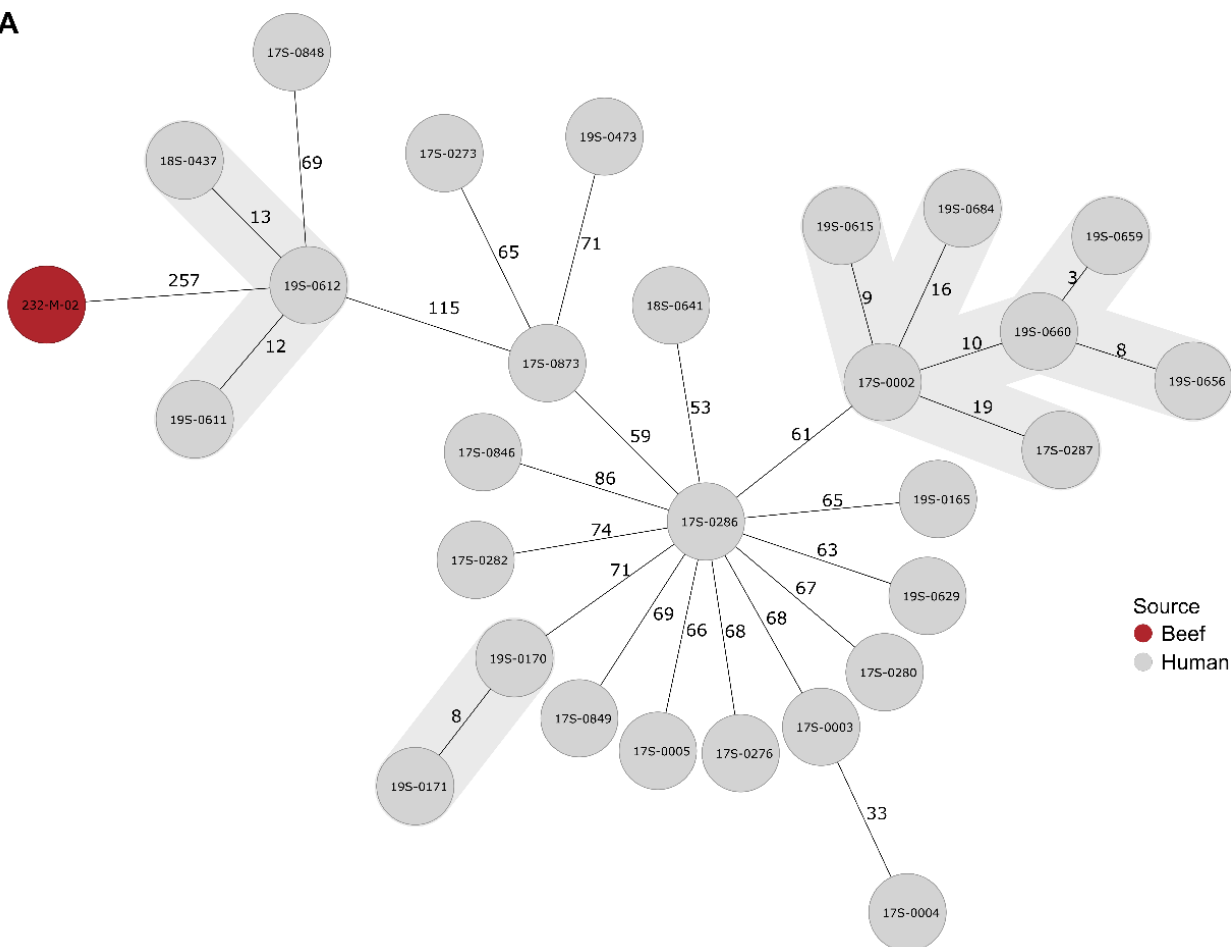
4.3 Discussion

A low prevalence of MRSA was recovered from Canadian retail pork and beef in this study, which contrasts with earlier studies of Canadian retail meat that have reported 7.7% and 9.6% MRSA contamination in retail pork and 5.6% in retail beef^{118,119}. It should be noted that previous studies screened and processed fresh retail meat samples, whereas MRSA selection in this study was performed on meat rinsates that had been frozen; therefore, the low prevalence of MRSA detected could be reflective of a potential loss of viability that occurred during storage.

Two livestock-associated STs were recovered from retail pork: ST398 and ST9. ST398 belongs to CC398, which is the predominant LA-MRSA clone found in Europe and North America, while ST9 belongs to CC9, which is predominant in Asia¹⁷⁶. While it was unsurprising to detect ST398 in retail meat samples, the isolation of a common Asian clone that has not been detected in Canadian human clinical isolates was unexpected.

LA-MRSA ST398 has long been associated with pigs and pig farming, with initial reports of this association coming from France¹⁷⁵. Since then, this strain has been reported in North

A



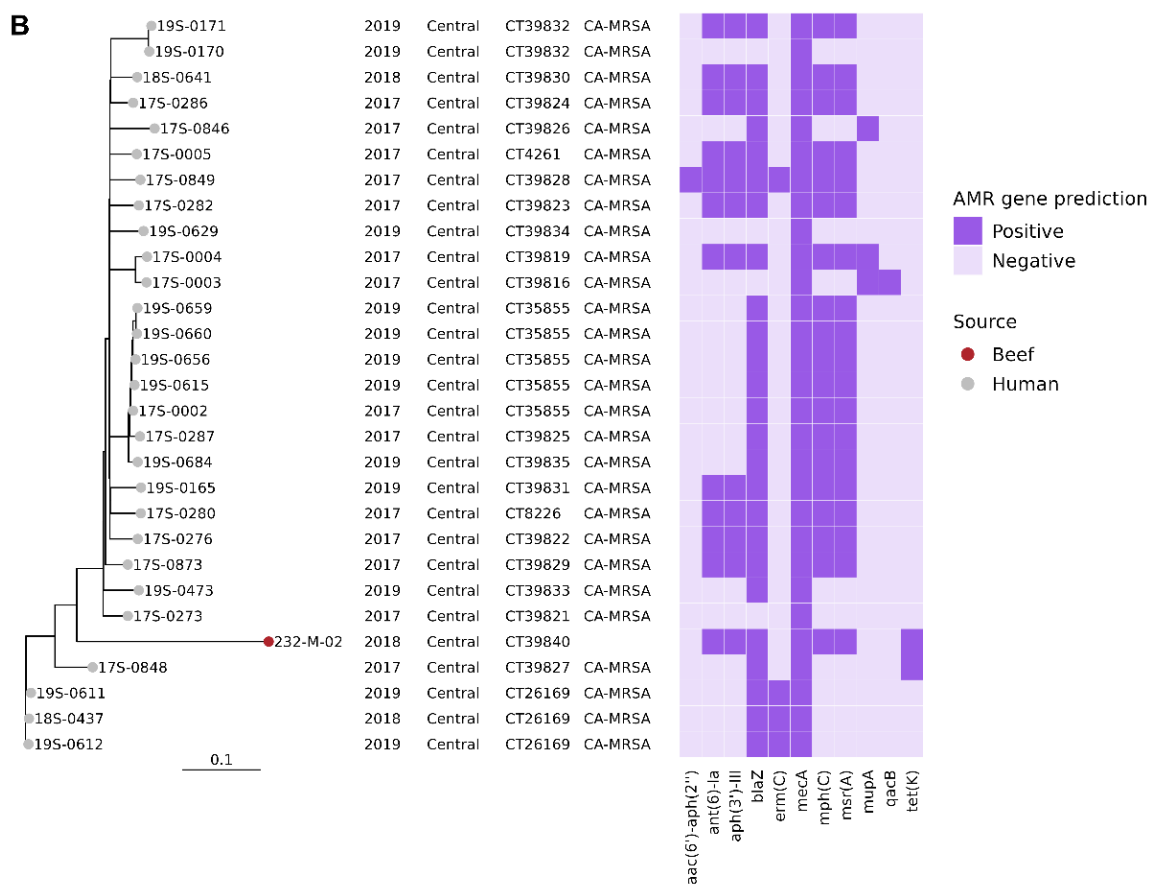


Figure 6. Phylogenetic analyses of 232-M-02 and human clinical t008/ST8 isolates. (A) cgMLST minimum spanning tree presented on a logarithmic scale. Grey highlight indicates clusters containing ≤ 24 allelic differences. (B) Unrooted maximum likelihood tree generated from the SNV analysis. 78.64% of the core genome was used in the analysis and 1612 variant sites were used to generate the phylogeny.

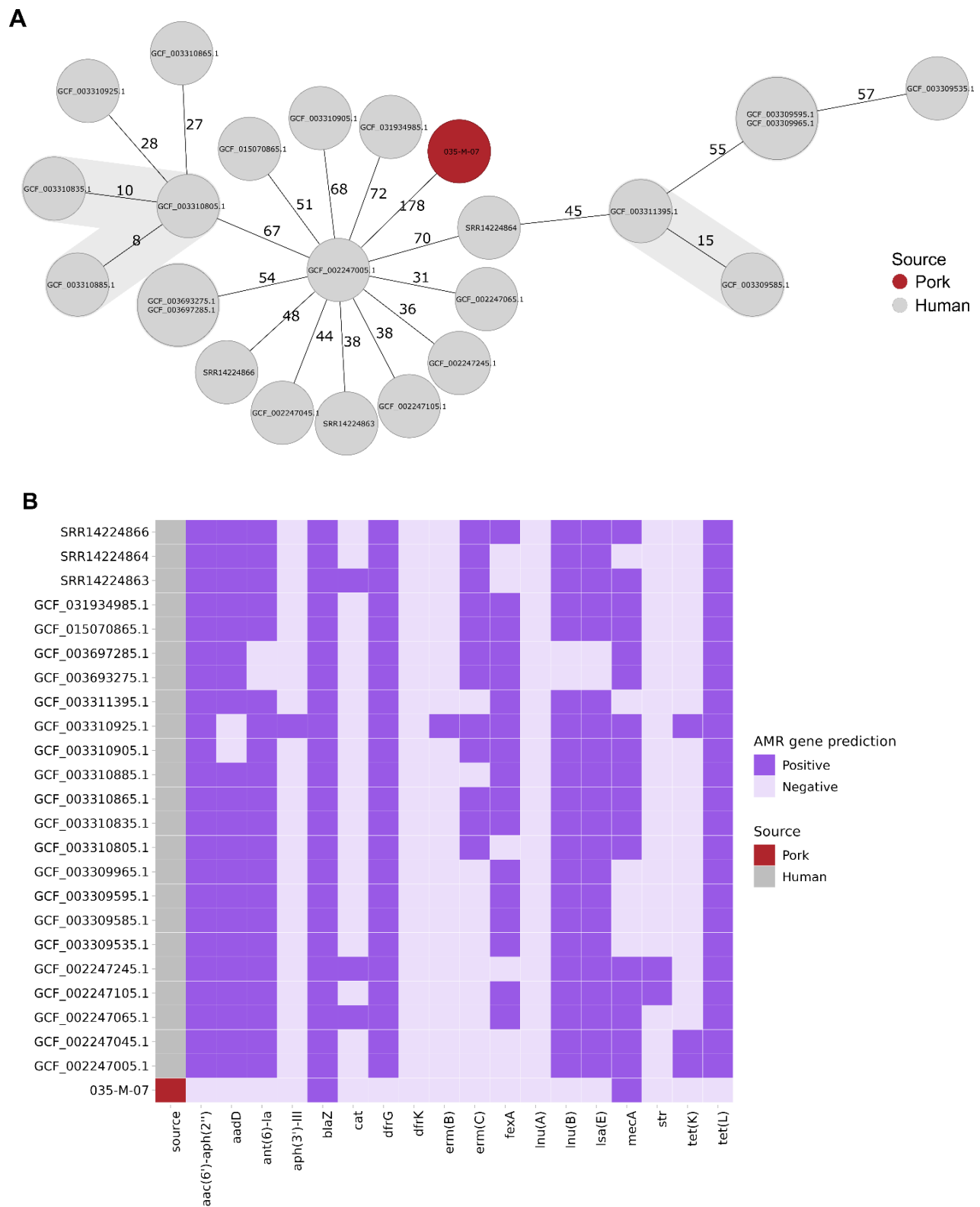


Figure 7. Comparison of 035-M-07 to publicly available human *S. aureus* t899/ST9. (A) cgMLST minimum spanning tree presented on a logarithmic scale. Grey highlight indicates clusters containing ≤ 24 allelic differences. (B) Heatmap of predicted ARGs and their sources of isolation.

America, having been recovered from pigs and pig farmers in Canada and the US^{174,177,178}. Furthermore, this strain has been isolated from both American and Canadian retail pork, with t034 also being detected in select strains from Canadian retail pork samples^{119,178}. Despite human cases being associated with occupational exposure to pigs^{174,177}, MRSA ST398 has been recovered from an individual in Canada with no known exposure to pigs or pig farms¹⁷⁹, indicating other routes of transmission.

Of the genomic comparisons performed in this study, t034/ST398 showed the closest linkages to human clinical MRSA. Despite this, its unique cgMLST CT and lack of clustering with other human clinical isolates does not support any direct transmission events. Furthermore, the pairwise SNV distances observed between 099-M-10 and human clinical isolates ranging from 69-93 SNVs are beyond SNV thresholds commonly used to define transmission in other studies^{180,181}.

While no close genetic relatedness through cgMLST or SNV analysis was observed in this study, identical ARG profiles were observed across select t034/ST398 isolates. Of note was the presence of tetracycline resistance gene *tet(M)*, which has been previously been used as a marker of livestock-associated CC398¹⁸². This gene was observed in the majority (6/8), but not all human isolates, and those that harboured this strain clustered more closely with 099-M-10 than those without the gene. The human isolates that lacked this tetracycline resistance gene were identified as CA-MRSA and shared identical ARG profiles with each other. Despite no evidence of direct transmission through cgMLST or SNV analysis, some human clinical isolates collected through CNISP appeared to be more closely related to 099-M-10 than others.

LA-MRSA ST9 is considered a dominant LA-MRSA strain in Asia¹⁸³, but it has also been isolated from different sources in Europe^{175,184}. Reports of this ST in North America are extremely limited, however, one study reported ST9 in fair pigs in the US¹⁸⁵ and another reported *S. aureus* CC9 from both pigs and workers at industrial hog farm¹⁸⁶. Beyond the ST, t899 has been identified as a major *spa* type among ST9 isolates¹⁸³, with one study suggesting that MRSA ST9-SCCmecXII-t899 evolved independently in China and is distinct from other ST9 lineages identified elsewhere¹⁸⁷. Identifying this *spa* type and ST9 in Canadian retail meat points to an origin in Asia, but sources of contamination are unclear.

Similar to the previous comparison, no close relationships between 035-M-07 and human t899/ST9 isolates were observed through cgMLST. However, unlike the previous comparison,

035-M-07 harboured strikingly few ARGs in contrast to the human t899/ST9 included in this study. Elsewhere, this strain has been associated with multidrug resistance (MDR) conferred through the MGE MDR_{ST9}¹⁸³. Predicted MDR can be observed with human isolates but not 035-M-07, further distinguishing it from the publicly available t899/ST9 of human origin. Without additional sampling, detailed epidemiological information, or reports of t899/ST9, it is challenging to understand the significance of this strain in the context of the Canadian One-Health continuum. Its presence indicates that t899/ST9 is present in some Canadian reservoirs; however, the importance of this strain remains ambiguous at this time. While no MRSA infections belonging to this ST were recorded through CNISP surveillance, it is important to note that non-BSI infections are not captured. Therefore, less severe infection types more commonly associated with LA-MRSA, such as skin and soft tissue infections, may be caused by this livestock-associated clone in Canada, but remain unrecorded by national surveillance efforts that focus just on BSIs.

The last strain identified in this study, t008/ST8, is a community-associated strain also known as CMRSA10/USA300. Previous Canadian studies have isolated t008/CMRSA5 from a retail pork sample¹¹⁹, and another study isolated MRSA ST8 from Canadian retail chicken meat¹¹⁷. In the United States, MRSA ST8 has been isolated from several meat types, including from beef^{188–190}. In humans, previous CNISP surveillance data from 2008-2012 showed this strain to cause BSI that were identified as both community-acquired and hospital-associated¹⁹¹. CMRSA10 first emerged in Canada in 2001, where most isolates were community-associated and it quickly became the second most common MRSA clone collected through CNISP, causing both HA and CA-MRSA infections¹⁹². Recent CNISP data shows that CMRSA10 remains an important lineage in Canada contributing to both HA and CA-MRSA, where it was identified as 27.0% of HA isolates, and 48.9% of CA isolates¹³⁴.

In this study, 232-M-02 is distinct from human clinical CA-MRSA t008/ST8 by both cgMLST and SNV analysis. While it does not share an ARG profile with any human isolates, it shares six of its seven ARGs with 11/28 human clinical isolates. Interestingly, 232-M-02 only shares its seventh ARG, *tet(K)*, with one human isolate, and this ARG has been associated with LA-MRSA in CC398¹⁹³. While the *spa*/ST alone suggest that this MRSA contamination is of human origin, the distinct genomic nature of this isolate coupled with the presence of *tet(K)* could indicate an association to livestock, however further sampling and epidemiological data is required

to determine a specific source of contamination.

Overall, this study identified a low prevalence of MRSA in Canadian retail meat, with two isolates being identified as LA-MRSA (ST398, ST9) and one as an important human clinical CA-MRSA (t008/ST8) clone in Canada. This study provides an updated perspective into MRSA as a One-Health pathogen, with relationships observed to human clinical isolates through *spa* and sequencing typing methods. At a more granular level, the genomic relationships observed through cgMLST and SNV analysis do not support any direct transmission event, which could be a reflection of isolate inclusion criteria for CNISP surveillance. Future work should include the investigation of MRSA in livestock, abattoir, and meat processing facilities to further understand important reservoirs and the potential transmission of this pathogen in Canada through a One-Health perspective.

Chapter 5: Genomic characterization of carbapenemase-producing-*Pseudomonas* spp. and *Acinetobacter* spp. in Canadian retail meat and linkages to human clinical infections in Canada

5.1 Introduction

CR-*A. baumannii* and *P. aeruginosa* are among the highest priority AMR pathogens for the prioritization of new antimicrobials identified by the World Health Organization¹⁸. These pathogens were estimated to be among the top six contributors to AMR-associated deaths in 2019, while CR-*A. baumannii* was estimated to be among the top seven pathogens in AMR-attributable deaths¹⁴. *A. baumannii* can cause a variety of infections, including ventilator-associated pneumonia, BSI, skin and soft tissue infections, urinary tract infections, and meningitis. Their success as nosocomial pathogens can be attributed to several factors, including their virulence factors and antimicrobial resistance mechanisms, and their ability to colonize humans and survive in hospital environments¹⁹⁴. *A. baumannii* belongs to a complex of six organisms, known as the ACB complex. Along with *A. baumannii* and *A. calcoaceticus*, this complex includes *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter seifertii*, and *Acinetobacter dijkschoorniae*. While most species in the complex are associated with human infection, *A. calcoaceticus* is often associated with environmental reservoirs¹⁹⁴.

P. aeruginosa cause a similar spectrum of infections as *A. baumannii*, including BSI, respiratory infections including ventilator-associated pneumonia and infections associated with cystic fibrosis, urinary tract infections, skin and soft tissue infections, and ear infections¹⁹⁵. Importantly, *P. aeruginosa* are known for their ability to form biofilms, which are associated with chronic infections^{196,197}. Like *A. baumannii*, *P. aeruginosa* can express several virulence factors and can harbour AMR mechanisms including those which are intrinsic to the species, acquired ARGs, and adaptive mutations. Intrinsic mechanisms include reduced membrane permeability through porins, an intrinsic AmpC cephalosporinase that hydrolyses most β -lactams, and broad-spectrum efflux pumps¹⁹⁵. These mechanisms can result in a CR phenotype, however their potential to acquire carbapenemases is of greater concern¹⁹⁸. In Canada, CP-*P. aeruginosa* are relatively rare, with previous reports from CANWARD finding 12.1% of *P. aeruginosa* isolates to be CR, with only 4.3% of these isolates (20/466) harbouring a carbapenemase¹⁹⁹.

Both *A. baumannii* and *P. aeruginosa* can be found throughout the environment. While

often studied as a nosocomial pathogen, *A. baumannii* has been involved in community-acquired infections²⁰⁰. Non-clinical sources of *A. baumannii* include soil²⁰¹, meat²⁰², fruits and vegetables²⁰³, wildlife and livestock²⁰⁴, and wastewater²⁰⁵. Additionally, non-*baumannii* *Acinetobacter* spp. are ubiquitous in nature and associated with environmental reservoirs such as soil, sewage and wastewater, and seawater²⁰⁶. They have also been isolated from livestock, companion animals, and lice in addition to human carriage²⁰⁶. AMR can be detected among these environmental reservoirs, with AMR *Acinetobacter* spp. having associations with environmental reservoirs, including livestock^{207,208}. With their ubiquitous nature, associations with community-acquired infections, animal and food reservoirs, and AMR, this genus has been proposed as an important One-Health pathogen²⁰⁹, with recent studies having identified both similarities and differences in *Acinetobacter* spp. from environmental and clinical sources^{207,210,211}. In the Canadian context, however, further work is required to understand the significance of *Acinetobacter* spp. within the One-Health continuum.

Similar to *Acinetobacter* spp., *Pseudomonas* spp. are also ubiquitous in nature and associated with water and aquatic environments. They have been isolated from sewage, surface water, and other aquatic environments^{212,213}, from healthy animals, including livestock, companion animals, and wild animals²¹⁴, agricultural soil²¹⁵, vegetables, seafood, meat²¹⁶, and from humans as opportunistic pathogens¹⁹⁹. *Pseudomonas* spp. have also been involved in community-acquired infections²¹⁷, and have been discussed through a One-Health lens with livestock identified as a potential reservoir for the spread of AMR pseudomonads²¹⁸.

In this study, retail pork and beef were screened for CPOs, and among the organisms isolated were *Acinetobacter* spp. and *Pseudomonas* spp. Here, the prevalence of CP members of these genera in Canadian retail meat are investigated, along with their genomic characterization. When available, linkages to human clinical infections in Canada are investigated through genomic comparisons.

5.2 Results

5.2.1 Prevalence of carbapenemase-producing *Acinetobacter* spp. and *Pseudomonas* spp. isolated from Canadian retail meat

CR-*Pseudomonas* spp. were recovered from 316/318 (99.4%) retail meat rinsates. In one rinsate, *Pseudomonas* was detected using MALDI-TOF MS but was not maintained for further

phenotypic carbapenemase screening (Figure 8). This isolate was selected from a retail pork rinsate purchased in Western Canada in 2016. In total, 3151 CR-*Pseudomonas* spp. isolates from 315 retail meat rinsates were screened for phenotypic carbapenemase production and 18 CP-*Pseudomonas* spp. were detected (Appendix C Table C1). One CP-*Pseudomonas* spp. was not available for subsequent analysis and was removed from the collection, leaving 17. Only one representative isolate from rinsates harboring the same species was included to avoid possible duplication, resulting in a final isolate collection consisting of 14 unique CP-*Pseudomonas* spp. from 13 meat rinsates.

A total of 20 CR-*Acinetobacter* spp. isolates were recovered from 318 retail meat rinsates (Table 4) but phenotypic carbapenemase detection was not performed due to the poor performance of mCIM phenotypic testing associated with this genus^{219,220}. After filtering for possible duplicate isolates, the final isolate collection consisted of 19 unique CR-*Acinetobacter* spp., 13/19 of which harboured a carbapenemase identified through WGS. The species and ARGs detected in carbapenemase-negative *Acinetobacter* spp. can be found in Appendix F Table F1.

Both *Pseudomonas* spp. and *Acinetobacter* spp. that harboured carbapenemases were recovered from retail meat rinsates. CP-*Pseudomonas* spp. were detected phenotypically in 4.01% (4/99) of retail beef samples and 4.1% (9/218) of retail pork samples, for an overall prevalence of 4.1% (13/317) in Canadian retail meat. *Acinetobacter* spp. that harboured carbapenemases were recovered at the same rate of 4.1% (13/318) as with CP-*Pseudomonas* spp., however the majority of positive rinsates were retail beef (10.1%, 10/99) rather than retail pork (1.4%, 3/219) (Fisher's Exact Test: $p = 3.3e-05$, odds ratio = 10.8, 95% CI: 2.9 – 60.5). Interestingly, over half (7/13) of the CP-*Acinetobacter* spp. originated from retail beef purchased in Central Canada in 2018 (Table 4). Eight of 14 *Pseudomonas* spp. were also recovered from 2018 samples, although they were found across different products and regions. Of note were the three retail meat rinsates (143, 161, and 225) that harboured both a CP-*Pseudomonas* spp. and CP-*Acinetobacter* spp. Both 143 and 225 are beef rinsates that were purchased in Western Canada in 2017 and Central Canada in 2018, respectively. The last rinsate, 161, is from pork purchased in Central Canada in 2018. Combined, these two genera were isolated from 7.2% (23/318) of retail meat rinsates.

5.2.2 Carbapenemases and other resistance in *Acinetobacter* spp. isolated from retail meat

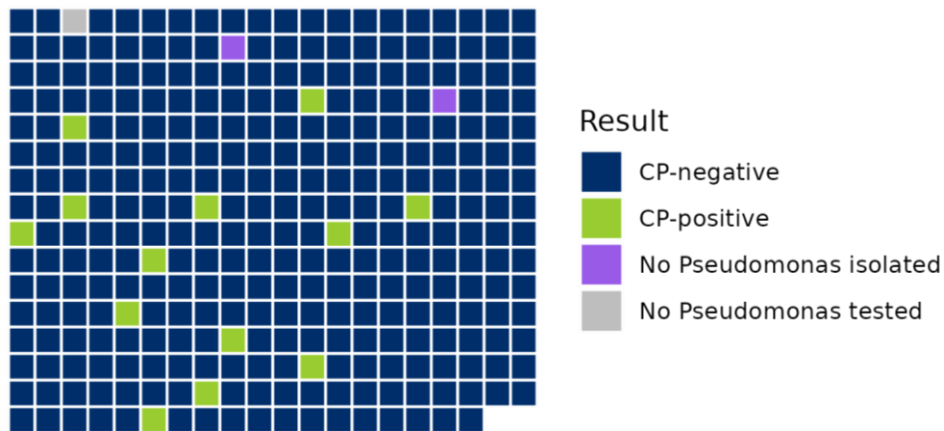


Figure 8. Overview of *Pseudomonas* sampling and testing from 318 retail meat rinsates. Retail meat rinsates are arranged from 1-318, left to right, with colours indicating whether *Pseudomonas* spp. were isolated from a given rinsate and tested for carbapenemase production.

Table 4. Species distribution of CP-*Pseudomonas* spp. and CP-*Acinetobacter* spp. retail meat rinsates and associated epidemiological data.

Source (n rinsates)	Region (n rinsates)	Year (n rinsates)	Rinsate number	Species
Beef (12/99)	Central (8/64)	2017 (1/10)	148	<i>P. fluorescens</i>
				<i>P. paracarnis</i>
		2018 (7/26)	160	<i>A. nematophilus</i>
			225	<i>A. gyllenbergii</i>
				<i>P. fluorescens</i>
			245	<i>A. pittii</i>
			252	<i>A. nematophilus</i>
			260	<i>A. pittii</i>
			292	<i>A. guillouiae</i>
			297	<i>A. baumannii</i>
	West (4/35)	2017 (2/9)	143	<i>A. pittii</i>
				<i>P. fluorescens</i>
		2018 (2/26)	156	<i>P. fluorescens</i>
			192	<i>A. gyllenbergii</i>
			271	<i>A. bereziniae</i>
Pork (11/219)	Central (8/123)	2016 (3/61)	72	<i>P. synxantha</i>
			83	<i>P. paracarnis</i>
			95	<i>A. bereziniae</i>
		2017 (0/14)	-	
		2018 (5/48)	161	<i>A. baumannii</i>
				<i>P. otitidis</i>
			272	<i>P. shahriarae</i>
			288	<i>P. shahriarae</i>
			289	<i>A. nematophilus</i>
			306	<i>P. fluorescens</i>
	West (3/96)	2016 (0/51) ¹	-	
		2017 (0/13)	-	
		2018 (3/32)	173	<i>P. paracarnis</i>
			186	<i>P. synxantha</i>
			249	<i>P. shahriarae</i>

¹50 rinsates are included in the investigation of *Pseudomonas* spp.

As described above, 19 CR-*Acinetobacter* spp. were recovered from 318 retail meat rinsates, and carbapenemases were detected in 68.4% (13/19) of isolates, all of which were identified as *bla*OXA-type. The 13 CP isolates were identified through WGS as *A. baumannii*, *Acinetobacter bereziniae*, *Acinetobacter guillouiae*, *Acinetobacter gyllenbergii*, *Acinetobacter nematophilus*, and *A. pittii* (Table 5).

Carbapenemase genes identified with AMRFinderPlus⁴⁸ were all *bla*OXA-type carbapenemases (Table 5), which can be found intrinsically in *Acinetobacter* spp. Nine different carbapenemases were detected from the 13 CP-*Acinetobacter* spp., which can be further classified into five different subfamilies. While there was some *bla*OXA diversity within isolates of the same species, *bla*OXA subfamilies were stratified by their species (Table 6). The majority of carbapenemases all had a high amino acid identity to their reference sequence ($\geq 98.93\%$) with the exception of *bla*OXA-1190 from *A. nematophilus* which only had an identity of 91.3% to its reference sequence (Table 6).

Beyond carbapenemases, select isolates harboured additional resistance genes related to efflux (*adeD*, *adeE*, *amvA*), fosfomycin resistance (*abaF*), aminoglycoside resistance (*ant(3'')-IIa*, *aph(3')-VIa*, *aac(6')-Ih*, *ant(3'')-IIc*), cephalosporin resistance (*bla*ADC-type), chloramphenicol resistance (*cpxE*), and tetracycline resistance (*tet(39)*). Additionally, nickel resistance (*nreB*) was predicted in *A. baumannii* and *A. pittii*, and mercury resistance (*mer* genes) was predicted in one *A. nematophilus* isolate (Table 5). While plasmids were detected in 10/13 CP isolates, only the resistance gene *tet(39)* from 260-O-05 was located on the plasmid AC603, which was predicted to be non-mobile.

5.2.3 Genomic linkages between carbapenemase-producing *Acinetobacter calcoaceticus*-*Acinetobacter baumannii* complex isolated from retail meat and human clinical isolates in Canada

There were five isolates that were identified as members of the ACB complex, and on the basis of available Canadian human clinical isolates with the same ST, genomic comparisons could be made to 4/5 of the ACB complex retail meat isolates. The fifth isolate, *A. baumannii* PA ST203, had no corresponding human clinical isolates of this ST available for genomic comparison. Genomic comparisons were made to one human clinical *A. baumannii* collected from CNISP and 9 human clinical *A. pittii* isolates collected through CANWARD.

Table 5. STs and AMR profiles of unique CP-*Acinetobacter* spp. isolates in this study.

Isolate name	Species	Pasteur ST	Oxford ST	ARG ¹	Metal tolerance gene
161-C-13	<i>A. baumannii</i>	203	714	<i>abaF</i> , <i>amvA</i> , <i>ant(3'')-iIa</i> , <i>blaADC-6</i> , <i>blaOXA-98</i> , <i>cxpE</i>	<i>nreB</i>
297-C-02	<i>A. baumannii</i>	1362	-	<i>amvA</i> , <i>ant(3'')-iIa</i> , <i>blaADC-154</i> , <i>blaOXA-78</i> , <i>cxpE</i>	<i>nreB</i>
095-O-09	<i>A. bereziniae</i>	-	-	<i>blaOXA-229</i>	
271-O-04	<i>A. bereziniae</i>	-	-	<i>blaOXA-355</i>	
292-O-06	<i>A. guillouiae</i>	-	-	<i>adeE</i> , <i>aph(3')-vIa</i> , <i>blaOXA-275</i>	
192-O-02	<i>A. gyllenbergii</i>	-	-	<i>aac(6')-ih</i> , <i>ant(3'')-iIc</i> , <i>blaOXA-671</i>	
225-O-01	<i>A. gyllenbergii</i>	2535	-	<i>aac(6')-ih</i> , <i>ant(3'')-iIc</i> , <i>blaOXA-671</i>	
160-O-05	<i>A. nematophilus</i>	-	-	<i>blaOXA-1190</i>	
252-O-05	<i>A. nematophilus</i>	1258	-	<i>blaOXA-1190</i>	<i>merA</i> , <i>merD</i> , <i>merE</i> , <i>merR</i> , <i>merT</i>
289-O-02	<i>A. nematophilus</i>	1258	-	<i>blaOXA-1190</i>	
143-C-03	<i>A. pittii</i>	214	2010	<i>adeD</i> , <i>adeE</i> , <i>amvA</i> , <i>ant(3'')-iIa</i> , <i>blaADC-245</i> , <i>blaOXA-506</i> , <i>cxpE</i>	<i>nreB</i>
245-C-06	<i>A. pittii</i>	214	2010	<i>adeD</i> , <i>adeE</i> , <i>amvA</i> , <i>ant(3'')-iIa</i> , <i>blaADC-245</i> , <i>blaOXA-506</i> , <i>cxpE</i>	<i>nreB</i>
260-O-05	<i>A. pittii</i>	119	1638	<i>amvA</i> , <i>ant(3'')-iIa</i> , <i>blaADC-150</i> , <i>blaOXA-421</i> , <i>cxpE</i> , <i>tet(39)</i>	<i>nreB</i>

¹carbapenemases have been bolded

Table 6. Summary of carbapenemases detected in unique CP-*Acinetobacter* spp. in this study.

Species (n)	Gene subfamily	Carbapenemase (n)	Identity to the reference (%) ¹	Accession
<i>A. baumannii</i> (2)	<i>bla</i> OXA-51	<i>bla</i> OXA-78 (1)	100	WP_005139262.1
		<i>bla</i> OXA-98 (1)	100	WP_001021777.1
<i>A. bereziniae</i> (2)	<i>bla</i> OXA-229	<i>bla</i> OXA-229 (1)	100	WP_063861840.1
		<i>bla</i> OXA-355 (1)	99.64	WP_063862726.1
<i>A. guillouiae</i> (1)	<i>bla</i> OXA-274	<i>bla</i> OXA-275 (1)	98.93	WP_004816501.1
<i>A. gyllenbergii</i> (2)	<i>bla</i> OXA-286	<i>bla</i> OXA-671 (2)	100	WP_081401608.1
<i>A. nematophilus</i> (3)	<i>bla</i> OXA-229	<i>bla</i> OXA-1190 (3)	91.3	WP_139419258.1
<i>A. pittii</i> (3)	<i>bla</i> OXA-213	<i>bla</i> OXA-421 (1)	100	WP_017386798.1
		<i>bla</i> OXA-506 (2)	100	WP_032007272.1

¹percent amino acid identity to the top reference sequence

The first comparison consisted of a single human clinical and retail meat isolate for *A. baumannii* PA ST1362. cgMLST analysis showed 14 allelic differences between the two isolates, which falls beyond the default threshold of 9 allelic differences to be considered as part of the same cgMLST cluster by Ridom SeqSphere+¹⁴³. Furthermore, unique complex types were detected for both the human clinical (CT8074) and the retail meat isolate (CT8073), further indicating a lack of close relationship at the level of cgMLST (Figure 9A). At the SNV level, the retail meat and the human clinical isolate differed by 62 SNVs, with a high core genome of 94.53%. Both isolates originated from Central Canada and shared identical ARGs, showing some level of similarity (Figure 9B).

The remaining two comparisons were performed on *A. pittii*, for which no cgMLST scheme was available for comparison through Ridom SeqSphere+¹⁴³. The *A. pittii* retail meat isolate 260-O-05 was isolated from retail beef purchased in 2018 in Central Canada. CANWARD human clinical isolates used in this genomic comparison all belonged to the same Pasteur ST as 260-O-05 (PA ST119), however only one of the eight clinical PA ST119 had the same OX ST (OX ST1638) as the meat isolate (Figure 10). Unsurprisingly, the relationship between 260-O-05 and the human clinical isolate N18-4136 (PA ST119/OX ST1638) was the closest relationship observed using pairwise SNV distances, where 481 SNVs were identified between the two isolates. Conversely, 260-O-05 differed by over >4500 SNVs with all other CANWARD OX ST1923 isolates available for comparison. Comparison amongst just the OX ST1923 isolates showed a closer relationship with SNVs ranging from 48-206. There was a greater level of similarity when ARGs were evaluated, with 260-O-05 sharing six of its seven ARGs with all human clinical CANWARD isolates, and the seventh gene, *tet(39)*, was the only ARG unique to the meat isolate. All *A. pittii* within this ST shared the same *blaOXA* allele: *blaOXA-421* (Figure 10).

In contrast to the previous comparison, only one human clinical CANWARD isolate, N23-02685, was available for comparisons to the remaining two *A. pittii* retail meat isolates (143-C-03 and 260-O-05) belonging to PA ST214/OX ST2010. All three isolates were isolated from different years and regions (Figure 11). The two retail meat isolates had fewer SNVs between them, with 52 SNVs being identified between the two isolates. Relative to the human clinical CANWARD isolate, 143-C-03 shared 93 SNV differences with N23-02685, while 245-C-06 shared 69 SNV differences. Furthermore, the core genome of the maximum likelihood tree was much higher at

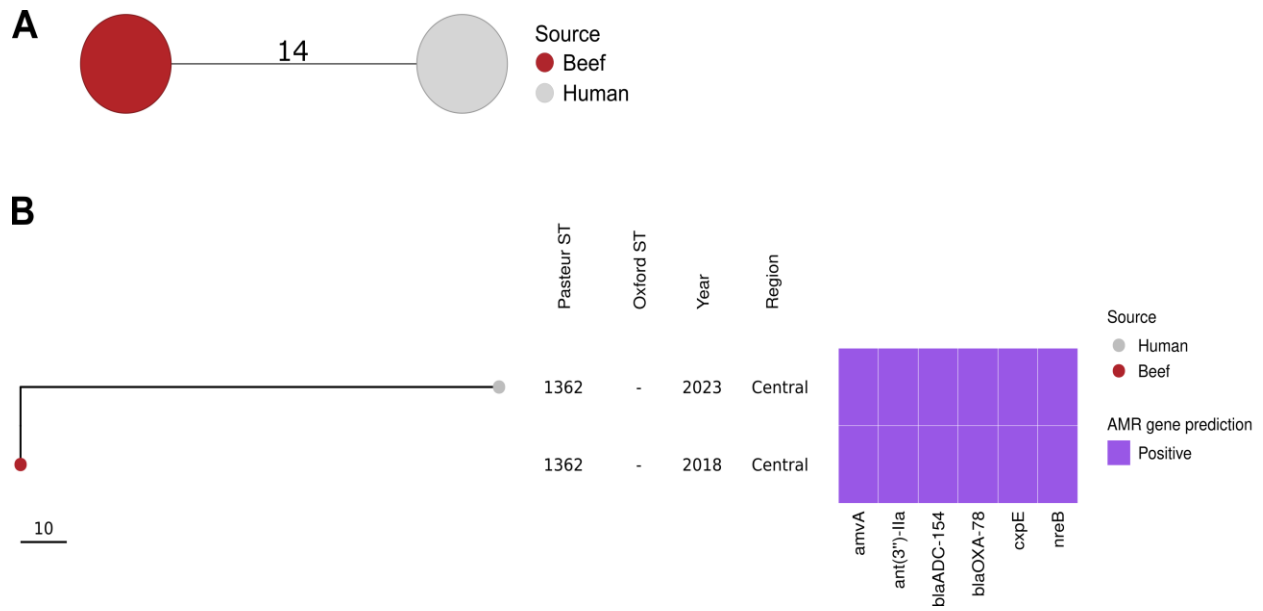


Figure 9. Phylogenetic analyses of 297-C-02 and a human clinical ST1362. (A) cgMLST minimum spanning tree presented on a logarithmic scale. (B) Unrooted maximum likelihood tree generated from the SNV analysis. 94.53% of the core genome was used in the analysis and 63 variant sites were used to generate the phylogeny.

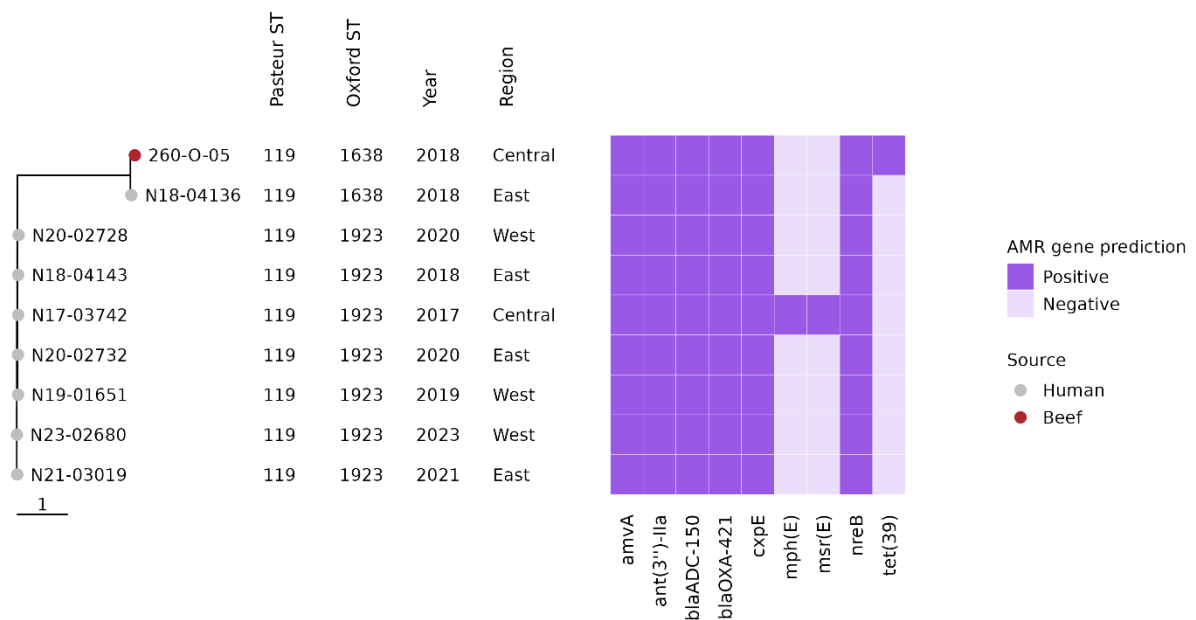


Figure 10. Unrooted maximum likelihood tree of genomic comparison of *A. pittii* PA ST119 collected from rinsates and human clinical samples. The phylogeny was generated from 5092 variant sites, and 82.07% of the core genome was used for the analysis.

96.66%, increasing the reliability of these results. Last, there were no differences in the ARGs identified across the three isolates. Isolates in this comparison showed no epidemiological similarity through collection year or region. Rather, retail meat and human clinical isolates in this comparison had identical STs (Paster and Oxford), identical ARG profiles, and relatively close genomic relationships despite the human clinical isolate having been collected 5 and 6 years after the retail meat rinsates (Figure 11).

5.2.4 Carbapenemases in *Pseudomonas* spp. isolated from retail meat

Fourteen unique CP-*Pseudomonas* isolates from 13 different retail meat rinsates were recovered with the most common species being *Pseudomonas fluorescens*, followed by *Pseudomonas paracarnis* and *Pseudomonas shahriarae*, *Pseudomonas synxantha*, and *Pseudomonas otitidis* (Table 7). In the majority of isolates (11/14), *bla*PFM-type carbapenemases were identified, with *bla*PFM allele types 1, 4, and 3 being the same within a given species. *bla*POM-1 was the only other carbapenemase identified and was detected in *P. otitidis*. All identified carbapenemases had high amino acid identities ($\geq 99.6\%$) to their reference sequences (Table 8). Interestingly, despite repeated testing for phenotypic carbapenemase-production *in vitro*, no carbapenemase genes were identified in *P. paracarnis* isolates 083-O-01 and 173-C-08 using default AMRFinderPlus⁴⁸ parameters. Lowering the default AMRFinderPlus threshold eventually identified *bla*PDC at 58.24% identity to the reference sequence in both isolates (Appendix F Table F2), which may be a novel gene with carbapenemase activity.

No other ARGs were detected in *Pseudomonas* spp. beyond these carbapenemases, however mercury resistance (*mer* genes) was detected in 5/14 isolates, and a gene related to copper resistance (*copR*) was detected in 2/14 isolates. Plasmids were detected in 6/14 isolates, however neither ARG nor metal tolerance genes across isolates were determined to be plasmid-located.

Curiously, 148-O-07 and 148-O-8, despite originating from the same rinsate and harbouring the same resistance genes, were identified as two different species: *P. fluorescens* and *P. paracarnis*, respectively. While it is possible that these are in fact two different species from the same rinsate, sharing an identical ARG profile and *mer* operon is cause for speculation, especially when the two other *P. paracarnis* isolates did not harbour any carbapenemase detected with AMRFinderPlus⁴⁸. 148-O-07 and 148-O-08 had average nucleotide identity (ANI) scores of 95.26 and 95.09, respectively, which is close to the default ANI threshold of 95 used in GTDB

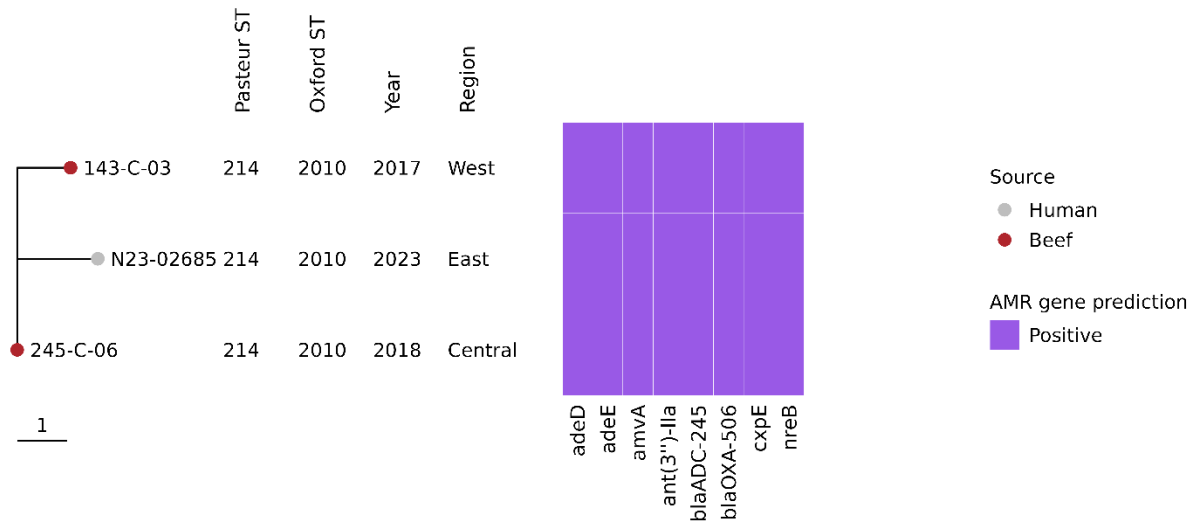


Figure 11. Unrooted maximum likelihood tree of genomic comparison of *A. pittii* PA ST214 collected from rinsates and human clinical samples. The phylogeny was generated from 107 variant sites, and 96.66% of the core genome was used for the analysis.

Table 7. Resistance genes detected in unique CP-*Pseudomonas* spp. in this study.

Isolate Name	Species	ARG	Metal tolerance gene
143-O-04	<i>P. fluorescens</i>	<i>bla</i> PFM-3	
156-O-07	<i>P. fluorescens</i>	<i>bla</i> PFM-3	
148-O-07	<i>P. fluorescens</i>	<i>bla</i> PFM-3	<i>merD, merE, merF, merP, merR, merT</i>
225-C-10	<i>P. fluorescens</i>	<i>bla</i> PFM-3	
306-O-09	<i>P. fluorescens</i>	<i>bla</i> PFM-3	
161-C-06	<i>P. otitidis</i>	<i>bla</i> POM-1	
083-O-01	<i>P. paracarnis</i>	-	
148-O-08	<i>P. paracarnis</i>	<i>bla</i> PFM-3	<i>merD, merE, merF, merP, merR, merT</i>
173-C-08	<i>P. paracarnis</i>	-	<i>copR</i>
249-C-06	<i>P. shahriarae</i>	<i>bla</i> PFM-4	<i>copR, merD, merE, merF, merP, merR, merT</i>
272-C-04	<i>P. shahriarae</i>	<i>bla</i> PFM-4	<i>merD, merE, merF, merP, merR, merT</i>
288-C-05	<i>P. shahriarae</i>	<i>bla</i> PFM-4	<i>merD, merE, merF, merP, merR, merT</i>
072-O-02	<i>P. synxantha</i>	<i>bla</i> PFM-1	
186-C-03	<i>P. synxantha</i>	<i>bla</i> PFM-1	

Table 8. Summary of carbapenemases detected in unique CP-*Pseudomonas* spp. in this study.

Species	Carbapenemase	Identity to reference (%)¹	Accession
<i>P. fluorescens</i> (5)	<i>bla</i> PFM-3 (5)	100	WP_156404660.1
<i>P. otitidis</i> (1)	<i>bla</i> POM-1 (1)	100	WP_044403015.1
<i>P. paracarnis</i> (3)	<i>bla</i> PFM-3 (1)	100	WP_156404660.1
	None identified (2)	-	-
<i>P. shahriarae</i> (3)	<i>bla</i> PFM-4 (3)	99.6 - 100	WP_198799270.1
<i>P. synxantha</i> (2)	<i>bla</i> PFM-1 (2)	100	WP_148044470.1

¹percent amino acid identity to the top reference sequence

-Tk. MALDI-TOF MS results were consistent across both isolates, where the top prediction for both isolates was *P. synxantha*, and scores were 2.12 and 2.17 for 148-O-07 replicates, and 2.09 and 2.11 for 148-O-08 replicates, indicating a secure genus with probable species identification. In contrast, ANI scores for other *P. paracarnis* isolates 083-O-01 and 173-C-08 were 99.22 and 99.14, respectively, but other *P. fluorescens* isolates had lower ANI scores ranging between 95.31 – 95.41. Ultimately, it is possible that one or both of the isolates originating from rinsate 148 were incorrectly identified through *in silico* speciation, however further investigation is required to distinguish the two isolates.

5.3 Discussion

Overall, there were 27 unique CP isolates recovered from 23 retail meat rinsates, 13 of which were *Acinetobacter* spp. and 14 *Pseudomonas* spp. Two of the *Acinetobacter* species identified were *A. baumannii* and *A. pittii*, both of which belong to the ACB complex²²¹. *A. pittii* is a less notorious pathogen than *A. baumannii*, however there are increasing examples of *A. pittii* involved in nosocomial outbreaks and as an important AMR pathogen^{222–225}. Beyond the ACB complex, *A. bereziniae* was another species of clinical importance in this collection and has been involved in MDR nosocomial infections²²⁶. Reports of infections involving the remaining species are not common, however they do exist for *A. guillouiae* and *A. gyllenbergii* despite being limited in number^{227,228}. The final species identified, *A. nematophilus*, was only recently identified as a species in 2023²²⁹, therefore it is unsurprising that there are no published reports identifying human cases. This species is closely related to both *A. guillouiae* and *A. bereziniae*, therefore it is possible that it was previously identified as either of these species prior to its identification²²⁹.

Acinetobacter spp. and CP-*Acinetobacter* spp. have also been reported from various meat products. CP-*Acinetobacter* spp. have been recovered from retail pork and beef purchased in the US¹²⁶. In Portugal, retail pork harboured the same CR-*Acinetobacter* spp. identified in this study, with the exception of *A. nematophilus*²³⁰. Another study from Iran evaluated various meat types and found 20% of samples harboured *A. baumannii*, 17.94% of which displayed phenotypic resistance to imipenem²³¹.

In Canada, CR-*Acinetobacter* are not a common nosocomial pathogen captured by hospital surveillance systems, with only 93 CP-*Acinetobacter* spp. reported through CNISP from 2010-2016²³². Due to the limited availability of this genus, CANWARD isolates were also considered

for genomic comparisons. This collection is also limited where a previous update of the CANWARD study reported that 171 *A. baumannii* were tested from 2007-2016²⁸. Despite the limited availability of CP-*Acinetobacter* spp. collected through these two surveillance systems, there was one *A. baumannii* from CNISP and nine *A. pittii* from CANWARD which were sequenced, had a corresponding ST to retail meat isolates, and were available for genomic comparisons.

While there was no evidence of potential direct transmission observed at the highest levels of investigation for the four retail meat isolates included in genomic comparisons, the level of relatedness was consistent for comparisons of PA ST1362 and PA ST214, where closest SNV distances ranged from 62-93 SNVs to retail meat isolates across the three comparisons. In the last comparison of PA ST119, relationships were more distant. Of note, it appeared that both STs were important in identifying isolate relationships, where isolates with the fewest SNVs between them shared both Pasteur and Oxford STs (Figure 10). Other studies have identified different discriminatory powers between Oxford and Pasteur schemes, with Oxford generally being regarded as the more discriminatory method, and Pasteur as more suitable for identifying clonality^{233,234}.

While *A. baumannii* and *A. pittii* isolates from retail meat and human clinical isolates shared the same STs, it is important to note that the core SNV distances are well above existing thresholds used for identifying relatedness and potential transmission. There is no single consensus on using SNV thresholds for *Acinetobacter* spp., with different thresholds set for different studies. To identify clonal clades in a phylogeny, 20 core SNVs has been employed⁸⁶, whereas to distinguish outbreak and non-outbreak samples, and identify transmission events, more stringent thresholds of 2.5 SNVs and 10 SNVs have been used, respectively^{235,236}. If the same thresholds are applied to isolates in this study, the genetic distance is too great to be considered part of a single transmission event, however isolates belonging to the same STs with high similarities in ARGs do show a degree of relatedness.

Similarities in the ARGs were detected across isolates, which were largely identical in retail meat and clinical samples, contrasting with a previous study that found distinct ARG profiles in clinical isolates compared to those associated with livestock²⁰⁷. Identical ARG profiles were observed within PA ST1362 and PA ST214 comparisons, respectively. Additionally, all ARGs

present in PA ST1362 were also identified in PA ST214, with exception of different *bla*ADC and *bla*OXA variants. Interestingly, in the comparison of PA ST119, the isolate from retail meat harboured *tet*(39), which was plasmid-located and unique in this comparison. This gene was first identified from *Acinetobacter* spp. from trout farms and sewage in Denmark, and from a clinical isolate from the Netherlands²³⁷. It was also detected in 20/171 *Acinetobacter* isolates from bulk tank milk²³⁸, and has been plasmid-located in a clinical *A. baumannii*²³⁹ and *Acinetobacter colistiniresistens* from a healthy fecal sample²⁴⁰.

Taken together, this study provides insight into potential relationships within the farm-to-fork continuum for *Acinetobacter* spp., despite the limited number of human clinical isolates available. The inclusion of publicly available isolates from human and environmental sources could further aid in determining a possible environmental or clinical association with these isolates, although the STs identified are not common. PA ST1362 was first identified from chicken farms in Norway²⁴¹ and as of June 2025, there are only 8 isolate records of *A. pittii* PA ST214 in the PubMLST isolate collection⁴⁰. In this study, the majority of human clinical isolates belonged to PA ST119, which are more commonly reported in the literature. *A. pittii* PA ST119 has been reported through several case reports, including carbapenemase-harboursing strains^{242–244}, and was the predominant metallo- β -lactamase-producing *Acinetobacter* strain (18/48) isolated from five hospitals in Japan²⁴⁵. This clone appears to be the most clinically significant among those isolated from retail meat in the present study, however a potential source of retail meat contamination remains unclear.

While the *Pseudomonas* species isolated in this study may not hold the same clinical importance as *P. aeruginosa*, select species have still been involved in important clinical infections. *P. fluorescens* is an opportunistic pathogen that has been isolated from several sites of infection; however, it is most commonly associated with BSI, particularly after exposure to contaminated medical equipment²⁴⁶. This species has also been involved in a nosocomial outbreak, where a contaminated water dispenser was identified as the outbreak source²⁴⁷. *P. otitidis* is another known opportunistic pathogen, where it was first identified as the causative agent of ear infections²⁴⁸. It has also caused more serious infections such as bacteremia, including in one instance where carbapenemase production was detected^{249,250}. The other species isolated do not appear to have high associations with clinical settings or human infections, although *P. shahriarae* is a member

of the *Pseudomonas gessardii* subgroup of the *P. fluorescens* group²⁵¹. The type strain for this species, however, was isolated from the rhizosphere of wheat, having environmental origins²⁵¹. *P. synxantha* has been investigated for its potential to produce useful bioactive compounds²⁵², and *P. paracarnis* has been noted as a spoilage organism in meat²⁵³. Beyond the recent identification of *P. paracarnis* in 2021, *Pseudomonas* are generally known as food spoilage organisms and have been isolated from retail meat²⁵⁴. Carbapenem resistance and carbapenemase production has also been detected from pseudomonads from retail meat, such as a CR-*Pseudomonas* spp. from retail chicken in Norway²⁵⁵. Another study found CP-*P. otitidis* in retail pork and chicken in Hong Kong²⁵⁶. Using non-specific culture methods, one study found a prevalence of 87.5% of *Pseudomonas* spp., including *P. fluorescens*, among other non-*aeruginosa* species in retail chicken and pork. Notably, no ARGs were detected in these samples, but *P. aeruginosa* was detected at a prevalence of 11% of the tested food samples. In the same study, *P. aeruginosa* carried *blaOXA* genes in all isolates, although a possible CR-phenotype was not investigated²¹⁶. This lack of distinction between CR and CP isolates remains a limitation of many retail meat surveillance studies; however, it is clear that *Pseudomonas* spp. are a known contaminant of retail meat.

Common across both genera were the species-associated carbapenemases identified, with *blaOXA* being intrinsic in many *Acinetobacter* spp.²⁵⁷ and *blaPFM* and *blaPOM* being intrinsic in some *Pseudomonas* species^{258,259}. The often mobilizable nature of carbapenemases is a major concern surrounding these genes⁸⁴, therefore intrinsic chromosomal carbapenemases are less attractive candidates of study. With their persistent association with *Acinetobacter* spp., OXA-type β -lactamases have been widely reported²⁵⁷. In contrast, the *Pseudomonas*-associated carbapenemases identified in this study are not frequently studied, especially with the rarity of infections caused by their host organisms. POM (*P. otitidis* metallo- β -lactamase) is a metallo- β -lactamase associated with *P. otitidis* and has a higher substrate specificity for penicillins and carbapenems than for cephalosporins²⁵⁹. PFM (*P. fluorescens* metallo- β -lactamase) can be found in some members of the *P. fluorescens* complex but are not harboured by all species²⁵⁸. This carbapenemase shares moderate amino acid identities with other B2 species-associated carbapenemases like Sfh-1 in *Serratia fonticola* (71%) and CphA1 in *Aeromonas hydrophila* (53%)²⁵⁸.

Unresolved in this dataset are the two *P. paracarnis* that did not test positive for

carbapenemases *in silico* using default thresholds from AMRFinderPlus⁴⁸. The closest match to a β -lactamase detected in these isolates was a β -lactamase with 58.24% amino acid identity to a *blaPDC* reference sequence. This gene is an AmpC-type β -lactamase found in *P. aeruginosa*, with some variants contributing to decreased susceptibility to imipenem²⁶⁰ which may be contributing to the carbapenem hydrolysis observed with the CIMTris assays. Without additional investigation, this CP-phenotype cannot be attributed to any one cause, as this *ampC* ortholog may have weak carbapenemase activity, or this gene may in fact encode a novel carbapenemase. Future work with these isolates should include an in-depth analysis of the *Pseudomonas* resistome to search for species-specific carbapenemase orthologs, which remains unresolved in two isolates from this study. Additionally, laboratory-based approaches are necessary to validate carbapenemase activity of potential carbapenemases identified through *in silico* methods.

This unresolved discrepancy highlights an unexpected result from the CP-*Pseudomonas* dataset: the overall lack of resistance genes beyond carbapenemases. While genes conferring metal tolerance were detected, there were no ARGs beyond the specified carbapenemases. A similar phenomenon was observed in a study of environmental pseudomonads, where ARGs were not readily detected using a PCR-based approach, but phenotypic testing showed 51% of their isolates to be MDR²⁶¹. *Pseudomonas* spp. are often associated with an MDR phenotype which is contributed to in part by the abundance of efflux pumps they can harbour²⁶². While there were no additional ARGs detected using default thresholds in this study, lowering the minimum threshold required by AMRFinderPlus⁴⁸ showed multiple genes related to efflux in both *P. paracarnis* isolates. (Appendix F Table F2). This discrepancy highlights the limitation of using a reference-based method for ARG detection, especially when investigating species-specific ARGs in uncommon species.

Overall, this study identified CP-*Acinetobacter* spp. of clinical importance, with one *A. baumannii* and two *A. pittii* isolates showing a genomic relationship to a clinical isolate through sequence typing and ARG profile; and a third *A. pittii* showing genomic relatedness based on sequence typing. Additionally, clinically important pseudomonads *P. fluorescens* and *P. otitidis* were identified, although no clinical isolates were available for further genomic comparisons. Carbapenemases across the dataset were species-associated, demonstrating a lack of highly mobile carbapenemases in retail meat samples in this study, but demonstrate the potential AMR threat of

these ubiquitous organisms. While current molecular approaches may capture the *bla*OXA variants found in *Acinetobacter* spp., *bla*PFM-type and *bla*POM-1 from *Pseudomonas* spp. would not be included in routine clinical panels. This emphasizes the need for phenotypic testing, especially in instances of unresolved species identification.

Chapter 6: Genomic characterization of carbapenemase-producing *Aeromonas* spp. isolated from Canadian retail meat

6.1 Introduction

CPOs are amongst the highest priority AMR pathogens for human health in Canada and globally^{13,24}. While not commonly considered under the CPO umbrella, the genus *Aeromonas* has garnered increased attention in recent years as an emerging opportunistic human pathogen and is increasingly associated with harbouring carbapenemases^{263–266}. The genus is comprised of 36 species, with *Aeromonas caviae*, *Aeromonas dhakensis*, *Aeromonas veronii*, and *Aeromonas hydrophila* being the most commonly reported from clinical infections²⁶⁷. *Aeromonas* spp. have caused a wide variety of infections in humans, however it is most commonly associated with gastrointestinal infections, wound and soft-tissue infections, and less commonly, as the causative agent of bacteremia and septicemia^{266,267}. Healthcare-associated and hospital-acquired *Aeromonas* spp. infections have been reported²⁶⁸, but human infections are typically associated with exposure to contaminated food or water^{266,269,270}.

Aeromonas spp. can be found ubiquitously throughout the environment, although they are heavily associated with water and aquatic environments²⁷¹. Their aquatic nature allows for their presence in a broad range of environments such as wastewater and hospital effluent^{272,273}, tap water²⁷⁴, bottled water²⁷⁴, well water²⁷⁰, and surface water²⁷³ including water used for irrigation where they may contaminate food crops²⁷⁵. Select species of this genus are known as fish pathogens²⁷⁶, where they have caused significant losses to the aquaculture industry²⁷⁷ and have also been isolated from fish and seafood^{278–280}. Beyond their association with aquatic environments, *Aeromonas* spp. have also been isolated from food products including dairy, fresh produce, and meat^{279,281–283}. This genus has also been isolated from animal-related sectors, such as from environmental samples on a Texas dairy farm²⁸⁴, feces of livestock²⁸⁵, animal carcasses²⁸⁶, and abattoir environmental samples²⁸⁶. Their widespread distribution lends the genus to be studied through a One-Health framework, particularly as vectors for the transmission and dissemination of ARGs.

Aeromonas spp. can harbour a diversity of ARGs, including carbapenemases, among other high priority resistance determinants such as *mcr* which confers resistance to one of the last-resort drugs colistin. Alarming, the co-occurrence of *mcr* and clinically relevant carbapenemases

(*bla*NDM-1 and *bla*KPC-2) in *Aeromonas* spp. has been reported in hospital wastewater^{265,272}. Furthermore, this genus can have a complex mobilome which allows for the carriage and possible dissemination of ARGs^{272,287}. *Aeromonas* spp. have been studied as reservoirs that may contribute to the spread of AMR across bacterial species and One-Health sectors^{288–290}, although the impact of this genus in the Canadian context is not entirely understood.

As part of a broader CPO surveillance study, Canadian retail pork and beef were screened for CPOs, which resulted in a large proportion of CR-*Aeromonas* spp. To understand the One-Health implications posed by these opportunistic pathogens from retail meat, all unique CP-*Aeromonas* isolates were further characterized using WGS and bioinformatic analysis.

6.2 Results

6.2.1 Prevalence and diversity of carbapenemase-producing *Aeromonas* spp. in Canadian retail meat

There were 217/318 (68.2%) retail meat rinsates that harboured one or more CR-*Aeromonas* isolates. There were 2 rinsates in which *Aeromonas* spp. were detected but not maintained for further characterization. From these 215 rinsates, 541 CR-*Aeromonas* isolates were selected and tested for carbapenemase production using CIMTris (Figure 12) (Appendix C Table C1). After phenotypic testing, 407/541 (75.2%) of the isolates were determined to be CP-*Aeromonas* spp.

To remove potential duplicate strains within the collection, a maximum likelihood tree of MLST alleles was performed on all 407 isolates (Appendix G Figure G1). In instances where more than one isolate clustered together in the MLST phylogeny, originated from the same retail meat rinsate, and harboured the same ARG profile (excluding metal resistance), only one representative isolate was used in subsequent analyses (Figure 13). This resulted in the removal of 96 potential duplicate isolates, and a final collection of 311 unique isolates from 189 meat rinsates, 27 of which harboured two different species.

CP-*Aeromonas* spp. were abundant in this retail meat collection with 189/316 (59.8%) of the rinsates testing positive (62.5% (137/217) in retail pork and 52.5% (52/99) in retail beef (Fisher's Exact Test: $p = 0.083$, odds ratio = 1.5, 95% CI: 0.9 – 2.6) (Table 9). CP-*Aeromonas* spp. were recovered from all combinations of years, regions, and products included in this study.

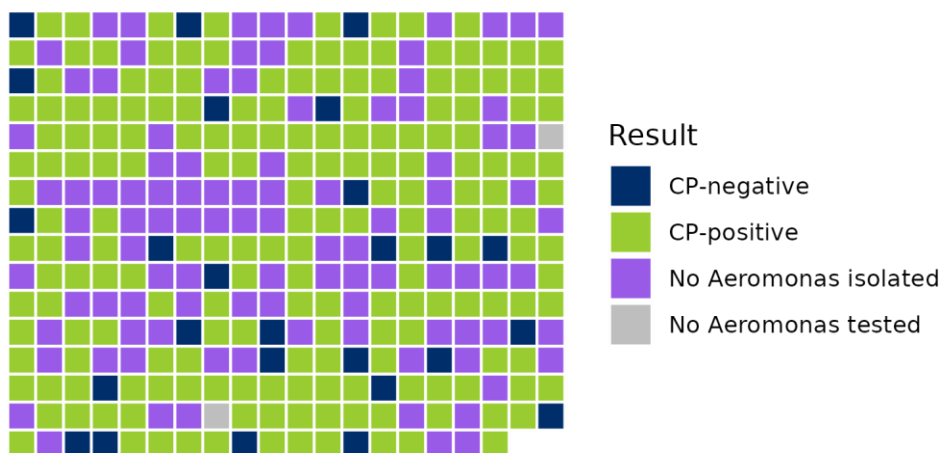


Figure 12. Overview of *Aeromonas* sampling and testing from 318 retail meat rinsates. Retail meat rinsates are arranged from 1-318, left to right, with colours indicating whether *Aeromonas* spp. were isolated from a given rinsate and tested for carbapenemase production.

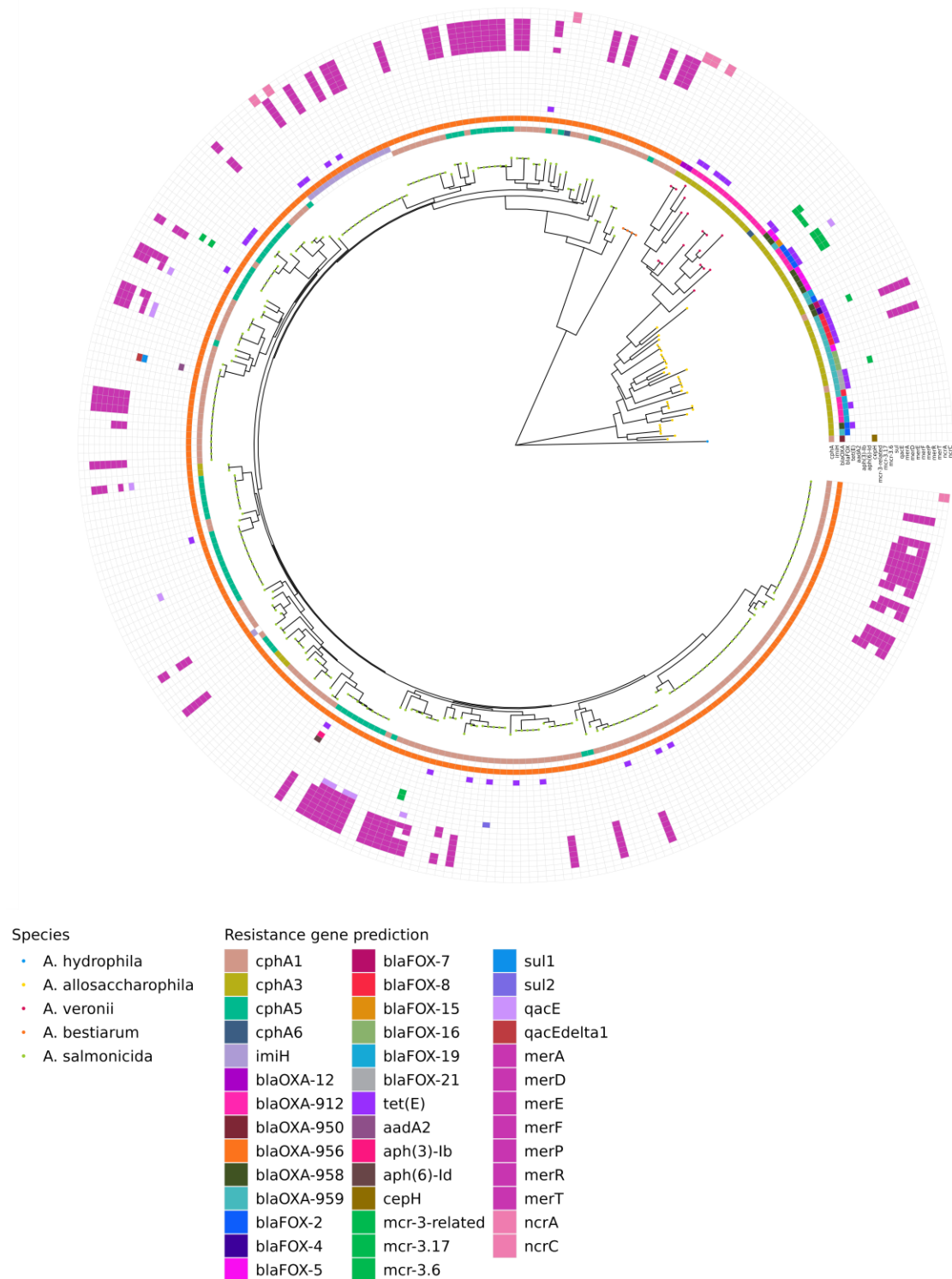


Figure 13. Midpoint-rooted maximum likelihood tree of 311 unique isolates generated from their concatenated MLST alleles. Unique colours are assigned to each gene variant presented in a given column. Individual columns are assigned to *mcr* variants, *mer*, and *ncr* genes, which are grouped by colour.

Table 9. Epidemiological data associated with retail meat rinsates with CP-*Aeromonas* spp.

Source	Province	Year
Beef (52/99)	Central (40/64)	2017 (5/10)
		2018 (35/54)
	West (12/35)	2017 (1/9)
		2018 (11/26)
Pork (137/217)	Central (81/121)	2016 (43/60)
		2017 (7/14)
		2018 (31/47)
	West (56/96)	2016 (32/51)
		2017 (9/13)
		2018 (15/32)

Minimal differences were observed in the species isolated from CP-*Aeromonas*-positive pork and beef (Table 10). Across all 316 rinsates, *Aeromonas salmonicida* was the most prevalent (169, 53.5%), followed by *Aeromonas allosaccharophila* (28, 8.9%), *A. veronii* (14, 4.4%), and *Aeromonas bestiarum* (4, 1.3%). A single retail pork sample was positive for *A. hydrophila* (1, 0.3%).

6.2.2 Carbapenemases and other resistance genes detected in the *Aeromonas* collection

There were only two types of carbapenemase genes detected across the selected *Aeromonas* spp.: the metallo- β -lactamases *imiH* and *cphA*, both of which are members of the *cphA* gene family. All species harboured *cphA*-type carbapenemases, while *imiH* was only detected in a small proportion (16/255; 6.3%) of *A. salmonicida* isolates. There appeared to be some phylogenetic association with these carbapenemase genes and their host strains, as 15/19 isolates harbouring *imiH* clustered closely together in the phylogenetic tree (Figure 13). Across species, *cphA1* was the most common *cphA* allele observed, with all species harbouring this carbapenemase variant with the exception of *A. veronii*. The remaining *cphA* alleles detected were *cphA3* in *A. allosaccharophila*, *A. salmonicida*, and *A. veronii*; *cphA5* in *A. salmonicida*; and *cphA6* in *A. salmonicida* and *A. veronii* (Table 11). Of note is one isolate, 117-C-17, which harboured two copies of *cphA1* on the same contig (data not shown).

While there were several ARGs detected across species, genes conferring β -lactam resistance were the most common type observed. In addition to the carbapenemases, all isolates harboured *blaOXA*, which included *blaOXA*-12, *blaOXA*-912, *blaOXA*-950, *blaOXA*-956, *blaOXA*-958, or *blaOXA*-959. These variants were highly conserved within some species, where *A. salmonicida* and *A. bestiarum* harboured exclusively *blaOXA*-956 and had highly similar amino acid similarity (Figure 14). In other species, multiple variants were predicted despite a more limited number of isolates, such as three different variants within 34 *A. allosaccharophila* isolates, and two variants within 17 *A. veronii*. Cephalosporinases were only observed in two species: *A. allosaccharophila*, where 100% of isolates harboured *blaFOX* variants, and *A. hydrophila*, which harboured *cepH*. Resistance to aminoglycosides (*aadA2*, *aph(3)-Ib*, *aph(6)-Id*), colistin (*mcr*), sulfonamide (*sul*), tetracycline (*tet(E)*), and disinfectants (*qacE*), was observed at lower rates across the dataset (Figure 13, Table 12). *A. salmonicida*, the most common species, harboured all ARG types with exception of cephalosporinases. Interestingly, despite the limited number of *A.*

Table 10. Comparison of species isolated from CP-*Aeromonas*-positive pork and beef rinsates.

Species	Positive pork rinsates ¹ n=137	Positive beef rinsates ¹ n=52	Fisher's Exact Test
<i>A. allosaccharophila</i>	20, 14.6 %	8, 15.4%	p = 1
<i>A. bestiarum</i>	3, 2.2%	1, 1.9%	p = 1
<i>A. hydrophila</i>	1, 0.73%	0, 0.0%	p = 1
<i>A. salmonicida</i>	123, 89.8%	46, 88.5%	p = 0.79
<i>A. veronii</i>	10, 7.3%	4, 7.7%	p = 1

¹Reflect rinsates with ≥ 2 different CP-*Aeromonas* species.

Table 11. Summary of carbapenemases detected across unique CP-*Aeromonas* spp. in this study.

Species (n)	Carbapenemase (n)	Identity to reference (%) ¹	Accession (n)
<i>A. allosaccharophila</i> (34)	<i>cphA1</i> (2)	96.85	WP_063844282.1 (1)
		98.82	WP_063844285.1 (1)
	<i>cphA3</i> (32)	96.06 – 99.21	WP_063844281.1 (32)
<i>A. bestiarum</i> (4)	<i>cphA1</i> (4)	94.47	WP_063844282.1 (4)
<i>A. hydrophila</i> (1)	<i>cphA1</i> (1)	96.85	WP_063844282.1 (1)
<i>A. salmonicida</i> (255)	<i>cphA1</i> (168)	94.47 – 96.44	WP_063844282.1 (3)
		95.24 – 98.81	WP_063844283.1 (165)
	<i>cphA3</i> (5)	95.26 – 99.61	WP_063844281.1 (5)
	<i>cphA5</i> (65)	96.80 – 98.41	WP_063865210.1 (65)
	<i>cphA6</i> (1)	98.43	WP_063865211.1 (1)
	<i>imiH</i> (16)	96.05 - 98.43	WP_063865292.1 (16)
<i>A. veronii</i> (17)	<i>cphA3</i> (16)	97.64 – 99.21	WP_063844281.1 (16)
	<i>cphA6</i> (1)	98.82	WP_063865211.1 (1)

¹percent amino acid identity to the top reference sequence

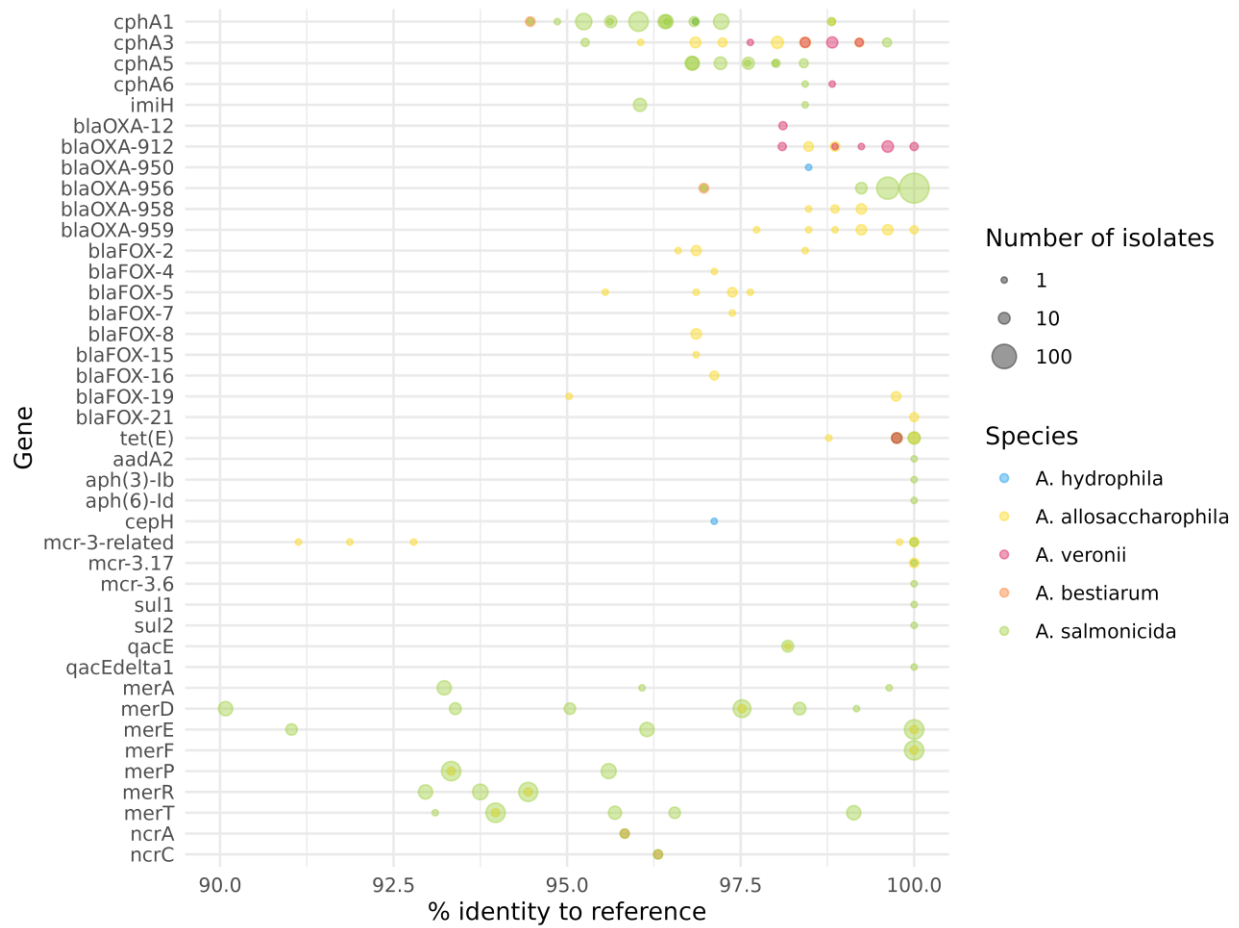


Figure 14. Bubble chart showing the diversity of ARG amino acid sequences across *Aeromonas* spp. from this study.

Table 12. Summary of the predicted AMR phenotypes for all unique CP-*Aeromonas* spp based off ARGs and metal tolerance genes.

Resistance type	<i>A. allosaccharophila</i> n = 34	<i>A. bestiarum</i> n = 4	<i>A. hydrophila</i> n = 1	<i>A. salmonicida</i> n = 255	<i>A. veronii</i> n = 17
Aminoglycoside	-	-	-	2, 0.8%	-
β-lactam					
Carbapenemases	34, 100 %	4, 100 %	1, 100 %	255, 100 %	17, 100 %
Cephalosporinases	34, 100%	-	1, 100%	-	-
Oxacillinases	34, 100%	4, 100%	1, 100%	255, 100%	17, 100%
Colistin	8, 23.5%	-	-	2, 0.8%	-
Sulfonamide	-	-	-	2, 0.8%	-
Tetracycline	16, 47.1%	-	-	19, 7.5%	6, 35.3%
Disinfectant	1, 2.9%	-	-	11, 4.3%	-
Metal	2, 5.9%	3, 75.0%	-	73, 32.4%	-

allosaccharophila in this collection, this species harboured resistance genes for all but two classes of antimicrobials detected across species. Importantly, this species was an important source of *mcr* genes in this dataset, with 8/34 *A. allosaccharophila* harbouring one or more *mcr* gene, or 8/10 of total *mcr*-harbouring isolates in this collection, with the two remaining species being *A. salmonicida*. The colistin resistance genes detected in this dataset were the *mcr*-3-related lipid A phosphoethanolamine transferase gene, *mcr*-3.17, and *mcr*-3.6. There were 6 isolates that carried the *mcr*-3-related gene in addition either *mcr*-3.17 (n=5) or *mcr*-3.6 (n=1) (Figure 13). Furthermore, three *A. allosaccharophila* had *mcr*-3-related genes with 91.13-92.79 % amino acid identity to the reference, indicating the potential for a novel variant that is not represented in the AMRFinderPlus⁴⁸ database used (Figure 14).

Last, metal resistance was detected in *A. allosaccharophila*, *A. bestiarum*, and *A. salmonicida* and was conferred either through *mer* genes conferring mercury resistance, or nickel resistance conferred through *ncrA* and *ncrC*. Interestingly, nickel resistance was detected in most *A. bestiarum* (3/4), but was only detected sporadically in *A. salmonicida* isolates (4/255). Mercury resistance (*mer* genes) seemed to be more common among some clusters in the phylogenetic tree, indicating a potential relationship to ST, however it was also detected in sporadic isolates across *A. bestiarum* and *A. salmonicida* (Figure 13).

6.2.3 Mobile genetic elements detected in carbapenemase-producing *Aeromonas* spp.

Both plasmids and other MGEs were common in this dataset, with plasmids predicted in 57.2% (178/311) of isolates and other transposable elements (insertion sequence elements and transposons) predicted in 98.4% (306/311) of isolates (Figure 15). Despite the abundance of plasmids detected in this dataset, plasmids carrying ARGs were only detected in six isolates, five of which were *A. salmonicida*, and the last in an *A. allosaccharophila* (Table 13). Only one plasmid was predicted to be conjugative, determined by the presence of *mobH* and *mpfF*. This plasmid, AE564, carried resistance to aminoglycosides (*aph(3)-Ib*, *aph(6)-Id*), and tetracycline (*tet(E)*). Three *A. salmonicida* harboured the same plasmid, AC079, which carried resistance to disinfectants (*qacE*) and the remaining two plasmids carried resistance to sulfonamides, disinfectants, and aminoglycosides; and resistance to tetracycline, respectively. Furthermore, mercury resistance located on plasmids was detected in 43.6% (31/71) of samples that harboured mercury resistance genes (Appendix G Table G1). Due to the limitations of short read sequencing

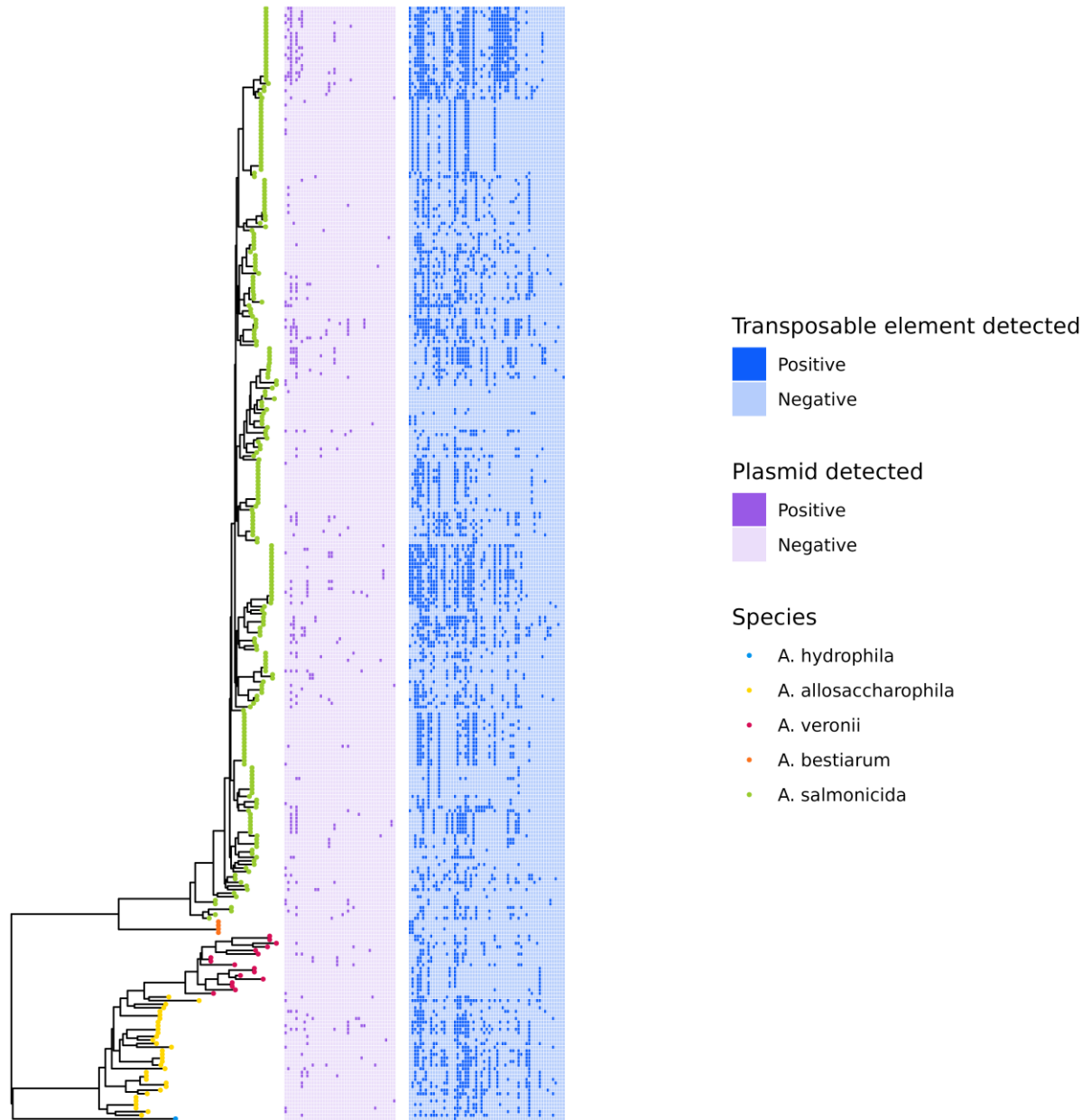


Figure 15. Midpoint-rooted maximum likelihood tree of 311 unique CP-*Aeromonas* spp. generated from their concatenated MLST alleles with their detected MGEs. A list of MGEs displayed with the phylogeny can be found in Appendix G Table G2.

Table 13. Summary of plasmids detected in unique CP-*Aeromonas* spp. that carried ARGs.

Isolate	Species	Plasmid	ARGs	Features	Mobility prediction
056-C-05	<i>A. salmonicida</i>	AC079	<i>qacE</i>	-	Non-mobilizable
077-C-03	<i>A. salmonicida</i>	AC260	<i>sul1, qacEdelta1, aadA2</i>	-	Non-mobilizable
134-C-13	<i>A. salmonicida</i>	AC079	<i>qacE</i>	-	Non-mobilizable
168-C-15	<i>A. salmonicida</i>	AC079	<i>qacE</i>	-	Non-mobilizable
284-C-08	<i>A. allosaccharophila</i>	AC217	<i>tet(E)</i>	-	Non-mobilizable
307-C-08	<i>A. salmonicida</i>	AE564	<i>aph(3)-Ib, aph(6)-Id, tet(E)</i>	<i>mobH, mpfF</i>	Conjugative

in MGE detection, no further investigation was performed.

6.3 Discussion

In this study, a high prevalence of CP-*Aeromonas* spp. were recovered from Canadian retail pork and beef. To our knowledge, this is the first Canadian study to identify and characterize CPOs from retail meat. The isolation of *Aeromonas* from retail meat, however, is not novel, with a previous study reporting *Aeromonas* spp. in 4/4 ground pork and 15/19 ground beef purchased in Eastern Canada²⁸¹. While the previous study did not investigate potential AMR in their isolates, a recent Canadian study found AMR *Aeromonas* spp. in retail mussel and oyster shellstock, where *Aeromonas* spp. harbouring *cphA* were identified²⁷⁸. In other countries, *Aeromonas* spp. have been isolated from various retail meat sources, including chicken, beef, lamb, and pork, among other food products^{291–293}. Thus, the presence of *Aeromonas* spp. in food products is widely reported and its status as an emerging enteric pathogen has been previously discussed²⁶⁶.

Within the context of AMR and the farm-to-fork continuum, this study originally aimed at isolating Gram-negative organisms harbouring carbapenemases of high clinical significance. *Aeromonas* spp. were confirmed to harbour carbapenemases by an initial phenotypic screening test, followed by *in silico* identification of the carbapenemase genes *cphA* and *imiH* via WGS. These are genetically similar species-associated carbapenemases encoded in the chromosome^{294–296} making them lesser of a potential threat than other mobile plasmid-encoded carbapenemases such as KPC plasmids with diverse host ranges²⁹⁷. Furthermore, the presence of *cphA* alone has less of an impact on the overall resistance of its host than the aforementioned carbapenemase due to its ability to hydrolyze carbapenems but not other β -lactams²⁹⁸. Nevertheless, the presence of *cphA* from infection-causing *Aeromonas* spp. has been previously reported as an important contributor to AMR^{299,300}.

In the clinical setting, *cphA*-harbouring *Aeromonas* spp. have proven difficult to characterize due to discrepancies between antimicrobial susceptibility testing, phenotypic carbapenemase testing, and molecular testing²⁹⁹. These challenges are present on top of the existing challenge of accurate species-level identification of *Aeromonas* spp. using routine diagnostic methods like MALDI-TOF MS or the VITEK2^{299,301}. False susceptibility to carbapenems in *cphA*-harbouring *Aeromonas* spp. has led to empirical carbapenem treatment in one organ transplant patient, where the patient ultimately died after *A. hydrophila* was repeatedly

isolated from a surgical drain³⁰⁰. A similar case was observed elsewhere, where a patient with sepsis died from *A. dhakensis* that showed resistance and upregulated carbapenemase expression only after treatment with meropenem³⁰². Therefore, the threat of *cphA*-harbouring *Aeromonas* spp. can be attributed, in part, to the difficulty of accurate identification and characterization of resistance in this genus.

While *cphA* was detected across species in this study, the human clinical significance of these species vary. *A. hydrophila* and *A. veronii* are the most clinically relevant²⁶⁶ and commonly reported species isolated in this study. Reports of *A. allosaccharophila*, *A. bestiarum*, and *A. salmonicida*, are less common and exist primarily as case reports or as minor findings of larger studies (described below). The most common species identified in this study, *A. salmonicida*, has been implicated in serious infections including endocarditis, peritonitis, and bacteremia^{303–305}. Reports of the remaining two species are even rarer, with *A. bestiarum* having been isolated from a wound infection, and *A. allosaccharophila* isolated from stool of a diarrheal patient^{299,306}. All species isolated in this study have therefore been reported in humans, including two species of clinical importance and *A. salmonicida* which while rare, has had serious clinical manifestations.

In Canada, AMR *Aeromonas* spp. are not captured as part of the routine surveillance of humans or livestock, therefore it is challenging to understand the significance of these results in the context of the farm-to-fork continuum^{22,307}. While AMR *Aeromonas* spp. could be manifesting in Canada as foodborne disease in the community, these infections are often self-limiting and no antimicrobial therapy is required³⁰⁸. As many *Aeromonas* spp. are ubiquitous in nature, they have the potential for harbouring and transmitting ARGs between different bacteria and sectors. In particular, *Aeromonas* spp. have been highlighted as reservoirs of the acquired colistin resistance gene *mcr-3*, which has been suggested to originate in this genus and spread to other genera via transposable elements²⁸⁸. In this study, *mcr-3-related* genes and *mcr-3* variants were detected in 23.5% of *A. allosaccharophila* and 0.8% of *A. salmonicida*. While these genes were not determined to be plasmid-located, there were a high prevalence of other MGEs in this isolate collection. This finding is not novel in North America, as a previous US-based study of 5,169 animal samples isolated 14 *mcr-3/7*-positive *Aeromonas* spp., or a prevalence of 0.29% in meat. Authors from this study noted the genetic context of *mcr* genes, which were located near insertion sequences and could suggest the mobility of these genes despite their location in the chromosome³⁰⁹. This

suggests that *Aeromonas* spp. from retail meat may serve as a vector for the transmission of important ARG between One-Health sectors, although the genomic context of these high priority ARGs should be investigated in future work.

In summary, a high prevalence of CP-*Aeromonas* spp. was recovered from retail pork and beef, including two clinically relevant species (*A. hydrophila*, *A. veronii*)²⁶⁷, and other species (*A. allosaccharophila*, *A. bestiarum*, *A. salmonicida*) that have caused rare yet serious infections in humans^{299,303–306}. The understanding of this genus as an emerging human pathogen continues to evolve, harbouring additional interest due to their intrinsic carbapenemase resistance which proves challenging to detect accurately and treat empirically. This study clearly shows that retail meat is a source of CphA-producing *Aeromonas* spp.; however, it remains unclear if this is an important point of exposure for humans in Canada due to a lack of published epidemiological and surveillance data on this pathogen. More broadly, AMR *Aeromonas* spp. from retail meat may serve as a vector for the transmission of important ARGs between sectors of the One-Health continuum in Canada, and merits further emphasis in surveillance efforts across sectors.

Chapter 7: Final summary and discussion of thesis findings, limitations, future directions, and conclusions

7.1 Final summary and discussion of thesis findings

Through the use of WGS, this work provides a novel perspective to farm-to-fork surveillance, targeting nosocomial AMR pathogens in place of traditional foodborne organisms routinely targeted through One-Health surveillance efforts^{307,310,311}. In this study, three priority AMR pathogens important for human health were investigated: *C. difficile* (Chapter 3, p35), MRSA (Chapter 4, p44), and CPOs (Chapters 5 & 6, p55 &76). These AMR pathogens are opportunistic^{171,221,246,266,312}, meaning that pathogen acquisition or colonization will not necessarily result in an active infection. Due to the nature of these pathogens, their ability to colonize their hosts, and the spectrum of infections they can cause, identifying farm-to-fork linkages through outbreak investigations is challenging. While farm-to-fork transmission has been described with other organisms³¹³, identifying direct transmission was not the targeted outcome of the present study. Rather, this study identified retail meat as a source of exposure for priority AMR pathogens and a potential reservoir for important ARGs. By leveraging CIPARS, CNISP, and CANWARD surveillance, this work highlights the farm-to-fork continuum as a potential route of transmission for AMR pathogens of clinical significance in Canada.

While the recovery of both *C. difficile* and MRSA from retail meat samples were low (0.9%), genomic linkages to human clinical infections were evident. Using MLST as an initial method of comparison, retail meat harboured strains previously identified in human clinical infections in Canada across all three pathogens when genomic comparisons were possible. Additionally, in several instances across *C. difficile* and MRSA comparisons, human clinical infections were identified as community-associated, increasing the potential of retail meat as a source of these infections outside of the hospital setting. Using pathogen-specific typing methods such as PCR ribotyping for *C. difficile* and *spa* typing for MRSA further revealed relatedness. WGS-based analysis then allowed for an investigation of relatedness using the highest levels of resolution, where clusters of interest identified through cgMLST could be examined through SNV analysis. Using these methods, the closest relationships observed were with *C. difficile* ST10/RT015 (p39), which had CA-CDI fall within SNV thresholds of genomic relatedness to the retail meat isolate, further supporting the notion of a farm-to-fork CDI linkage. Comparisons to *C.*

difficile ST1/RT027 (p38), a hypervirulent clone, showed similar levels of relatedness with human clinical isolates clustering with the retail meat isolate through cgMLST allelic thresholds. MRSA showed moderate degrees of relatedness between retail meat and human clinical isolates, although no isolates clustered through cgMLST nor met existing SNV thresholds used to identify transmission. MRSA from retail meat showed two isolates that belonged to livestock-associated STs in North America (ST398) (p47) and Asia (ST9) (p51), the latter of which has previously not been recovered through CNISP surveillance. The third isolate, ST8 (p50), is associated with human CA-MRSA infections. Overall, these two AMR pathogens were rare in retail meat, although despite this rarity, genomic comparisons to human clinical isolates demonstrated a level of similarity which has not previously been investigated in Canada.

Higher proportions of retail meat samples were found to contain CPOs, including CP-*Aeromonas* spp. (59.8%), CP-*Pseudomonas* spp. (4.1%), and CP-*Acinetobacter* spp. (4.1%). No CPE were recovered, which is unsurprising given the rarity of these organisms in previous international retail meat surveillance studies^{121,122,125,126} and a Canadian One-Health study¹³⁰. All carbapenemases identified in *Acinetobacter* spp., *Pseudomonas* spp., and *Aeromonas* spp. were chromosomal and species-associated which included *bla*OXA-type carbapenemases in *Acinetobacter* spp. (p61), *bla*PFM and *bla*POM in *Pseudomonas* spp. (p68), and *cphA* variants in *Aeromonas* spp (p83). Importantly, this study revealed carbapenemases in retail meat that would not be detected using traditional molecular methods commonly used in the laboratory. With the rarity of CP-*Acinetobacter* spp. infections reported in Canada, only select members of the ACB complex could be investigated in genomic CPO comparisons. As with *C. difficile* and MRSA investigations, comparison at the level of MLST showed STs from retail meat that have previously been involved in CP-*Acinetobacter* infections in Canada. cgMLST and SNV analysis did not reveal high levels of relatedness, although there were similarities in ARG profiles where some isolates shared identical ARGs. Additionally, genomic relationships through SNV analysis were closer when isolates shared both PA and OX STs.

The genomic relationships identified in this study cannot be used to identify a single transmission event, however they show a relationship between agri-food and human sources. Even when genomic comparisons showed a high degree of similarity as observed with *C. difficile* ST10/RT015, there is no transmission pathway that can be identified from this data due to an

overall lack of detailed epidemiological information and the retrospective nature of this study. This study identified genomic similarities between retail meat and human isolates both in the resistance genes detected and with the typing methods utilized.

Beyond priority AMR species, this study shows the potential importance of emerging environmental pathogens such as *Aeromonas* spp., which may serve as reservoirs for the transmission of other clinically significant ARGs such as *mcr*, as previously noted^{288,309}. Additionally, the species-associated carbapenemases identified in environmental organisms may gain clinical importance or spread to other organisms over time. This phenomenon was observed with environmental *Kluyvera* species where the extended-spectrum β -lactamase *bla*CTX-M evolved prior to being mobilizable into other species³¹⁴. Environmental organisms can harbour intrinsic genes which confer AMR; however, the selection and spread of these organisms and their ARGs is a human-driven phenomenon. Continued surveillance and investigation of reservoirs could aid in curbing the emergence of these environmental organisms in the clinical setting.

It is important to note that this study is exploratory in nature, and does not serve itself as a risk assessment of retail meat consumption nor as an investigation of retail meat contamination sources. Rather, it shows that genomic linkages can be made between human infections reported through Canadian hospitals and AMR isolates collected from retail meat purchased in Canada. The isolation of AMR pathogens from the farm-to-fork continuum can imply that retail meat contamination is of farm or animal origin. While this is one potential source of contamination, contamination may be of human origin, which could occur throughout retail meat processing. Thus, sources of retail meat contamination remain ambiguous, where directionality cannot be identified.

When assessing the broader implications of these results, it is important to discern between the consumption and exposure to contaminated retail meat products, as the implications of these actions vary by organism. In this work, *C. difficile* presents the most obvious risk when consumed due to the fecal-oral transmission route of this organism and its heat-resistant endospore state^{312,315}. For the other pathogens in this study, the actual risk associated with the consumption of these organisms remains ambiguous without additional investigation into pathogenicity and virulence. In MRSA, staphylococcal enterotoxins are heat resistant³¹⁶ which could cause gastrointestinal illness. Due to the highly transmissible nature of this pathogen, colonization from an exposure may

pose a greater risk of future infection than actual consumption³¹⁷. While proper food handling and adherence to guidelines could mitigate the risk of transmission from an exposure, perfect adherence cannot be assumed for producers and the average consumer.

7.2 Study limitations

This study has limitations with both *in vitro* methods of selection, and organism-specific limitations that were encountered *in silico*. First, common across all isolates was the retail meat rinsates that were used for sample isolations. The retail meat rinsates used for pathogen isolation were convenience samples collected through routine CIPARS surveillance; therefore, the methods used for the retail meat rinse were optimized for CIPARS target organisms. Additionally, samples were stored frozen prior to isolation in this study. In an ideal scenario, isolations would have been performed immediately after the meat rinsates were produced to ensure maximum cell viability.

7.2.1 Limitations of pathogen prevalence versus quantification

The infectious dose is the inoculum of bacteria necessary to cause disease in a host. This study aimed only to capture pathogen prevalence across the retail meat sample collection rather than to quantify the number of pathogens in a given sample. In this way, results from this study cannot be used to infer the risk of consuming retail meat from the perspective of an infectious dose. Quantitative data could have provided insight into potential risk associated with retail meat exposure and consumption. While the exact dose is unknown, *C. difficile* in particular is thought to have a low infectious dose¹⁶⁵. The number of spores present within a sample is therefore of interest and has been investigated previously¹¹⁶. Ultimately, the data collected in this study can only determine a presence or absence of a given pathogen and cannot be used to infer risk relative to an infectious dose. To quantify AMR pathogens within a given sample, a different method of pathogen detection is required beyond those used in this study.

7.2.2 Limitations of CPO isolations

Colony selection for CPO isolations was particularly challenging in this study due to the broad selection criteria utilized. Two different technicians were involved in colony selection, aiming to select one colony of each morphology per chromID® plate type. Dividing isolations across two different technicians likely resulted in uneven sampling rates due to the subjective nature of colony selection, which could influence CPO isolation rates. Additionally, as described

in Section 2.4.3, three retail meat rinsates were processed using only OXA-48 bi-plates due to limited rinsate availability of those samples. The final prevalence of CPOs is therefore a reflection of unequal sampling. This could be mitigated in future studies by adhering to targeted colony selection criteria and sampling quotas. Common between both CPO investigations were the opportunistic pathogens isolated that are strongly associated with environmental reservoirs. As discussed previously, both the *Aeromonas* and *Pseudomonas* species isolated in this study are not commonly observed in clinical settings; however, common methods of identification, such as MALDI-TOF MS, may be biased towards clinical species, leading to misidentification of related organisms³¹⁸. Conversely, this study identified species at the highest level of discriminatory power using WGS. Species-level identifications for all sequenced CPOs was performed using an ANI-based approach, which is a WGS-based adaptation of DNA-DNA hybridization, the gold standard in taxonomic identification³¹⁹. Given the environmental nature of the species isolated in the *Pseudomonas* and *Aeromonas* genera, there may be inaccuracies with species-level identifications that are made with common clinical methods like MALDI-TOF MS and VITEK2, which would influence estimations of the clinical importance of some species due to species misidentification. As more studies adopt WGS-based bacterial identification and databases expand, the clinical significance of some isolates in this study may be re-evaluated.

7.2.3 Limitations of ARG identification using reference databases

This study was reliant on publicly available databases for ARG detection, which directly influences the ARGs that can be detected. Publicly available databases are known to be biased towards clinical and abundant pathogenic species³²⁰. This bias limits the analysis of environmental organisms not generally considered to be human pathogens. As observed with *Pseudomonas* spp., there were limited ARGs detected in these environmental isolates, with two instances where no carbapenemases were identified using default thresholds for AMRFinderPlus⁴⁸. While the approach used in this study provides a greater likelihood of ARG identification than PCR-based methods, it does not account for the identification of novel ARGs. Therefore, further investigation may be required to fully capture the diversity of ARGs in these isolates.

7.2.4 Limitations of CNISP and CANWARD datasets used for genomic comparisons

Genomic comparisons across all species in this study (*C. difficile*, MRSA, and *Acinetobacter* spp.) were limited by the infection types captured through CNISP and CANWARD

surveillance systems. For example, CDI are only captured through CNISP during a two-month period every year²², therefore there is potential that additional CA-CDI would have fit the selection criteria if CNISP sampling occurred year-round. In MRSA comparisons, only BSI are captured through CNISP, therefore MRSA that resulted in other infections such as skin and soft tissue infections would not be available for genomic comparisons in this study. Last, *Acinetobacter* spp. collected through CNISP and CANWARD are not limited to a single infection type, however CANWARD sampling from each participating site is limited to a set number of specific infection types per year^{28,321}. Thus, comparisons to human clinical infections in Canada could only be made to those captured through their appropriate hospital surveillance systems.

7.3 Future directions

This work focuses on the farm-to-fork continuum through the investigation of retail meat. Future work can expand the exploration of this continuum in Canada through other areas of the One-Health sector, with the aim of linking novel data to the data presented here. While this study focused on identifying linkages between retail meat and human sectors, sources of retail meat contamination can also be investigated. Through CIPARS, ongoing and future work will isolate and characterize priority AMR pathogens from animal and agri-food sectors, namely farm and abattoir sources. The investigation of these sources can provide additional insights into sources of retail meat contamination to gain a more holistic perspective of the farm-to-fork continuum and provide evidence-based data to be used in risk model assessments.

On a more granular level, future work with this isolate collection can focus on addressing some gaps and limitations discussed previously. Future analysis should include the investigation of virulence factors across pathogens which may change interpretations regarding clinical significance. The investigation of virulence factors was not within the scope of this study but could reveal important insights into the pathogenicity of these isolates.

7.4 Conclusions

Through phenotypic and genomic methods, this study showed that retail meat is a low source of select priority AMR pathogens in Canada, with demonstrated genomic linkages to human clinical infections of *C. difficile*, MRSA, and *Acinetobacter* spp. in Canada. The findings from this study revealed linkages to the human clinical sector that were previously unidentified, showing varying degrees of relatedness between AMR pathogens isolated from retail meat and those of

human origin. This work bridges the gap between One-Health sectors, and contributes to a holistic understanding of AMR in the Canadian farm-to-fork continuum. Additionally, this study did not detect any priority CPE, but showed that retail meat is a source of species-associated carbapenemases in *Acinetobacter* spp., *Aeromonas* spp., and *Pseudomonas* spp., some of which have caused important human infections globally. Resistance determinants in *Aeromonas* spp. also showed that this genus may be a reservoir for the transmission of high priority ARGs such as *mcr*. A wealth of information can be extracted from retail meat, allowing for the investigation into both AMR organisms and important transmittable ARGs. Future related work should investigate potential sources of retail meat contamination and aim to investigate transmission pathways of both organisms and mobile resistance genes from the farm-to-fork continuum in Canada.

References

1. Antimicrobial resistance: A silent pandemic. *Nat. Commun.* **15**, 6198 (2024).
2. Porooshat Dadgostar. Antimicrobial resistance: Implications and costs. *Infect. Drug Resist.* **12**, 3903–3910 (2019).
3. Prestinaci, F., Pezzotti, P. & Pantosti, A. Antimicrobial resistance: A global multifaceted phenomenon. *Pathog. Glob. Health* **109**, 309–318 (2015).
4. Coculescu, B. Antimicrobial resistance induced by genetic changes. *J. Med. Life* **2**, 114–123 (2009).
5. Tello, A., Austin, B. & Telfer, T. C. Selective pressure of antibiotic pollution on bacteria of importance to public health. *Environ. Health Perspect.* **120**, 1100–1106 (2012).
6. Davies, J. & Davies, D. Origins and evolution of antibiotic resistance. *Microbiol. Mol. Biol. Rev.* **74**, 417–433 (2010).
7. Zou, H.-Y. *et al.* Prevalence of antibiotic resistance genes in mining-impacted farmland environments. *Ecotoxicol. Environ. Saf.* **289**, 117651 (2025).
8. Gao, F.-Z. *et al.* Contaminant-degrading bacteria are super carriers of antibiotic resistance genes in municipal landfills: A metagenomics-based study. *Environ. Int.* **195**, 109239 (2025).
9. Qiu, D. *et al.* Response of microbial antibiotic resistance to pesticides: An emerging health threat. *Sci. Total Environ.* **850**, 158057 (2022).
10. Foysal, M. J., Neilan, B. A. & Timms, V. The impact of anthropogenic activities on antimicrobial and heavy metal resistance in aquatic environments. *Appl. Environ. Microbiol.* **91**, e02317-24 (2025).
11. Allel, K. *et al.* Global antimicrobial-resistance drivers: An ecological country-level study at the human–animal interface. *Lancet Planet. Health* **7**, e291–e303 (2023).
12. Collignon, P., Beggs, J. J., Walsh, T. R., Gandra, S. & Laxminarayan, R. Anthropological and socioeconomic factors contributing to global antimicrobial resistance: A univariate and multivariable analysis. *Lancet Planet. Health* **2**, e398–e405 (2018).
14. Council of Canadian Academies. *When antibiotics fail*. Ottawa (ON): The expert panel on the potential socio-economic impacts of antimicrobial resistance in Canada (2019).
14. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet* **399**, 629–655 (2022).
15. GBD 2021 Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance 1990–2021: A systematic analysis with forecasts to 2050. *Lancet* **404**, 1199–1226 (2024).
16. Diener, A., Wang, H. & Nkangu, M. Hospital and related resource costs associated with antimicrobial-resistant infections in Canada, 2019. *Can. Commun. Dis. Rep.* **48**, 529–539 (2022).
17. World Health Organization. *Global Action Plan on Antimicrobial Resistance*. <https://www.who.int/publications/i/item/9789241509763> (2015).

18. World Health Organization. *Prioritization of Pathogens to Guide Discovery, Research, and Development of New Antibiotics for Drug-Resistant Bacterial Infections, Including Tuberculosis*. <https://www.who.int/publications/i/item/WHO-EMP-IAU-2017.12> (2017).
19. World Health Organization. *WHO Bacterial Priority Pathogens List, 2024: Bacterial Pathogens of Public Health Importance to Guide Research, Development and Strategies to Prevent and Control Antimicrobial Resistance*. <https://www.who.int/publications/i/item/9789240093461> (2024).
20. Public Health Agency of Canada. *Pan-Canadian Action Plan on Antimicrobial Resistance*. (2023).
21. Health Infobase. The Canadian Nosocomial Infection Surveillance Program (CNISP). <https://health-infobase.canada.ca/cnisp/index.html> (2025).
22. Canadian Nosocomial Infection Surveillance Program. Healthcare-associated infections and antimicrobial resistance in Canadian acute care hospitals, 2018–2022. *Can. Commun. Dis. Rep.* **50**, 179–196 (2024).
23. Canadian Nosocomial Infection Surveillance Program. The Canadian Nosocomial Infection Surveillance Program: Keeping an eye on antimicrobial resistance in Canadian hospitals since 1995. *Can. Commun. Dis. Rep.* **48**, 506–511 (2022).
24. Public Health Agency of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS): About CIPARS. <https://www.canada.ca/en/public-health/services/surveillance/canadian-integrated-program-antimicrobial-resistance-surveillance-cipars/about-cipars.html> (2024).
25. Rudnick, W. *et al.* Overview of Canada’s Antimicrobial Resistance Network (AMRNet): A data-driven One Health approach to antimicrobial resistance surveillance. *Can Commun Rep* **48**, 522–528 (2022).
26. NML launches antimicrobial resistance surveillance network. <https://science.gc.ca/site/science/en/blogs/science-health/nml-launches-antimicrobial-resistance-surveillance-network> (2023).
27. Zhanel, G. G., Adam, H. J., & Canadian Antimicrobial Resistance Alliance (CARA) and CANWARD. Ten years of the CANWARD Study (2007–16). *J. Antimicrob. Chemother.* **74**, iv2–iv4 (2019).
28. Zhanel, G. G. *et al.* 42936 pathogens from Canadian hospitals: 10 years of results (2007–16) from the CANWARD surveillance study. *J. Antimicrob. Chemother.* **74**, iv5–iv21 (2019).
29. Public Health Agency of Canada. Canadian Antimicrobial Resistance Surveillance System (CARSS) report 2022. <https://www.canada.ca/en/public-health/services/publications/drugs-health-products/canadian-antimicrobial-resistance-surveillance-system-2024-executive-summary.html> (2024).
30. Health Infobase. Canadian Antimicrobial Resistance Surveillance System. <https://health-infobase.canada.ca/carss/amr/> (2024).
31. Garner, M. J. *et al.* An assessment of antimicrobial resistant disease threats in Canada. *PLOS ONE* **10**, e0125155 (2015).

32. Arbefeville, S. S., Timbrook, T. T. & Garner, C. D. Evolving strategies in microbe identification—a comprehensive review of biochemical, MALDI-TOF MS and molecular testing methods. *J. Antimicrob. Chemother.* **79**, i2–i8 (2024).
33. Singhal, N., Kumar, M., Kanaujia, P. K. & Viridi, J. S. MALDI-TOF mass spectrometry: An emerging technology for microbial identification and diagnosis. *Front. Microbiol.* **6**, 791 (2015).
34. Tsuchida, S. & Nakayama, T. MALDI-based mass spectrometry in clinical testing: Focus on bacterial identification. *Appl. Sci. Switz.* **12**, 2814 (2022).
35. Elbehiry, A. *et al.* How MALDI-TOF mass spectrometry technology contributes to microbial infection control in healthcare settings. *Vaccines* **10**, 1881 (2022).
36. Uelze, L. *et al.* Typing methods based on whole genome sequencing data. *One Health Outlook* **2**, 3 (2020).
37. Lakhundi, S. & Zhang, K. Methicillin-resistant *Staphylococcus aureus*: Molecular characterization, evolution, and epidemiology. *Clin. Microbiol. Rev.* **31**, e00020–e00118 (2018).
38. Simar, S. R., Hanson, B. M. & Arias, C. A. Techniques in bacterial strain typing: Past, present, and future. *Curr. Opin. Infect. Dis.* **34**, 339–345 (2021).
39. Abad-Fau, A., Sevilla, E., Martín-Burriel, I., Moreno, B. & Bolea, R. Update on commonly used molecular typing methods for *Clostridioides difficile*. *Microorganisms* **11**, 1752 (2023).
40. Jolley, K. A., Bray, J. E. & Maiden, M. C. J. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res.* **3**, (2018).
41. Maiden, M. C. J. *et al.* MLST revisited: The gene-by-gene approach to bacterial genomics. *Nat. Rev. Microbiol.* **11**, 728–736 (2013).
42. Rodriguez-R, L. M. *et al.* An ANI gap within bacterial species that advances the definitions of intra-species units. *mBio* **15**, e02696-23 (2023).
43. Stoesser, N. *et al.* Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. *mBio* **7**, e02162–e02215 (2016).
44. Frentrup, M. *et al.* A publicly accessible database for *Clostridioides difficile* genome sequences supports tracing of transmission chains and epidemics. *Microb. Genomics* **6**, 000410 (2020).
45. Bletz, S., Janezic, S., Harmsen, D., Rupnik, M. & Mellmann, A. Defining and evaluating a core genome multilocus sequence typing scheme for genome-wide typing of *Clostridium difficile*. *J. Clin. Microbiol.* **56**, e01987–e02017 (2018).
46. Kjær Hansen, S. *et al.* Using core genome multilocus sequence typing (cgMLST) for vancomycin-resistant *Enterococcus faecium* isolates to guide infection control interventions and end an outbreak. *J. Glob. Antimicrob. Resist.* **24**, 418–423 (2021).

47. Yousfi, K. *et al.* Source tracking based on core genome SNV and CRISPR typing of *Salmonella enterica* serovar Heidelberg isolates involved in foodborne outbreaks in Québec, 2012. *Front. Microbiol.* **11**, 1317 (2020).
48. Feldgarden, M. *et al.* AMRFinderPlus and the Reference Gene Catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. *Sci. Rep.* **11**, 12728 (2021).
49. Bharat, A. *et al.* Correlation between phenotypic and in silico detection of antimicrobial resistance in *Salmonella enterica* in Canada using Staramr. *Microorganisms* **10**, 292 (2022).
50. Florensa, A. F., Kaas, R. S., Clausen, P. T. L. C., Aytan-Aktug, D. & Aarestrup, F. M. ResFinder – an open online resource for identification of antimicrobial resistance genes in next-generation sequencing data and prediction of phenotypes from genotypes. *Microb. Genomics* **8**, 000748 (2022).
51. Zankari, E. *et al.* PointFinder: A novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J. Antimicrob. Chemother.* **72**, 2764–2768 (2017).
52. Papp, M. & Solymosi, N. Review and comparison of antimicrobial resistance gene databases. *Antibiotics* **11**, 339 (2022).
53. Partridge, S. R., Kwong, S. M., Firth, N. & Jensen, S. O. Mobile genetic elements associated with antimicrobial resistance. *Clin. Microbiol. Rev.* **31**, e00088-17 (2018).
54. Berbers, B. *et al.* Using a combination of short- and long-read sequencing to investigate the diversity in plasmid- and chromosomally encoded extended-spectrum beta-lactamases (ESBLs) in clinical *Shigella* and *Salmonella* isolates in Belgium. *Microb. Genomics* **9**, 000925 (2023).
55. Robertson, J. & Nash, J. H. E. MOB-suite: Software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microb. Genomics* **4**, e000206 (2018).
56. Lim, S. C., Knight, D. R. & Riley, T. V. *Clostridium difficile* and One Health. *Clin Microbiol Infect* **26**, 857–863 (2020).
57. Polage, C. R., Solnick, J. V. & Cohen, S. H. Nosocomial diarrhea: Evaluation and treatment of causes other than *Clostridium difficile*. *Clin. Infect. Dis.* **55**, 982–989 (2012).
58. Mullish, B. H. & Williams, H. R. *Clostridium difficile* infection and antibiotic-associated diarrhoea. *Clin. Med.* **18**, 237–241 (2018).
59. Finn, E., Andersson, F. L. & Madin-Warburton, M. Burden of *Clostridioides difficile* infection (CDI) - a systematic review of the epidemiology of primary and recurrent CDI. *BMC Infect. Dis.* **21**, 456 (2021).
60. Balsells, E. *et al.* Global burden of *Clostridium difficile* infections: A systematic review and meta-analysis. *J. Glob. Health* **9**, 010407 (2019).
61. Du, T. *et al.* Characterization of healthcare-associated and community-associated *Clostridioides difficile* infections among adults, Canada, 2015–2019. *Emerg. Infect. Dis.* **28**, 1128–1136 (2022).

62. Giles, J. & Roberts, A. *Clostridioides difficile*: Current overview and future perspectives. in *Advances in Protein Chemistry and Structural Biology* (ed. Donev, R.) vol. 129 215–245 (Academic Press, 2022).
63. Buddle, J. E. & Fagan, R. P. Pathogenicity and virulence of *Clostridioides difficile*. *Virulence* **14**, 2150452 (2023).
64. Knight, D. R., Elliott, B., Chang, B. J., Perkins, T. T. & Riley, T. V. Diversity and evolution in the genome of *Clostridium difficile*. *Clin. Microbiol. Rev.* **28**, 721–741 (2015).
65. Furuya-Kanamori, L. *et al.* Asymptomatic *Clostridium difficile* colonization: Epidemiology and clinical implications. *BMC Infect. Dis.* **15**, 516 (2015).
66. Sholeh, M. *et al.* Antimicrobial resistance in *Clostridioides (Clostridium) difficile* derived from humans: A systematic review and meta-analysis. *Antimicrob. Resist. Infect. Control* **9**, 158 (2020).
67. Eubank, T. A., Gonzales-Luna, A. J., Hurdle, J. G. & Garey, K. W. Genetic mechanisms of vancomycin resistance in *Clostridioides difficile*: A systematic review. *Antibiotics* **11**, 258 (2022).
68. Griffiths, D. *et al.* Multilocus sequence typing of *Clostridium difficile*. *J. Clin. Microbiol.* **48**, 770–778 (2010).
69. Neoh, H. min, Tan, X. E., Sapri, H. F. & Tan, T. L. Pulsed-field gel electrophoresis (PFGE): A review of the ‘gold standard’ for bacteria typing and current alternatives. *Infect. Genet. Evol.* **74**, 103935 (2019).
70. Chitnis, A. S. *et al.* Epidemiology of community-associated *Clostridium difficile* infection, 2009 through 2011. *JAMA Intern. Med.* **173**, 1359–1367 (2013).
71. Fawley, W. N. *et al.* Development and validation of an internationally-standardized, high-resolution capillary gel-based electrophoresis PCR-ribotyping protocol for *Clostridium difficile*. *PloS One* **10**, e0118150 (2015).
72. Guh, A. Y. *et al.* Trends in U.S. burden of *Clostridioides difficile* infection and outcomes. *N. Engl. J. Med.* **382**, 1320–1330 (2020).
73. Turner, N. A. *et al.* Methicillin-resistant *Staphylococcus aureus*: An overview of basic and clinical research. *Nat. Rev. Microbiol.* **17**, 203–218 (2019).
74. Strauß, L. *et al.* Origin, evolution, and global transmission of community-acquired *Staphylococcus aureus* ST8. *Proc. Natl. Acad. Sci.* **114**, E10596–E10604 (2017).
75. Simor, A. E. *et al.* Laboratory characterization of methicillin-resistant *Staphylococcus aureus* in Canadian hospitals: Results of 5 years of national surveillance, 1995–1999. *J. Infect. Dis.* **186**, 652–660 (2002).
76. Golding, G. R. *et al.* A preliminary guideline for the assignment of methicillin-resistant *Staphylococcus aureus* to a Canadian pulsed-field gel electrophoresis epidemic type using spa typing. *Can. J. Infect. Dis. Med. Microbiol.* **19**, 273–281 (2008).
77. Mellmann, A. *et al.* Automated DNA sequence-based early warning system for the detection of methicillin-resistant *Staphylococcus aureus* outbreaks. *PLoS Med.* **3**, e33 (2006).

78. Bartels, M. D. *et al.* Comparing whole-genome sequencing with sanger sequencing for *spa* typing of methicillin-resistant *Staphylococcus aureus*. *J. Clin. Microbiol.* **52**, 4305–4308 (2014).
79. Strommenger, B. *et al.* Assignment of *Staphylococcus* isolates to groups by *spa* typing, SmaI macrorestriction analysis, and multilocus sequence typing. *J. Clin. Microbiol.* **44**, 2533–2540 (2006).
80. Durand, G. *et al.* Routine whole-genome sequencing for outbreak investigations of *Staphylococcus aureus* in a national reference center. *Front. Microbiol.* **9**, 511 (2018).
81. Halat, D. H. & Moubareck, C. A. The current burden of carbapenemases: Review of significant properties and dissemination among Gram-negative bacteria. *Antibiotics* **9**, 186 (2020).
82. Diene, S. M. & Rolain, J. M. Carbapenemase genes and genetic platforms in Gram-negative bacilli: *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter* species. *Clin. Microbiol. Infect.* **20**, 831–838 (2014).
83. Papp-Wallace, K. M., Endimiani, A., Taracila, M. A. & Bonomo, R. A. Carbapenems: Past, present, and future. *Antimicrob. Agents Chemother.* **55**, 4943–4960 (2011).
84. Meletis, G. Carbapenem resistance: Overview of the problem and future perspectives. *Ther. Adv. Infect. Dis.* **3**, 15–21 (2016).
85. Queenan, A. M. & Bush, K. Carbapenemases: The versatile β -lactamases. *Clin. Microbiol. Rev.* **20**, 440–458 (2007).
86. Ambler, R. P. The structure of β -lactamases. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **289**, 321–331 (1980).
87. Rasmussen, B. A. & Bush, K. Carbapenem-hydrolyzing β -lactamases. *Antimicrob. Agents Chemother.* **41**, 223–232 (1997).
88. Bartoszko, J. J., Mitchell, R., Katz, K., Mulvey, M. & Mataseje, L. Characterization of extensively drug-resistant (XDR) carbapenemase-producing Enterobacterales (CPE) in Canada from 2019 to 2020. *Microbiol. Spectr.* **10**, e0097522 (2022).
89. Rood, I. G. H. & Li, Q. Review: Molecular detection of extended spectrum- β -lactamase- and carbapenemase-producing *Enterobacteriaceae* in a clinical setting. *Diagn. Microbiol. Infect. Dis.* **89**, 245–250 (2017).
90. Tamma, P. D. & Simner, P. J. Phenotypic detection of carbapenemase-producing organisms from clinical isolates. *J. Clin. Microbiol.* **56**, e01140-18 (2018).
91. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute (2024).
92. Pierce, V. M. *et al.* Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among *Enterobacteriaceae*. *J. Clin. Microbiol.* **55**, 2321–2333 (2017).
93. Cui, X., Zhang, H. & Du, H. Carbapenemases in *Enterobacteriaceae*: Detection and antimicrobial therapy. *Front. Microbiol.* **10**, 1823 (2019).

94. Uechi, K. *et al.* A modified carbapenem inactivation method, CIMTris, for carbapenemase production in *Acinetobacter* and *Pseudomonas* species. *J. Clin. Microbiol.* **55**, 3405–3410 (2017).
95. Jing, X. *et al.* The simplified carbapenem inactivation method (sCIM) for simple and accurate detection of carbapenemase-producing Gram-negative bacilli. *Front. Microbiol.* **9**, 2391 (2018).
96. Nordmann, P., Poirel, L. & Dortet, L. Rapid detection of carbapenemase-producing *Enterobacteriaceae*. *Emerg. Infect. Dis.* **18**, 1503–1507 (2012).
97. Tamma, P. D. *et al.* Comparison of 11 phenotypic assays for accurate detection of carbapenemase-producing *Enterobacteriaceae*. *J. Clin. Microbiol.* **55**, 1046–1055 (2017).
98. Mackenzie, J. S. & Jeggo, M. The One Health approach—why is it so important? *Trop. Med. Infect. Dis.* **4**, 88 (2019).
99. Velazquez-Meza, M. E., Galarde-López, M., Carrillo-Quiróz, B. & Alpuche-Aranda, C. M. Antimicrobial resistance: One Health approach. *Vet. World* **15**, 743–749 (2022).
100. Massé, J. *et al.* Prevalence of antimicrobial resistance and characteristics of *Escherichia coli* isolates from fecal and manure pit samples on dairy farms in the province of Québec, Canada. *Front. Vet. Sci.* **8**, 654125 (2021).
101. Kos, D. *et al.* Insight into antimicrobial resistance at a new beef cattle feedlot in western Canada. *mSphere* **8**, e00317-23 (2023).
102. Public Health Agency of Canada. *Canadian Antimicrobial Resistance Surveillance System (CARSS) Report 2022*. <https://www.canada.ca/en/public-health/services/publications/drugs-health-products/canadian-antimicrobial-resistance-surveillance-system-report-2022.html> (2022).
103. Bennani, H. *et al.* Overview of evidence of antimicrobial use and antimicrobial resistance in the food chain. *Antibiotics* **9**, 49 (2020).
104. Hammerum, A. M. *et al.* Danish Integrated Antimicrobial Resistance Monitoring and Research Program. *Emerg. Infect. Dis.* **13**, 1632–1639 (2007).
105. European Commission. Ban on antibiotics as growth promoters in animal feed enters into effect. https://ec.europa.eu/commission/presscorner/detail/en/ip_05_1687 (2005).
106. Health Canada. Responsible use of Medically Important Antimicrobials in animals. <https://www.canada.ca/en/public-health/services/antibiotic-antimicrobial-resistance/animals/actions/responsible-use-antimicrobials.html> (2021).
107. Teklemariam, A. D. *et al.* Human salmonellosis: A continuous global threat in the farm-to-fork food safety continuum. *Foods* **12**, 1756 (2023).
108. Dutil, L. *et al.* Ceftiofur resistance in *Salmonella enterica* serovar Heidelberg from chicken meat and humans, Canada. *Emerg. Infect. Dis.* **16**, 48–54 (2010).
109. Shaji, S., Selvaraj, R. K. & Shanmugasundaram, R. Salmonella infection in poultry: A review on the pathogen and control strategies. *Microorganisms* **11**, 2814 (2023).

110. Ou, Q. *et al.* A meta-analysis of the global prevalence rates of *Staphylococcus aureus* and methicillin-resistant *S. aureus* contamination of different raw meat products. *J. Food Prot.* **80**, 763–774 (2017).
111. Borji, S. *et al.* Global prevalence of *Clostridioides difficile* in 17,148 food samples from 2009 to 2019: A systematic review and meta-analysis. *J. Health Popul. Nutr.* **42**, 36 (2023).
112. Tan, D. T. *et al.* A *Clostridioides difficile* surveillance study of Canadian retail meat samples from 2016–2018. *Anaerobe* **74**, 102551 (2022).
113. Metcalf, D., Reid-Smith, R. J., Avery, B. P. & Weese, J. S. Prevalence of *Clostridium difficile* in retail pork. *Can. Vet. J.* **51**, 873–876 (2010).
114. Rodriguez-Palacios, A., Staempfli, H. R., Duffield, T. & Weese, J. S. *Clostridium difficile* in retail ground meat, Canada. *Emerg. Infect. Dis.* **13**, 485–487 (2007).
115. Visser, M. *et al.* Detection of *Clostridium difficile* in retail ground meat products in Manitoba. *Can. J. Infect. Dis. Med. Microbiol.* **23**, 28–30 (2012).
116. Weese, J. S., Avery, B. P., Rousseau, J. & Reid-Smith, R. J. Detection and enumeration of *Clostridium difficile* spores in retail beef and pork. *Appl. Environ. Microbiol.* **75**, 5009–5011 (2009).
117. Bernier-Lachance, J. *et al.* Prevalence and characteristics of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) isolated from chicken meat in the province of Quebec, Canada. *PLOS ONE* **15**, e0227183 (2020).
118. Weese, J. S., Avery, B. P. & Reid-Smith, R. J. Detection and quantification of methicillin-resistant *Staphylococcus aureus* (MRSA) clones in retail meat products. *Lett. Appl. Microbiol.* **51**, 338–342 (2010).
119. Weese, J. S., Reid-Smith, R., Rousseau, J. & Avery, B. Methicillin-resistant *Staphylococcus aureus* (MRSA) contamination of retail pork. *Can. Vet. J.* **51**, 749–752 (2010).
120. Feng, J. *et al.* Characterization of carbapenem-resistant *Enterobacteriaceae* cultured from retail meat products, patients, and porcine excrement in China. *Front. Microbiol.* **12**, 743468 (2021).
121. Ma, W.-Q. *et al.* Contamination of *Proteus mirabilis* harbouring various clinically important antimicrobial resistance genes in retail meat and aquatic products from food markets in China. *Front. Microbiol.* **13**, 1086800 (2022).
122. Murray, M. *et al.* Market chickens as a source of antibiotic-resistant *Escherichia coli* in a peri-urban community in Lima, Peru. *Front. Microbiol.* **12**, 635871 (2021).
123. Klees, S. *et al.* Prevalence and epidemiology of multidrug-resistant pathogens in the food chain and the urban environment in northwestern Germany. *Antibiotics* **9**, 708 (2020).
124. Sadek, M. *et al.* First genomic characterization of *bla*_{VIM-1} and *mcr-9*-coharbouring *Enterobacter hormaechei* isolated from food of animal origin. *Pathogens* **9**, 687 (2020).
125. Garcia-Graells, C. *et al.* First detection of a plasmid located carbapenem resistant *bla*_{VIM-1} gene in *E. coli* isolated from meat products at retail in Belgium in 2015. *Int. J. Food Microbiol.* **324**, 108624 (2020).

126. Ballash, G. A. *et al.* Antimicrobial resistant bacteria recovered from retail ground meat products in the US include a *Raoultella ornithinolytica* co-harboring *bla*_{KPC-2} and *bla*_{NDM-5}. *Sci. Rep.* **11**, 14041 (2021).
127. Janecko, N. *et al.* Carbapenem-resistant *Enterobacter* spp. in retail seafood imported from Southeast Asia to Canada. *Emerg. Infect. Dis.* **22**, 1675–1677 (2016).
128. Morrison, B. J. & Rubin, J. E. Carbapenemase producing bacteria in the food supply escaping detection. *PLoS ONE* **10**, e0126717 (2015).
129. Mangat, C. S. *et al.* Characterization of VCC-1, a novel Ambler class A carbapenemase from *Vibrio cholerae* isolated from imported retail shrimp sold in Canada. *Antimicrob. Agents Chemother.* **60**, 1819–1825 (2016).
130. Zaidi, S. E. Ze. *et al.* Genomic characterization of carbapenem-resistant bacteria from beef cattle feedlots. *Antibiotics* **12**, 960 (2023).
131. Collineau, L. *et al.* Integrating whole-genome sequencing data into quantitative risk assessment of foodborne antimicrobial resistance: A review of opportunities and challenges. *Front. Microbiol.* **10**, 1107 (2019).
132. Shafer, D. *et al.* Comparison of direct smear and chemical extraction methods for MALDI-TOF mass spectrometry identification of clinical relevant anaerobic bacteria. *Front. Lab. Med.* **1**, 27–30 (2017).
133. Uechi, K. *et al.* An improved carbapenem inactivation method, CIMTrisII, for carbapenemase production by Gram-negative pathogens. *J. Med. Microbiol.* **68**, 124–131 (2019).
134. CNISP. Healthcare-associated infections and antimicrobial resistance in Canadian acute care hospitals, 2017–2021. *Can. Commun. Dis. Rep.* **49**, 235–252 (2023).
135. Chen, S., Zhou, Y., Chen, Y. & Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, i884–i890 (2018).
136. Andrew, S. FastQC: A quality control tool for high throughput sequence data. <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/> (2010).
137. Truong, D. T. *et al.* MetaPhlAn2 for enhanced metagenomic taxonomic profiling. *Nat. Methods* **12**, 902–903 (2015).
138. National Microbiology Laboratory. refseq_masher. https://github.com/phac-nml/refseq_masher (2025).
139. Seemann, T. tseemann/shovill. <https://github.com/tseemann/shovill> (2025).
140. Mikheenko, A., Prijibelski, A., Saveliev, V., Antipov, D. & Gurevich, A. Versatile genome assembly evaluation with QUAST-LG. *Bioinformatics* **34**, i142–i150 (2018).
141. Camacho, C. *et al.* BLAST+: Architecture and applications. *BMC Bioinformatics* **10**, 421 (2009).
142. Seemann, T. mlst. <https://github.com/tseemann/mlst> (2025).
143. Jünemann, S. *et al.* Updating benchtop sequencing performance comparison. *Nat. Biotechnol.* **31**, 294–296 (2013).

144. Petkau, A. *et al.* SNVPhyl: A single nucleotide variant phylogenomics pipeline for microbial genomic epidemiology. *Microb. Genomics* **3**, e000116 (2017).
145. Paradis, E. & Schliep, K. ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2019).
146. Yu, G., Smith, D. K., Zhu, H., Guan, Y. & Lam, T. T. Y. ggtree: An r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. *Methods Ecol. Evol.* **8**, 28–36 (2017).
147. High Content Genomics & Bioinformatics - Germans Trias i Pujol Research Institute. spaTyper. <https://github.com/HCGB-IGTP/spaTyper>.
148. Manni, M., Berkeley, M. R., Seppey, M., Simão, F. A. & Zdobnov, E. M. BUSCO update: Novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. *Mol. Biol. Evol.* **38**, 4647–4654 (2021).
149. Chklovski, A., Parks, D. H., Woodcroft, B. J. & Tyson, G. W. CheckM2: A rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. *Nat. Methods* **20**, 1203–1212 (2023).
150. Chaumeil, P. A., Mussig, A. J., Hugenholtz, P. & Parks, D. H. GTDB-Tk: A toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* **36**, 1925–1927 (2020).
151. Quinlan, A. R. & Hall, I. M. BEDTools: A flexible suite of utilities for comparing genomic features. *Bioinformatics* **26**, 841–842 (2010).
152. Katoh, K. & Standley, D. M. MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
153. Capella-Gutiérrez, S., Silla-Martínez, J. M. & Gabaldón, T. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* **25**, 1972–1973 (2009).
154. Shen, W., Le, S., Li, Y. & Hu, F. SeqKit: A cross-platform and ultrafast toolkit for FASTA/Q file manipulation. *PLOS ONE* **11**, e0163962 (2016).
155. Page, A. J. *et al.* SNP-sites: Rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb. Genomics* **2**, e000056 (2016).
156. Minh, B. Q. *et al.* IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).
157. Hoang, D. T., Chernomor, O., Von Haeseler, A., Minh, B. Q. & Vinh, L. S. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* **35**, 518–522 (2018).
158. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing (2025).
159. Bauer, M. P. *et al.* *Clostridium difficile* infection in Europe: A hospital-based survey. *The Lancet* **377**, 63–73 (2011).

160. Banks, A., Brown, D. J., Mather, H., Coia, J. E. & Wiuff, C. Sentinel community *Clostridium difficile* infection (CDI) surveillance in Scotland, April 2013 to March 2014. *Anaerobe* **37**, 49–53 (2016).
161. Skally, M., Bennett, K., Humphreys, H. & Fitzpatrick, F. Rethinking *Clostridioides difficile* infection (CDI) surveillance definitions based on changing healthcare utilisation and a more realistic incubation period: Reviewing data from a tertiary-referral hospital, Ireland, 2012 to 2021. *Eurosurveillance* **29**, 2300335 (2024).
162. Turner, N. A. *et al.* Epidemiologic trends in *Clostridioides difficile* infections in a regional community hospital network. *JAMA Netw. Open* **2**, e1914149 (2019).
163. Knight, D. R. & Riley, T. V. Genomic delineation of zoonotic origins of *Clostridium difficile*. *Front. Public Health* **7**, 164 (2019).
164. Bolton, D. & Marcos, P. The environment, farm animals and foods as sources of *Clostridioides difficile* infection in humans. *Foods* **2023 Vol 12 Page 1094** **12**, 1094 (2023).
165. Warriner, K., Xu, C., Habash, M., Sultan, S. & Weese, S. J. Dissemination of *Clostridium difficile* in food and the environment: Significant sources of *C. difficile* community-acquired infection? *J. Appl. Microbiol.* **122**, 542–553 (2017).
166. Candel-Pérez, C., Santaella-Pascual, J., Ros-Berruezo, G. & Martínez-Graciá, C. Occurrence of *Clostridioides (Clostridium) difficile* in poultry giblets at slaughter and in retail pork and poultry meat in Southeastern Spain. *J. Food Prot.* **84**, 310–314 (2021).
167. Songer, J. G. *et al.* *Clostridium difficile* in retail meat products, USA, 2007. *Emerg. Infect. Dis.* **15**, 819–821 (2009).
168. Esfandiari, Z. *et al.* Prevalence and antibiotic resistance of *Clostridioides (Clostridium) difficile* in meat and meat products: A systematic review and meta-analysis. *Iran. J. Public Health* **52**, 2516–2527 (2023).
169. Knetsch, C. W. *et al.* Zoonotic transfer of *Clostridium difficile* harboring antimicrobial resistance between farm animals and humans. *J. Clin. Microbiol.* **56**, e01384-17 (2018).
170. Eyre, D. W. *et al.* Diverse sources of *C. difficile* infection identified on whole-genome sequencing. *N. Engl. J. Med.* **369**, 1195–1205 (2013).
171. Lee, A. S. *et al.* Methicillin-resistant *Staphylococcus aureus*. *Nat. Rev. Dis. Primer* **4**, 18033 (2018).
172. Shoaib, M. *et al.* MRSA compendium of epidemiology, transmission, pathophysiology, treatment, and prevention within one health framework. *Front. Microbiol.* **13**, 1067284 (2023).
173. Nichol, K. A. *et al.* Characterization of MRSA in Canada from 2007 to 2016. *J. Antimicrob. Chemother.* **74**, iv55–iv63 (2019).
174. Khanna, T., Friendship, R., Dewey, C. & Weese, J. S. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Vet. Microbiol.* **128**, 298–303 (2008).

175. Armand-Lefevre, L., Ruimy, R. & Andreumont, A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg. Infect. Dis.* **11**, 711–714 (2005).
176. Butaye, P., Argudín, M. A. & Smith, T. C. Livestock-associated MRSA and its current evolution. *Curr. Clin. Microbiol. Rep.* **3**, 19–31 (2016).
177. Smith, T. C. *et al.* Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in Midwestern U.S. swine and swine workers. *PLoS ONE* **4**, e4258 (2009).
178. Molla, B. *et al.* Epidemiology and genotypic characteristics of methicillin-resistant *Staphylococcus aureus* strains of porcine origin. *J. Clin. Microbiol.* **50**, 3687–3693 (2012).
179. Golding, G. R. *et al.* Livestock-associated methicillin-resistant *Staphylococcus aureus* sequence type 398 in humans, Canada. *Emerg. Infect. Dis.* **16**, 587–594 (2010).
180. Chow, A. *et al.* MRSA transmission dynamics among interconnected acute, intermediate-term, and long-term healthcare facilities in Singapore. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **64**, S76–S81 (2017).
181. Coll, F. *et al.* Definition of a genetic relatedness cutoff to exclude recent transmission of methicillin-resistant *Staphylococcus aureus*: A genomic epidemiology analysis. *Lancet Microbe* **1**, e328–e335 (2020).
182. Price, L. B. *et al.* *Staphylococcus aureus* CC398: Host adaptation and emergence of methicillin resistance in livestock. *mBio* **3**, e00305-11 (2012).
183. Yu, F. *et al.* Molecular evolution and adaptation of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) sequence type 9. *mSystems* **6**, e00492-21 (2021).
184. Dhup, V., Kearns, A. M., Pichon, B. & Foster, H. A. First report of identification of livestock-associated MRSA ST9 in retail meat in England. *Epidemiol. Infect.* **143**, 2989–2992 (2015).
185. Dressler, A. E. *et al.* Prevalence, antibiotic resistance and molecular characterisation of *Staphylococcus aureus* in pigs at agricultural fairs in the USA. *Vet. Rec.* **170**, 495 (2012).
186. Randad, P. R. *et al.* Transmission of antimicrobial-resistant *Staphylococcus aureus* clonal complex 9 between pigs and humans, United States. *Emerg. Infect. Dis.* **27**, 740–748 (2021).
187. Ji, X. *et al.* Comparative analysis of genomic characteristics, fitness and virulence of MRSA ST398 and ST9 isolated from China and Germany. *Emerg. Microbes Infect.* **10**, 1481–1494 (2021).
188. Pu, S., Han, F. & Ge, B. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* strains from Louisiana retail meats. *Appl. Environ. Microbiol.* **75**, 265–267 (2008).
189. Waters, A. E. *et al.* Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Clin. Infect. Dis.* **52**, 1227–1230 (2011).
190. Hanson, B. M. *et al.* Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J. Infect. Public Health* **4**, 169–174 (2011).

191. Simor, A. E. *et al.* Determinants of outcome in hospitalized patients with methicillin-resistant *Staphylococcus aureus* bloodstream infection: Results from national surveillance in Canada, 2008-2012. *Infect. Control Hosp. Epidemiol.* **37**, 390–397 (2016).
192. Simor, Andrew. E. *et al.* Methicillin-resistant *Staphylococcus aureus* colonization or infection in Canada: National surveillance and changing epidemiology, 1995-2007. *Infect. Control Hosp. Epidemiol.* **31**, 348–356 (2010).
193. Larsen, J. *et al.* Copresence of *tet(K)* and *tet(M)* in livestock-associated methicillin-resistant *Staphylococcus aureus* clonal complex 398 is associated with increased fitness during exposure to sublethal concentrations of tetracycline. *Antimicrob. Agents Chemother.* **60**, 4401–4403 (2016).
194. Moubareck, C. A. & Halat, D. H. Insights into *Acinetobacter baumannii*: A review of microbiological, virulence, and resistance traits in a threatening nosocomial pathogen. *Antibiotics* **9**, 119 (2020).
195. Pelegrin, A. C. *et al.* *Pseudomonas aeruginosa*: A clinical and genomics update. *FEMS Microbiol. Rev.* **45**, fuab026 (2021).
196. del Pozo, J. L. & Patel, R. The challenge of treating biofilm-associated bacterial infections. *Clin. Pharmacol. Ther.* **82**, 204–209 (2007).
197. Tuon, F. F., Dantas, L. R., Suss, P. H. & Tasca Ribeiro, V. S. Pathogenesis of the *Pseudomonas aeruginosa* biofilm: A review. *Pathogens* **11**, 300 (2022).
198. Rostami, S. *et al.* Investigating of four main carbapenem-resistance mechanisms in high-level carbapenem resistant *Pseudomonas aeruginosa* isolated from burn patients. *J. Chin. Med. Assoc.* **81**, 127–132 (2018).
199. McCracken, M. G. *et al.* Characterization of carbapenem-resistant and XDR *Pseudomonas aeruginosa* in Canada: Results of the CANWARD 2007–16 study. *J. Antimicrob. Chemother.* **74**, iv32–iv38 (2019).
200. Eveillard, M., Kempf, M., Belmonte, O., Pailhoriès, H. & Joly-Guillou, M.-L. Reservoirs of *Acinetobacter baumannii* outside the hospital and potential involvement in emerging human community-acquired infections. *Int. J. Infect. Dis.* **17**, e802–e805 (2013).
201. Houang, E. T. S. *et al.* Epidemiology and infection control implications of *Acinetobacter* spp. in Hong Kong. *J. Clin. Microbiol.* **39**, 228–234 (2001).
202. Elbehiry, A. *et al.* *Acinetobacter baumannii* as a community foodborne pathogen: Peptide mass fingerprinting analysis, genotypic of biofilm formation and phenotypic pattern of antimicrobial resistance. *Saudi J. Biol. Sci.* **28**, 1158–1166 (2021).
203. Ababneh, Q., Al-Rousan, E. & Jaradat, Z. Fresh produce as a potential vehicle for transmission of *Acinetobacter baumannii*. *Int. J. Food Contam.* **9**, 5 (2022).
204. Wilharm, G. *et al.* Relatedness of wildlife and livestock avian isolates of the nosocomial pathogen *Acinetobacter baumannii* to lineages spread in hospitals worldwide. *Environ. Microbiol.* **19**, 4349–4364 (2017).

205. Hubeny, J. *et al.* Characterization of carbapenem resistance in environmental samples and *Acinetobacter* spp. isolates from wastewater and river water in Poland. *Sci. Total Environ.* **822**, 153437 (2022).
206. Al Atrouni, A., Joly-Guillou, M.-L., Hamze, M. & Kempf, M. Reservoirs of non-*baumannii* *Acinetobacter* species. *Front. Microbiol.* **7**, 49 (2016).
207. Savin, M. *et al.* Tracing clinically-relevant antimicrobial resistances in *Acinetobacter baumannii-calcoaceticus* complex across diverse environments: A study spanning clinical, livestock, and wastewater treatment settings. *Environ. Int.* **186**, 108603 (2024).
208. Tobin, L. A. *et al.* Genomic analysis of diverse environmental *Acinetobacter* isolates identifies plasmids, antibiotic resistance genes, and capsular polysaccharides shared with clinical strains. *Appl. Environ. Microbiol.* **90**, e01654-23 (2024).
209. Hernández-González, I. L. & Castillo-Ramírez, S. Antibiotic-resistant *Acinetobacter baumannii* is a One Health problem. *Lancet Microbe* **1**, e279 (2020).
210. Stehling, E. G. *et al.* Genomic features and comparative analysis of a multidrug-resistant *Acinetobacter bereziniae* strain infecting an animal: A novel emerging one health pathogen? *World J. Microbiol. Biotechnol.* **40**, 63 (2024).
211. Sykes, E. M. E. *et al.* Phylogenomic and phenotypic analyses highlight the diversity of antibiotic resistance and virulence in both human and non-human *Acinetobacter baumannii*. *mSphere* **9**, e00741-23 (2024).
212. Mena, K. D. & Gerba, C. P. Risk assessment of *Pseudomonas aeruginosa* in water. in *Reviews of Environmental Contamination and Toxicology Vol 201* (ed. Whitacre, D. M.) 71–115 (Springer US, Boston, MA, 2009). doi:10.1007/978-1-4419-0032-6_3.
213. Devarajan, N. *et al.* Antibiotic resistant *Pseudomonas* spp. in the aquatic environment: A prevalence study under tropical and temperate climate conditions. *Water Res.* **115**, 256–265 (2017).
214. Ruiz-Roldán, L. *et al.* Antimicrobial resistance and virulence of *Pseudomonas* spp. among healthy animals: Concern about exolysin ExlA detection. *Sci. Rep.* **10**, 11667 (2020).
215. Licea-Herrera, J. I. *et al.* Agricultural soil as a reservoir of *Pseudomonas aeruginosa* with potential risk to public health. *Microorganisms* **12**, 2181 (2024).
216. Bloomfield, S. J., Palau, R., Holden, E. R., Webber, M. A. & Mather, A. E. Genomic characterization of *Pseudomonas* spp. on food: Implications for spoilage, antimicrobial resistance and human infection. *BMC Microbiol.* **24**, 20 (2024).
217. Cillóniz, C. *et al.* Community-acquired pneumonia due to multidrug- and non-multidrug-resistant *Pseudomonas aeruginosa*. *CHEST* **150**, 415–425 (2016).
218. Abd El-Ghany, W. A. *Pseudomonas aeruginosa* infection of avian origin: Zoonosis and one health implications. *Vet. World* **14**, 2155–2159 (2021).
219. Khuntayaporn, P., Thirapanmethee, K., Kanathum, P., Chitsombat, K. & Chomnawang, M. T. Comparative study of phenotypic-based detection assays for carbapenemase-producing *Acinetobacter baumannii* with a proposed algorithm in resource-limited settings. *PLoS ONE* **16**, e0259686 (2021).

220. Zhang, S. *et al.* The optimized carbapenem inactivation method for objective and accurate detection of carbapenemase-producing *Acinetobacter baumannii*. *Front. Microbiol.* **14**, (2023).
221. Visca, P., Seifert, H. & Towner, K. J. *Acinetobacter* infection – an emerging threat to human health. *IUBMB Life* **63**, 1048–1054 (2011).
222. Idzenga, D., Schouten, M. A. & Zanten, A. R. H. van. Outbreak of *Acinetobacter* genomic species 3 in a Dutch intensive care unit. *J. Hosp. Infect.* **63**, 485–487 (2006).
223. Brasiliense, D. M. *et al.* Emergence of carbapenem-resistant *Acinetobacter pittii* carrying the *bla_{OXA-72}* gene in the Amazon region, Brazil. *Diagn. Microbiol. Infect. Dis.* **93**, 82–84 (2019).
224. Horrevorts, A. *et al.* Clinical and epidemiological investigations of *Acinetobacter* genomospecies 3 in a neonatal intensive care unit. *J. Clin. Microbiol.* **33**, 1567–1572 (1995).
225. Pailhoriès, H. *et al.* Fortuitous diagnosis of NDM-1-producing *Acinetobacter pittii* carriage in a patient from France with no recent history of travel. *J. Antimicrob. Chemother.* **72**, 942–944 (2017).
226. Mo, X. M. *et al.* First identification of multidrug-resistant *Acinetobacter bereziniae* isolates harboring *bla_{NDM-1}* from hospitals in South China. *Heliyon* **9**, e12365 (2023).
227. Nemec, A. *et al.* *Acinetobacter beijerinckii* sp. nov. and *Acinetobacter gyllenbergii* sp. nov., haemolytic organisms isolated from humans. *Int. J. Syst. Evol. Microbiol.* **59**, 118–124 (2009).
228. Kim, H. J. *et al.* Septic shock due to unusual pathogens, *Comamonas testosteroni* and *Acinetobacter guillouiae* in an immune competent patient. *Acute Crit. Care* **30**, 180–183 (2015).
229. Machado, R. A. R. *et al.* *Acinetobacter nematophilus* sp. nov., *Alcaligenes nematophilus* sp. nov., *Enterobacter nematophilus* sp. nov., and *Kaistia nematophila* sp. nov., isolated from soil-norne nematodes and proposal for the elevation of *Alcaligenes faecalis* subsp. *faecalis*, *Alcaligenes faecalis* subsp. *parafoecalis*, and *Alcaligenes faecalis* subsp. *phenolicus* to the species level. *Taxonomy* **3**, 148–168 (2023).
230. Carvalho, A., Casquete, R., Silva, J. & Teixeira, P. Prevalence and antimicrobial susceptibility of *Acinetobacter* spp. isolated from meat. *Int. J. Food Microbiol.* **243**, 58–63 (2017).
231. Askari, N., Momtaz, H. & Tajbakhsh, E. Prevalence and phenotypic pattern of antibiotic resistance of *Acinetobacter baumannii* isolated from different types of raw meat samples in Isfahan, Iran. *Vet. Med. Sci.* **6**, 147–153 (2020).
232. Boyd, D. A. *et al.* Results from the Canadian Nosocomial Infection Surveillance Program for detection of carbapenemase-producing *Acinetobacter* spp. in Canadian hospitals, 2010–16. *J. Antimicrob. Chemother.* **74**, 315–320 (2019).
233. Tomaschek, F., Higgins, P. G., Stefanik, D., Wisplinghoff, H. & Seifert, H. Head-to-head comparison of two multi-locus sequence typing (MLST) schemes for characterization of *Acinetobacter baumannii* outbreak and sporadic isolates. *PLOS ONE* **11**, e0153014 (2016).

234. Gaiarsa, S. *et al.* Comparative analysis of the two *Acinetobacter baumannii* multilocus sequence typing (MLST) schemes. *Front. Microbiol.* **10**, 930 (2019).
235. Fitzpatrick, M. A., Ozer, E. A. & Hauser, A. R. Utility of whole-genome sequencing in characterizing *Acinetobacter* epidemiology and analyzing hospital outbreaks. *J. Clin. Microbiol.* **54**, 593–612 (2016).
236. Mangioni, D. *et al.* Genomic characterization of carbapenem-resistant *Acinetobacter baumannii* (CRAB) in mechanically ventilated COVID-19 patients and impact of infection control measures on reducing CRAB circulation during the second wave of the SARS-CoV-2 pandemic in Milan, Italy. *Microbiol. Spectr.* **11**, e00209-23 (2023).
237. Agersø, Y. & Guardabassi, L. Identification of Tet 39, a novel class of tetracycline resistance determinant in *Acinetobacter* spp. of environmental and clinical origin. *J. Antimicrob. Chemother.* **55**, 566–569 (2005).
238. Tamang, M. D. *et al.* Short communication: Genetic characterization of antimicrobial resistance in *Acinetobacter* isolates recovered from bulk tank milk. *J. Dairy Sci.* **97**, 704–709 (2014).
239. Hua, X. *et al.* Complete genome sequence of *Acinetobacter baumannii* A1296 (ST1469) with a small plasmid harbouring the *tet(39)* tetracycline resistance gene. *J. Glob. Antimicrob. Resist.* **11**, 105–107 (2017).
240. Muzahid, N. H. *et al.* Genomic and phenotypic characterization of *Acinetobacter colistiniresistens* isolated from the feces of a healthy member of the community. *Sci. Rep.* **13**, 12596 (2023).
241. Edvardsen, P. K. T. *Acinetobacter baumannii* in Norwegian chicken farms: A One Health perspective. (University of Oslo, 2020).
242. Ang, G. Y., Yu, C. Y., Cheong, Y. M., Yin, W.-F. & Chan, K.-G. Emergence of ST119 *Acinetobacter pittii* co-harboring NDM-1 and OXA-58 in Malaysia. *Int. J. Antimicrob. Agents* **47**, 168–169 (2016).
243. Kamolvit, W., Derrington, P., Paterson, D. L. & Sidjabat, H. E. A case of IMP-4-, OXA-421-, OXA-96-, and CARB-2-producing *Acinetobacter pittii* sequence type 119 in Australia. *J. Clin. Microbiol.* **53**, 727–730 (2015).
244. Li, P. & Fan, X. Pertussis-like syndrome caused by *Acinetobacter pittii* ST119. *Indian J. Pediatr.* **90**, 1051–1051 (2023).
245. Yamamoto, M. *et al.* Regional dissemination of *Acinetobacter* species harbouring metallo- β -lactamase genes in Japan. *Clin. Microbiol. Infect.* **19**, 729–736 (2013).
246. Scales, B. S., Dickson, R. P., Lipuma, J. J. & Huffnagle, G. B. Microbiology, genomics, and clinical significance of the *Pseudomonas fluorescens* species complex, an unappreciated colonizer of humans. *Clin. Microbiol. Rev.* **27**, 927–948 (2014).
247. Wong, V., Levi, K., Baddal, B., Turton, J. & Boswell, T. C. Spread of *Pseudomonas fluorescens* due to contaminated drinking water in a bone marrow transplant unit. *J. Clin. Microbiol.* **49**, 2093–2096 (2011).

248. Clark, L. L., Dajcs, J. J., McLean, C. H., Bartell, J. G. & Stroman, D. W. *Pseudomonas otitidis* sp. nov., isolated from patients with otic infections. *Int. J. Syst. Evol. Microbiol.* **56**, 709–714 (2006).
249. Mori, T. *et al.* *Pseudomonas otitidis* bacteremia in an immunocompromised patient with cellulitis: Case report and literature review. *BMC Infect. Dis.* **23**, 883 (2023).
250. Caixinha, A. L., Valsamidis, A. N., Chen, M. & Lindberg, M. *Pseudomonas otitidis* bacteraemia in a patient with COPD and recurrent pneumonia: Case report and literature review. *BMC Infect. Dis.* **21**, 868 (2021).
251. Girard, L. *et al.* The ever-expanding pseudomonas genus: Description of 43 new species and partition of the *Pseudomonas putida* group. *Microorganisms* **9**, 1766 (2021).
252. Mukherjee, K., Mandal, S., Mukhopadhyay, B., Mandal, N. C. & Sil, A. K. Bioactive compound from *Pseudomonas synxantha* inhibits the growth of *Mycobacteria*. *Microbiol. Res.* **169**, 794–802 (2014).
253. Lick, S. *et al.* *Pseudomonas paracarnis* sp. nov., isolated from refrigerated beef. *Int. J. Syst. Evol. Microbiol.* **71**, 004652 (2021).
254. Zhu, Y. *et al.* Microbial diversity of meat products under spoilage and its controlling approaches. *Front. Nutr.* **9**, 1078201 (2022).
255. Heir, E., Moen, B., Åsli, A. W., Sunde, M. & Langsrud, S. Antibiotic resistance and phylogeny of *Pseudomonas* spp. isolated over three decades from chicken meat in the Norwegian food chain. *Microorganisms* **9**, 207 (2021).
256. Wong, M. H., Chan, E. W. chi & Chen, S. Isolation of carbapenem-resistant *Pseudomonas* spp. from food. *J. Glob. Antimicrob. Resist.* **3**, 109–114 (2015).
257. Evans, B. A. & Amyes, S. G. B. OXA β -Lactamases. *Clin. Microbiol. Rev.* **27**, 241–263 (2014).
258. Poirel, L. *et al.* PFM-like enzymes are a novel family of subclass B2 metallo- β -lactamases from *Pseudomonas synxantha* belonging to the *Pseudomonas fluorescens* complex. *Antimicrob. Agents Chemother.* **64**, e01700-19 (2020).
259. Borgianni, L. *et al.* Biochemical characterization of the POM-1 metallo- β -lactamase from *Pseudomonas otitidis*. *Antimicrob. Agents Chemother.* **59**, 1755–1758 (2015).
260. Rodríguez-Martínez, J.-M., Poirel, L. & Nordmann, P. Extended-spectrum cephalosporinases in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **53**, 1766–1771 (2009).
261. Ramos, M. S. *et al.* Patterns of antimicrobial resistance and metal tolerance in environmental *Pseudomonas aeruginosa* isolates and the genomic characterization of the rare O6/ST900 clone. *Environ. Monit. Assess.* **195**, 713 (2023).
262. Wu, W., Huang, J. & Xu, Z. Antibiotic influx and efflux in *Pseudomonas aeruginosa*: Regulation and therapeutic implications. *Microb. Biotechnol.* **17**, e14487 (2024).
263. Gray, H. K. *et al.* Nosocomial infections by diverse carbapenemase-producing *Aeromonas hydrophila* associated with combination of plumbing issues and heat waves. *Am. J. Infect. Control* **52**, 337–343 (2024).

264. Sekizuka, T. *et al.* Potential KPC-2 carbapenemase reservoir of environmental *Aeromonas hydrophila* and *Aeromonas caviae* isolates from the effluent of an urban wastewater treatment plant in Japan. *Environ. Microbiol. Rep.* **11**, 589–597 (2019).
265. Xu, T. *et al.* First report of multidrug-resistant carbapenemase-producing *Aeromonas caviae* co-harboring *mcr-3.43* and *mcr-7.2*. *Microbiol. Spectr.* **12**, e03685-23. (2024).
266. Pessoa, R. B. G., Oliveira, W. F. de, Correia, M. T. dos S., Fontes, A. & Coelho, L. C. B. *Aeromonas* and human health disorders: Clinical approaches. *Front. Microbiol.* **13**, 868890 (2022).
267. Fernández-Bravo, A. & Figueras, M. J. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. *Microorganisms* **8**, 129 (2020).
268. Sakurai, A. *et al.* Clinical features, genome epidemiology, and antimicrobial resistance profiles of *Aeromonas* spp. causing human infections: A multicenter prospective cohort study. *Open Forum Infect. Dis.* **10**, ofad587 (2023).
269. Janda, J. M. & Abbott, S. L. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev.* **23**, 35–73 (2010).
270. Katz, M. J., Parrish, N. M., Belani, A. & Shah, M. Recurrent *Aeromonas* bacteremia due to contaminated well water. *Open Forum Infect. Dis.* **2**, ofv142 (2015).
271. Carusi, J., Kabuki, D. Y., de Seixas Pereira, P. M. & Cabral, L. *Aeromonas* spp. in drinking water and food: Occurrence, virulence potential and antimicrobial resistance. *Food Res. Int.* **175**, 113710 (2024).
272. Wu, Y. *et al.* *Aeromonas* spp. from hospital sewage act as a reservoir of genes resistant to last-line antibiotics. *Drug Resist. Updat.* **67**, 100925 (2023).
273. 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).
274. Didugu, H. *et al.* A study on the prevalence of *Aeromonas* spp. and its enterotoxin genes in samples of well water, tap water, and bottled water. *Vet. World* **8**, 1237–1242 (2015).
275. Kivanc, M., Yilmaz, M. & Demir, F. The occurrence of *Aeromonas* in drinking water, tap water and the Porsuk River. *Braz. J. Microbiol.* **42**, 126–131 (2011).
276. Beaz-Hidalgo, R. & Figueras, M. J. *Aeromonas* spp. whole genomes and virulence factors implicated in fish disease. *J. Fish Dis.* **36**, 371–388 (2013).
277. Abdelrahman, H. A. *et al.* Epidemiology and economic impact of disease-related losses on commercial catfish farms: A seven-year case study from Alabama, USA. *Aquaculture* **566**, 739206 (2023).
278. Rao, M., Teixeira, J. S., Flint, A. & Tamber, S. Hazard characterization of antibiotic-resistant *Aeromonas* spp. isolated from mussel and oyster shellstock available for retail purchase in Canada. *J. Food Prot.* **87**, 100374 (2024).
279. Alhazmi, M. I. Isolation of *Aeromonas* spp. from food products: Emerging *Aeromonas* infections and their significance in public health. *J. AOAC Int.* **98**, 927–929 (2015).

280. Yücel, N. & Balci, Ş. Prevalence of *Listeria*, *Aeromonas*, and *Vibrio* species in fish used for human consumption in Turkey. *J. Food Prot.* **73**, 380–384 (2010).
281. Singh, U. Isolation and identification of *Aeromonas* spp. from ground meats in Eastern Canada. *J. Food Prot.* **60**, 125–130 (1997).
282. Callister, S. M. & Agger, W. A. Enumeration and characterization of *Aeromonas hydrophila* and *Aeromonas caviae* isolated from grocery store produce. *Appl. Environ. Microbiol.* **53**, 249–253 (1987).
283. McMahon, M. A. S. & Wilson, I. G. The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Int. J. Food Microbiol.* **70**, 155–162 (2001).
284. Poole, T. L. *et al.* Whole-genome sequence of *Aeromonas* spp. isolated from a dairy farm in central Texas. *Microbiol. Res.* **14**, 161–176 (2023).
285. Gray, S. J. *Aeromonas hydrophila* in livestock: Incidence, biochemical characteristics and antibiotic susceptibility. *J. Hyg. (Lond.)* **92**, 365–375 (1984).
286. Fontes, M. C., Saavedra, M. J., Martins, C. & Martínez-Murcia, A. J. Phylogenetic identification of *Aeromonas* from pigs slaughtered for consumption in slaughterhouses at the North of Portugal. *Int. J. Food Microbiol.* **146**, 118–122 (2011).
287. Piotrowska, M. & Popowska, M. Insight into the mobilome of *Aeromonas* strains. *Front. Microbiol.* **6**, 494 (2015).
288. McDonald, N. L., Wareham, D. W. & Bean, D. C. *Aeromonas* and *mcr-3*: A critical juncture for transferable polymyxin resistance in Gram-negative bacteria. *Pathogens* **13**, 921 (2024).
289. Dubey, S. *et al.* *Aeromonas* species isolated from aquatic organisms, insects, chicken, and humans in India show similar antimicrobial resistance profiles. *Front. Microbiol.* **13**, 1008870 (2022).
290. Ling, Z. *et al.* Chromosome-mediated *mcr-3* variants in *Aeromonas veronii* from chicken meat. *Antimicrob. Agents Chemother.* **61**, e01272-17 (2017).
291. Elbehiry, A. *et al.* Proteomic characterization and discrimination of *Aeromonas* species recovered from meat and water samples with a spotlight on the antimicrobial resistance of *Aeromonas hydrophila*. *MicrobiologyOpen* **8**, e782 (2019).
292. Ibrahim, A. & Mac Rae, I. C. Incidence of *Aeromonas* and *Listeria* spp. in red meat and milk samples in Brisbane, Australia. *Int. J. Food Microbiol.* **12**, 263–269 (1991).
293. Shen, Y. *et al.* Prevalence and genetic analysis of *mcr-3*-positive *Aeromonas* species from humans, retail meat, and environmental water samples. *Antimicrob. Agents Chemother.* **62**, e00404-18 (2018).
294. Chen, P. L., Ko, W. C. & Wu, C. J. Complexity of β -lactamases among clinical *Aeromonas* isolates and its clinical implications. *J. Microbiol. Immunol. Infect.* **45**, 398–403 (2012).
295. Niumsup, P. *et al.* Genetic linkage of the penicillinase gene, *amp*, and *blrAB*, encoding the regulator of β -lactamase expression in *Aeromonas* spp. *J. Antimicrob. Chemother.* **51**, 1351–1358 (2003).

296. Bertran, X. *et al.* Taxonomic identification of different species of the genus *Aeromonas* by whole-genome sequencing and use of their species-specific β -lactamases as phylogenetic markers. *Antibiotics* **10**, 354 (2021).
297. Stoesser, N. *et al.* Genomic epidemiology of complex, multispecies, plasmid-borne *bla*_{KPC} carbapenemase in Enterobacterales in the United Kingdom from 2009 to 2014. *Antimicrob. Agents Chemother.* **64**, e02244-19 (2020).
298. Segatore, B., Massidda, O., Satra, G., Setacci, D. & Amicosantel, G. High specificity of *cphA*-encoded metallo- β -lactamase from *Aeromonas hydrophila* AE036 for carbapenems and its contribution to β -lactam resistance. *Antimicrob. Agents Chemother.* **37**, 1324–1328 (1993).
299. Sinclair, H. A. *et al.* Genotypic and phenotypic identification of *Aeromonas* species and CphA-mediated carbapenem resistance in Queensland, Australia. *Diagn. Microbiol. Infect. Dis.* **85**, 98–101 (2016).
300. Hilt, E. E. *et al.* Carbapenem resistant *Aeromonas hydrophila* Carrying *bla*_{cphA7} isolated from two solid organ transplant patients. *Front. Cell. Infect. Microbiol.* **10**, 563482 (2020).
301. Morinaga, Y. *et al.* Identification error of *Aeromonas aquariorum*: A causative agent of septicemia. *Diagn. Microbiol. Infect. Dis.* **76**, 106–109 (2013).
302. Murata, M. *et al.* The rapid induction of carbapenem-resistance in an *Aeromonas dhakensis* blood isolate. *Jpn. J. Infect. Dis.* **69**, 439–441 (2016).
303. Salehi, M. R. *et al.* Endocarditis with *Aeromonas salmonicida*. *IDCases* **18**, e00625 (2019).
304. Yang, X. *et al.* *Aeromonas salmonicida* peritonitis after eating fish in a patient undergoing CAPD. *Perit. Dial. Int.* **28**, 316–317 (2008).
305. Moore, C. A., Khalid, M. F., Patel, P. D. & Goldstein, J. S. *Aeromonas salmonicida* bacteremia associated with chronic well water consumption in a patient with diabetes. *J. Glob. Infect. Dis.* **9**, 82–84 (2017).
306. Li, F. *et al.* Distribution, virulence-associated genes and antimicrobial resistance of *Aeromonas* isolates from diarrheal patients and water, China. *J. Infect.* **70**, 600–608 (2015).
307. Public Health Agency of Canada. Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) 2022 executive summary: Key and integrated findings. <https://www.canada.ca/en/public-health/services/publications/drugs-health-products/canadian-integrated-program-antimicrobial-resistance-surveillance-2022-executive-summary.html> (2024).
308. Igbiosa, I. H., Igumbor, E. U., Aghdasi, F., Tom, M. & Okoh, A. I. Emerging *Aeromonas* species infections and their significance in public health. *Sci. World J.* **2012**, 625023 (2012).
309. Wang, Y., Hou, N., Rasooly, R., Gu, Y. & He, X. Prevalence and genetic analysis of chromosomal *mcr-3/7* in *Aeromonas* from U.S. animal-derived samples. *Front. Microbiol.* **12**, 667406 (2021).
310. Narvaez-Bravo, C., Taboada, E. N., Mutschall, S. K. & Aslam, M. Epidemiology of antimicrobial resistant *Campylobacter* spp. isolated from retail meats in Canada. *Int. J. Food Microbiol.* **253**, 43–47 (2017).

311. Public Health Agency of Canada. FoodNet Canada 2019 integrated findings report. <https://www.canada.ca/en/public-health/services/publications/food-nutrition/foodnet-canada-2019-integrated-findings-report.html> (2024).
312. Czepiel, J. *et al.* *Clostridium difficile* infection: Review. *Eur. J. Clin. Microbiol. Infect. Dis.* **38**, 1211–1221 (2019).
313. Wilson, D. *et al.* Farm-to-fork investigation of an outbreak of Shiga toxin-producing *Escherichia coli* O157. *Microb. Genomics* **4**, e000160 (2018).
314. Livermore, D. M. *et al.* CTX-M: Changing the face of ESBLs in Europe. *J. Antimicrob. Chemother.* **59**, 165–174 (2007).
315. Rodriguez-Palacios, A. & LeJeune, J. T. Moist-heat resistance, spore aging, and superdormancy in *Clostridium difficile*. *Appl. Environ. Microbiol.* **77**, 3085–3091 (2011).
316. Cieza, M. Y. R., Bonsaglia, E. C. R., Rall, V. L. M., Santos, M. V. dos & Silva, N. C. C. Staphylococcal enterotoxins: Description and importance in food. *Pathogens* **13**, 676 (2024).
317. Blagojevic, C. *et al.* Long-term risk of infection among patients colonized with antimicrobial-resistant pathogens: A population-wide cohort study. *Open Forum Infect. Dis.* **11**, ofae712 (2024).
318. Rahi, P. & Vaishampayan, P. Editorial: MALDI-TOF MS application in microbial ecology studies. *Front. Microbiol.* **10**, 02954 (2020).
319. Richter, M. & Rosselló-Móra, R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 19126–19131 (2009).
320. Cooper, A. L., Wong, A., Tamber, S., Blais, B. W. & Carrillo, C. D. Analysis of antimicrobial resistance in bacterial pathogens recovered from food and human sources: Insights from 639,087 bacterial whole-genome sequences in the NCBI Pathogen Detection Database. *Microorganisms* **12**, 709 (2024).
321. Infection Prevention and Control Canada. CNISP Protocols & Publications. <https://ipac-canada.org/resource-centre/infection-control-resources/cnisp-protocols-publications/> (2025).

Appendix A: List of retail meat rinsates used in this study

Table A1. Retail meat rinsate number and associated retail meat data.

Rinsate number	Date of collection	Commodity	Product description	Country of origin	Region of purchase
1	2016-01-04	Pork	Loin center chops	Unknown	Central
2	2016-01-11	Pork	Loin center chops	Unknown	Central
3	2016-01-11	Pork	Chops	Canada	West
4	2016-01-18	Pork	Loin center chops	Canada	Central
5	2016-01-18	Pork	Chops	Unknown	West
6	2016-01-25	Pork	Loin center chops	Unknown	Central
7	2016-01-31	Pork	Shoulder	Canada	West
8	2016-02-01	Pork	Shoulder	Unknown	West
9	2016-02-01	Pork	Chops	Unknown	West
10	2016-02-01	Pork	Chops	Canada	West
11	2016-02-15	Pork	Loin center chops	Unknown	Central
12	2016-02-15	Pork	Loin center chops	Canada	West
13	2016-02-22	Pork	Loin center chops	Unknown	Central
14	2016-02-29	Pork	Loin center chops	Canada	Central
15	2016-02-29	Pork	Chops	Canada	West
16	2016-03-01	Pork	Chops	Canada	West
17	2016-03-07	Pork	Chops	Canada	West
18	2016-03-14	Pork	Cubes	Canada	West
19	2016-03-14	Pork	Ground	Canada	West
20	2016-03-14	Pork	Chops	Canada	West
21	2016-04-11	Pork	Chops	Unknown	Central
22	2016-04-12	Pork	Chops	Canada	West
23	2016-04-12	Pork	Shoulder	Canada	West
24	2016-04-12	Pork	Shoulder	Canada	West
25	2016-04-19	Pork	Sirloin chops	Canada	Central
26	2016-04-19	Pork	Rib chops	Canada	Central
27	2016-04-25	Pork	Loin center chops	Unknown	Central
28	2016-04-25	Pork	Shoulder chops	Canada	West
29	2016-04-25	Pork	Loin center chops	Canada	West
30	2016-04-25	Pork	Loin center chops	Unknown	West
31	2016-04-26	Pork	Loin center chops	Canada	West
32	2016-04-26	Pork	Ground	Canada	West
33	2016-05-02	Pork	Chops	Unknown	Central
34	2016-05-09	Pork	Loin center chops	Unknown	Central
35	2016-05-09	Pork	Loin center chops	Canada	West
36	2016-05-09	Pork	Shoulder	Canada	West
37	2016-05-16	Pork	Chops	Unknown	Central
38	2016-05-30	Pork	Loin center chops	Unknown	Central
39	2016-05-30	Pork	Rib chops	Canada	West
40	2016-06-06	Pork	Chops	Unknown	Central
41	2016-06-06	Pork	Shoulder	Canada	Central
42	2016-06-06	Pork	Rib chops	Unknown	Central
43	2016-06-13	Pork	Loin center chops	Unknown	Central
44	2016-06-13	Pork	Ground	Unknown	Central
45	2016-06-13	Pork	Loin center chops	Canada	Central
46	2016-06-13	Pork	Shoulder chops	Unknown	Central
47	2016-06-13	Pork	Tenderloin	Canada	West

48	2016-06-13	Pork	Tenderloin	Canada	West
49	2016-06-13	Pork	Tenderloin	Canada	West
50	2016-07-04	Pork	Shoulder chops	Canada	Central
51	2016-07-04	Pork	Chops	Canada	West
52	2016-07-11	Pork	Loin center chops	Canada	Central
53	2016-07-11	Pork	Sirloin chops	Unknown	Central
54	2016-07-11	Pork	Loin center chops	Unknown	Central
55	2016-07-11	Pork	Chops	Canada	West
56	2016-07-18	Pork	Chops	Unknown	Central
57	2016-07-18	Pork	Loin center chops	Unknown	Central
58	2016-07-18	Pork	Shoulder chops	Unknown	Central
59	2016-07-25	Pork	Loin center chops	Unknown	Central
60	2016-07-25	Pork	Rib chops	Unknown	Central
61	2016-07-25	Pork	Chops	Canada	West
62	2016-07-25	Pork	Tenderloin	Canada	West
63	2016-07-25	Pork	Chops	Canada	West
64	2016-08-01	Pork	Loin center chops	Unknown	Central
65	2016-08-06	Pork	Loin center chops	Canada	Central
66	2016-08-07	Pork	Chops	Unknown	Central
67	2016-08-08	Pork	Rib chops	Unknown	Central
68	2016-08-08	Pork	Loin center chops	Canada	West
69	2016-08-08	Pork	Chops	Canada	West
70	2016-08-15	Pork	Shoulder	Canada	Central
71	2016-08-15	Pork	Loin center chops	Canada	Central
72	2016-08-22	Pork	Loin center chops	Canada	Central
73	2016-08-22	Pork	Chops	Canada	West
74	2016-08-29	Pork	Loin center chops	Canada	Central
75	2016-08-28	Pork	Loin center chops	Unknown	Central
76	2016-08-29	Pork	Shoulder	Canada	West
77	2016-08-29	Pork	Shoulder	Unknown	West
78	2016-09-12	Pork	Chops	Canada	Central
79	2016-09-12	Pork	Chops	Canada	West
80	2016-09-12	Pork	Chops	Canada	West
81	2016-09-19	Pork	Loin center chops	Unknown	Central
82	2016-09-19	Pork	Sirloin chops	Unknown	Central
83	2016-09-19	Pork	Tenderloin	Unknown	Central
84	2016-09-19	Pork	Chops	Unknown	West
85	2016-09-26	Pork	Loin center chops	Unknown	Central
86	2016-09-26	Pork	Loin center chops	Canada	Central
87	2016-09-26	Pork	Shoulder	Canada	Central
88	2016-10-03	Pork	Loin center chops	Unknown	Central
89	2016-10-03	Pork	Chops	Canada	West
90	2016-10-03	Pork	Tenderloin	Canada	West
91	2016-10-17	Pork	Loin center chops	Canada	Central
92	2016-10-16	Pork	Chops	Canada	Central
93	2016-10-17	Pork	Chops	Unknown	Central
94	2016-10-24	Pork	Chops	Unknown	Central
95	2016-10-24	Pork	Chops	Unknown	Central
96	2016-10-24	Pork	Chops	Canada	West
97	2016-10-31	Pork	Chops	Canada	West
98	2016-11-14	Pork	Ground	Canada	Central
99	2016-11-14	Pork	Loin center chops	Unknown	Central
100	2016-11-12	Pork	Chops	Canada	Central

101	2016-11-13	Pork	Chops	Canada	Central
102	2016-11-14	Pork	Chops	Unknown	West
103	2016-11-21	Pork	Shoulder	Unknown	West
104	2016-11-28	Pork	Chops	Unknown	Central
105	2016-11-28	Pork	Shoulder chops	Canada	West
106	2016-11-28	Pork	Loin center chops	Canada	West
107	2016-11-28	Pork	Roast	Canada	West
108	2016-12-05	Pork	Loin center chops	Canada	Central
109	2016-12-05	Pork	Chops	Canada	Central
110	2016-12-05	Pork	Chops	Unknown	West
111	2016-12-12	Pork	Chops	Canada	Central
112	2016-12-12	Pork	Chops	Canada	West
113	2017-01-09	Pork	Rib chops	Unknown	Central
114	2017-01-09	Beef	Ground (lean)	Unknown	Central
115	2017-01-09	Pork	Loin center chops	Unknown	Central
116	2017-01-09	Beef	Ground (extra-lean)	Unknown	West
117	2017-01-09	Pork	Chops	Canada	West
118	2017-01-09	Pork	Chops	Canada	West
119	2017-01-09	Pork	Chops	Canada	Central
120	2017-01-09	Beef	Ground (lean)	Canada	Central
121	2017-01-09	Pork	Rib chops	Canada	West
122	2017-01-09	Beef	Ground (extra-lean)	Canada	West
123	2017-01-16	Beef	Ground (lean)	Unknown	Central
124	2017-01-16	Pork	Chops	Unknown	Central
125	2017-01-16	Beef	Ground (lean)	Canada	West
126	2017-01-16	Pork	Loin center chops	Canada	West
127	2016-01-17	Beef	Ground (lean)	Canada	West
128	2016-01-17	Pork	Chops	Canada	West
129	2017-01-16	Pork	Loin center chops	Canada	Central
130	2017-01-16	Pork	Tenderloin	Canada	Central
131	2017-01-16	Beef	Ground (extra-lean)	Canada	Central
132	2017-01-23	Beef	Ground (medium)	Unknown	Central
133	2017-01-23	Pork	Loin center chops	Canada	Central
134	2017-01-30	Beef	Ground (medium)	Unknown	Central
135	2017-01-30	Pork	Loin center chops	Unknown	Central
136	2017-01-30	Beef	Ground (extra-lean)	Canada	West
137	2017-01-30	Pork	Loin center chops	Canada	West
138	2017-02-06	Pork	Rib chops	Unknown	Central
139	2017-02-06	Beef	Ground (lean)	Unknown	Central
140	2017-02-06	Beef	Ground (extra-lean)	Canada	Central
141	2017-02-06	Pork	Chops	Canada	Central
142	2017-02-06	Pork	Chops	Canada	West
143	2017-02-06	Beef	Ground (regular)	Canada	West
144	2017-02-06	Pork	Chops	Canada	West
145	2017-02-14	Beef	Ground (lean)	Canada	West
146	2017-02-14	Pork	Chops	United States & Canada	West
147	2017-02-20	Pork	Loin center chops	Unknown	Central

148	2017-02-20	Beef	Ground (lean)	Unknown	Central
149	2017-02-19	Beef	Ground (extra-lean)	Canada	Central
150	2017-02-19	Pork	Rib chops	Canada	Central
151	2017-02-19	Pork	Shoulder chops	Canada	Central
152	2017-02-20	Pork	Chops	Canada	West
153	2017-02-20	Beef	Ground (lean)	Canada	West
154	2017-02-20	Pork	Chops	Unknown	West
155	2017-12-11	Pork	Chops	Unknown	Central
156	2017-12-11	Beef	Ground (extra-lean)	Unknown	West
157	2017-12-11	Pork	Chops	Canada	West
158	2017-12-11	Pork	Chops	Canada	West
159	2018-01-08	Pork	Chops	Unknown	Central
160	2018-01-08	Beef	Ground (medium)	Unknown	Central
161	2018-01-08	Pork	Loin center chops	Unknown	Central
162	2018-01-08	Beef	Ground (sirloin)	Canada	West
163	2018-01-08	Pork	Tenderloin	Canada	West
164	2018-01-08	Pork	Loin center chops	Canada	West
165	2018-01-08	Beef	Ground (lean)	Canada	West
166	2018-01-08	Pork	Rib chops	Canada	West
167	2018-01-08	Pork	Rib chops	Canada	West
168	2018-01-15	Beef	Ground (extra-lean)	Unknown	Central
169	2018-01-15	Pork	Loin center chops	Unknown	Central
170	2018-01-22	Beef	Ground (medium)	Unknown	Central
171	2018-01-22	Pork	Loin center chops	Unknown	Central
172	2018-01-22	Beef	Ground (lean)	Unknown	Central
173	2018-01-22	Pork	Chops	Canada	West
174	2018-01-23	Beef	Ground (lean)	Canada	West
175	2018-01-23	Pork	Loin center chops	Canada	West
176	2018-01-29	Beef	Ground (lean)	Unknown	Central
177	2018-01-29	Beef	Ground (lean)	Unknown	Central
178	2018-01-29	Pork	Loin center chops	Unknown	Central
179	2018-02-05	Beef	Ground (extra-lean)	Canada	West
180	2018-02-05	Pork	Chops	Canada	West
181	2018-02-05	Pork	Rib chops	Canada	West
182	2018-02-05	Pork	Chops	Canada	West
183	2018-02-12	Beef	Ground (extra-lean)	Unknown	Central
184	2018-02-12	Pork	Chops	Canada	Central
185	2018-02-12	Beef	Ground (lean)	Unknown	Central
186	2018-02-12	Pork	Breast bones	Canada	West
187	2018-02-12	Beef	Ground (extra-lean)	Unknown	West
188	2018-02-12	Pork	Chops	Canada	West
189	2018-02-19	Pork	Rib chops	Canada	Central
190	2018-02-19	Beef	Ground (medium)	Unknown	Central
191	2018-02-19	Pork	Chops	Unknown	Central
192	2018-02-19	Beef	Ground (lean)	Canada	West
193	2018-02-19	Pork	Chops	Canada	West
194	2018-02-19	Beef	Ground (lean)	Canada	West

195	2018-02-26	Beef	Ground (lean)	Unknown	Central
196	2018-02-26	Pork	Loin center chops	Canada	Central
197	2018-03-05	Beef	Ground (lean)	Canada	West
198	2018-03-05	Pork	Chops	Canada	West
199	2018-03-12	Beef	Ground (medium)	Unknown	Central
200	2018-03-12	Pork	Cubes	Unknown	Central
201	2018-03-12	Pork	Rib chops	Unknown	Central
202	2018-03-12	Beef	Ground (lean)	Unknown	Central
203	2018-03-12	Pork	Chops	Unknown	Central
204	2018-03-12	Pork	Chops	Canada	West
205	2018-03-12	Beef	Ground (extra-lean)	Unknown	West
206	2018-03-19	Pork	Loin center chops	Unknown	Central
207	2018-03-19	Beef	Ground (extra-lean)	Unknown	Central
208	2018-03-19	Beef	Ground (regular)	Canada	West
209	2018-03-19	Pork	Chops	Canada	West
210	2018-03-19	Beef	Stew chunks	Canada	West
211	2018-04-16	Pork	Loin center chops	Unknown	Central
212	2018-04-16	Beef	Ground (lean)	Unknown	Central
213	2018-04-16	Beef	Ground (regular)	Canada	West
214	2018-04-16	Pork	Chops	Canada	West
215	2018-04-16	Pork	Rib chops	Canada	West
216	2018-04-16	Beef	Ground	Canada	West
217	2018-04-23	Pork	Loin center chops	Unknown	Central
218	2018-04-23	Beef	Ground (lean)	Unknown	Central
219	2018-04-23	Pork	Rib chops	Unknown	Central
220	2018-04-30	Beef	Ground (lean)	Unknown	Central
221	2018-04-30	Pork	Chops	Unknown	Central
222	2018-04-30	Beef	Ground	Unknown	Central
223	2018-04-30	Beef	Ground (lean)	Canada	West
224	2018-04-30	Pork	Chops	Canada	West
225	2018-05-07	Beef	Ground (lean)	Unknown	Central
226	2018-05-07	Pork	Loin center chops	Unknown	Central
227	2018-05-07	Beef	Ground (medium)	Unknown	Central
228	2018-05-07	Pork	Rib chops	Unknown	Central
229	2018-05-14	Beef	Ground (medium)	Unknown	Central
230	2018-05-14	Pork	Loin center chops	Unknown	Central
231	2018-05-14	Pork	Rib chops	Unknown	Central
232	2018-05-14	Beef	Ground (extra-lean)	Unknown	Central
233	2018-05-14	Pork	Chops	Canada	West
234	2018-05-14	Beef	Ground (extra-lean)	Canada	West
235	2018-05-28	Pork	Loin center chops	Unknown	Central
236	2018-05-28	Beef	Ground (medium)	Unknown	Central
237	2018-05-29	Beef	Ground (extra-lean)	Canada	West
238	2018-05-29	Beef	Stew chunks	Canada	West
239	2018-05-29	Pork	Shoulder	Canada	West
240	2018-05-29	Pork	Chops	Canada	West
241	2018-06-04	Beef	Ground (medium)	Unknown	Central
242	2018-06-04	Pork	Loin center chops	Unknown	Central

243	2018-06-04	Beef	Ground (lean)	Unknown	Central
244	2018-06-11	Pork	Chops	Unknown	Central
245	2018-06-11	Beef	Ground (extra-lean)	Unknown	Central
246	2018-06-11	Pork	Tenderloin	Canada	Central
247	2018-06-10	Pork	Chops	Canada	West
248	2018-06-11	Beef	Ground (regular)	Canada	West
249	2018-06-11	Pork	Tenderloin	Canada	West
250	2018-06-11	Pork	Chops	Canada	West
251	2018-06-11	Pork	Shoulder	Canada	West
252	2018-06-18	Beef	Ground (extra-lean)	Unknown	Central
253	2018-06-18	Pork	Loin center chops	Unknown	Central
254	2018-06-18	Beef	Ground (lean)	Unknown	Central
255	2018-07-09	Beef	Ground (extra-lean)	Canada	West
256	2018-07-09	Pork	Chops	Canada	West
257	2018-07-16	Pork	Loin center chops	Unknown	Central
258	2018-07-16	Beef	Ground (lean)	Unknown	Central
259	2018-07-23	Pork	Rib chops	Unknown	Central
260	2018-07-23	Beef	Ground (extra-lean)	Unknown	Central
261	2018-07-23	Pork	Loin center chops	Unknown	Central
262	2018-07-23	Beef	Ground (extra-lean)	Canada	West
263	2018-07-23	Pork	Rib chops	Canada	West
264	2018-07-24	Beef	Ground (lean)	Canada	West
265	2018-07-24	Pork	Chops	Canada	West
266	2018-07-24	Pork	Tenderloin	Canada	West
267	2018-07-30	Pork	Loin center chops	Unknown	Central
268	2018-07-30	Beef	Ground (extra-lean)	Unknown	Central
269	2018-07-30	Beef	Ground (extra-lean)	Canada	West
270	2018-07-30	Pork	Chops	Canada	West
271	2018-07-30	Beef	Ground (lean)	Unknown	West
272	2018-08-13	Pork	Tenderloin	Canada	Central
273	2018-08-13	Beef	Ground (medium)	Unknown	Central
274	2018-08-13	Pork	Loin center chops	Unknown	Central
275	2018-08-13	Beef	Ground (medium)	Unknown	Central
276	2018-08-13	Beef	Ground (lean)	Unknown	West
277	2018-08-13	Beef	Ground (regular)	Unknown	West
278	2018-08-13	Pork	Chops	Unknown	West
279	2018-08-20	Beef	Ground (extra-lean)	Unknown	Central
280	2018-08-20	Pork	Loin center chops	Unknown	Central
281	2018-08-20	Beef	Ground (extra-lean)	Unknown	Central
282	2018-09-10	Beef	Ground (extra-lean)	Unknown	Central
283	2018-09-10	Pork	Loin center chops	Unknown	Central
284	2018-09-10	Pork	Rib chops	Unknown	Central
285	2018-09-17	Beef	Ground (medium)	Unknown	Central
286	2018-09-17	Pork	Loin center chops	Canada	Central

287	2018-09-24	Beef	Ground (extra-lean)	Unknown	Central
288	2018-09-24	Pork	Chops	Canada	Central
289	2018-09-24	Pork	Chops	Unknown	Central
290	2018-09-24	Beef	Ground (lean)	Unknown	Central
291	2018-10-01	Beef	Ground (extra-lean)	Unknown	Central
292	2018-10-01	Beef	Ground (medium)	Unknown	Central
293	2018-10-01	Pork	Loin center chops	Unknown	Central
294	2018-10-09	Beef	Ground (lean)	Unknown	Central
295	2018-10-09	Pork	Loin center chops	Unknown	Central
296	2018-10-09	Pork	Rib chops	Unknown	Central
297	2018-10-15	Beef	Ground (lean)	Unknown	Central
298	2018-10-15	Pork	Loin center chops	Unknown	Central
299	2018-10-22	Beef	Ground (lean)	Unknown	Central
300	2018-10-22	Beef	Ground (lean)	Unknown	Central
301	2018-10-29	Beef	Ground (lean)	Unknown	Central
302	2018-10-29	Beef	Ground (extra-lean)	Unknown	Central
303	2018-11-05	Pork	Loin center chops	Canada	Central
304	2018-11-05	Beef	Ground (lean)	Unknown	Central
305	2018-11-19	Beef	Ground (lean)	Unknown	Central
306	2018-11-19	Pork	Loin center chops	Unknown	Central
307	2018-11-26	Beef	Ground (medium)	Unknown	Central
308	2018-11-26	Beef	Ground (lean)	Unknown	Central
309	2018-11-26	Pork	Loin center chops	Unknown	Central
310	2018-11-26	Pork	Loin center chops	Unknown	Central
311	2018-12-03	Beef	Ground (medium)	Unknown	Central
312	2018-12-03	Pork	Loin center chops	Unknown	Central
313	2018-12-03	Beef	Ground (lean)	Unknown	Central
314	2018-12-10	Beef	Ground (medium)	Canada	Central
315	2018-12-10	Beef	Ground (lean)	Unknown	Central
316	2018-12-10	Pork	Loin center chops	Unknown	Central
317	2018-12-10	Beef	Ground (extra-lean)	Unknown	West
318	2018-12-10	Pork	Chops	Canada	West

Appendix B: Additional methods and methods comparison

Table B1.1. Primers for *C. difficile* PCR ribotyping.

Primer	Sequence (5'-3')	Size (bp)
16S-FAM forward primer	GTG CGG CTG GAT CAC CTC CT	20
23S reverse primer	CCC TGC ACC CTT AAT AAC TTG ACC	24

Table B1.2. *C. difficile* PCR ribotyping reaction.

Component	Volume (ul)
2X HotStar Taq Master Mix	12.5
16S-FAM forward primer (10μM)	0.25
23S reverse primer (10μM)	0.25
H ₂ O (autoclaved)	10.0
DNA	2.00

Table B1.3. *C. difficile* PCR ribotyping cycling conditions.

Cycles (n)	Temperature	Length	Step
1	95 °C	15 minutes	Initial denaturation
24	95 °C	60 seconds	Denaturation
	57 °C	60 seconds	Annealing
	72 °C	30 seconds	Extension
1	72 °C	30 minutes	Final extension
	4 °C	∞	Storage

Table B2. Comparison of methods for phenotypic carbapenemase detection.

Organism (n)	Carbapenemase gene ¹	β-lactamase gene ¹	mCIM		cimTRIS		cimTRIS II ²	
			Correctly identified	Incorrectly identified	Correctly identified	Incorrectly identified	Correctly identified	Incorrectly identified
<i>Aeromonas allosaccharophila</i> ^a (1)	cphA4	blaFOX-3, ampS	1		1			1
<i>Aeromonas caviae</i> (1)	NDM	TEM	1		1		1	
<i>Aeromonas hydrophila</i> (1)	GES	TEM	1		1			1
<i>Aeromonas hydrophila</i> (1)	GES	TEM, OXA-1	1		1		1	
<i>Aeromonas hydrophila</i> (1)	Negative	TEM		1		1	1	
<i>Aeromonas hydrophila</i> (2)	Negative		1	1		2	1	1
<i>Aeromonas sp.</i> (1)	Negative	CTX-M, TEM, OXA-1	1		1		1	
<i>Aeromonas sp.</i> (1)	Negative	TEM		1		1		1
<i>Aeromonas sp.</i> (1)	Negative			1		1	1	
<i>Aeromonas veronii</i> ³ (1)	Negative	blaCEPH-A3, ampS		1	1		1	
<i>Aeromonas veronii</i> (1)	Negative			1		1	1	
<i>Pseudomonas aeruginosa</i> (1)	AIM, DIM			1		1		1

<i>Pseudomonas aeruginosa</i> (2)	CAM-1		2		2		2	
<i>Pseudomonas aeruginosa</i> (1)	GES	CTX-M, SHV, TEM, OXA-1		1		1		1
<i>Pseudomonas aeruginosa</i> (2)	GES		1	1	2			2
<i>Pseudomonas aeruginosa</i> (1)	GES; AIM, DIM,		1		1			1
<i>Pseudomonas aeruginosa</i> (2)	IMP		2		2		2	
<i>Pseudomonas aeruginosa</i> (1)	IMP; VIM			1	1		1	
<i>Pseudomonas aeruginosa</i> (2)	KPC		2		2		2	
<i>Pseudomonas aeruginosa</i> (1)	NDM	CTX-M, TEM, OXA-1	1		1		1	
<i>Pseudomonas aeruginosa</i> (2)	NDM		2		2		2	
<i>Pseudomonas aeruginosa</i> (1)	NDM; GES		1		1		1	
<i>Pseudomonas aeruginosa</i> (1)	NDM; IMP		1		1		1	
<i>Pseudomonas aeruginosa</i> (1)	NDM; VIM	OXA1	1		1		1	
<i>Pseudomonas aeruginosa</i> (1)	NDM; VIM		1		1		1	
<i>Pseudomonas aeruginosa</i> (1)	Negative	CMY2	1		1		1	
<i>Pseudomonas aeruginosa</i> (1)	Negative	CTX-M	1		1		1	
<i>Pseudomonas aeruginosa</i> (2)	Negative	OXA-1	2		2		2	
<i>Pseudomonas aeruginosa</i> (2)	Negative	TEM	2		2		2	
<i>Pseudomonas aeruginosa</i> (2)	Negative		2		1	1	2	
<i>Pseudomonas aeruginosa</i> (1)	OXA-48		1		1		1	
<i>Pseudomonas aeruginosa</i> (2)	VIM		2		2		2	
<i>Pseudomonas putida</i> (1)	Negative	CMY2	1		1		1	
<i>Pseudomonas putida</i> (1)	Negative		1		1		1	
<i>Pseudomonas putida</i> (1)	OXA-48			1		1		1
<i>Pseudomonas putida</i> (1)	VIM	OXA-1	1		1		1	
<i>Pseudomonas putida</i> (2)	VIM		2		2		2	
<i>Pseudomonas putida</i> (1)	Negative		1		1		1	
<i>Pseudomonas sp.</i> (1)	OXA-48		1		1		1	
<i>Pseudomonas stutzeri</i> (1)	Negative		1		1		1	

¹ genes determined by PCR unless specified

² cimTRIS II modified from the published protocol¹³³ to use 10 ug meropenem disks in place of 5 ug meropenem disks

³ genes determined via whole-genome sequencing using starAMR⁴⁹

Appendix C: Summary of carbapenemase detection results

Table C1. Results of phenotypic carbapenemase testing by genus.

Genus	Positive	Negative or indeterminate	Total
<i>Aeromonas</i> ¹	407	134	541
<i>Citrobacter</i>	0	1	1
<i>Escherichia</i>	0	3	3
<i>Hafnia</i>	0	4	4
<i>Proteus</i>	0	1	1
<i>Providencia</i>	0	1	1
<i>Pseudomonas</i> ¹	18	3134	3151
<i>Raoultella</i>	0	2	2
<i>Serratia</i>	0	20	20
<i>Yersinia</i>	0	1	1
Not identifiable	0	121	121

¹Totals may reflect duplicate isolates from a given retail meat rinsate.

Appendix D: Publicly available MRSA used in genomic comparisons

Table D1. List of publicly available MRSA t899/ST9 used for genomic comparison.

NCBI Accession	Country of origin	Isolate source	Year of isolation
GCF_002247005.1	Taiwan	Human	2002
GCF_002247045.1	Taiwan	Human	2004
GCF_002247065.1	Taiwan	Human	2012
GCF_002247105.1	Taiwan	Human	2010
GCF_002247245.1	Taiwan	Human	2002
GCF_003309535.1	China	Human	2015
GCF_003309585.1	China	Human	2015
GCF_003309595.1	China	Human	2015
GCF_003309965.1	China	Human	2015
GCF_003310805.1	China	Human	2015
GCF_003310835.1	China	Human	2015
GCF_003310865.1	China	Human	2015
GCF_003310885.1	China	Human	2015
GCF_003310905.1	China	Human	2015
GCF_003310925.1	China	Human	2015
GCF_003311395.1	China	Human	2015
GCF_003693275.1	China	Human	2010
GCF_003697285.1	China	Human	2010
GCF_015070865.1	China	Human	2017
GCF_031934985.1	China	Human	2020
SRR14224863	China	Human	NA
SRR14224864	China	Human	NA
SRR14224866	China	Human	NA

Appendix E: Read and assembly quality of sequences

Table E1. Read and assembly quality of *C. difficile* from retail meat after fastp trimming.

Isolate name	Average base coverage	Assembly N50	Contigs (n)	GC (%)
063-A-02	157.63	56613	173	28.39
169-A-04	207.31	71093	145	28.22
180-A-01	182.85	59281	169	28.18

Table E2. Trimmed read and assembly quality of MRSA from retail meat.

Isolate name	Average base coverage	Assembly N50	Contigs (n)	GC (%)
099-M-10	145.58	152524	61	32.84
232-M-02	75.46	167274	53	32.65
035-M-07	146.78	325416	28	32.69

Table E3. Trimmed read and assembly quality of all sequences in Chapters 5 and 6.

Organism	Average base coverage mean (median, min - max)	Assembly N50 median, min - max	Contigs (n) median, min-max	BUSCO median, min-max	CheckM2 median, min-max
<i>Acinetobacter</i>	65.81 (62.98, 46.19 - 127.37)	113910.5, 43718 - 239247	86.5, 40 - 293	99.7, 99.2 - 100	100, 99.99 - 100
<i>Aeromonas</i>	78.42 (74.17, 40.7 - 161.61)	123715, 31753 - 461773	100, 27 - 319	99.7, 98.1 - 100	100, 100 - 100
<i>Pseudomonas</i>	74.11 (75.48, 45.05 - 88.06)	119282, 81184 - 167723	112, 82 - 156	100, 99.7 - 100	100, 100 - 100

Appendix F: Supplementary data - Chapter 5

Table F1. Species and resistance genes detected in carbapenemase-negative *Acinetobacter* spp.

Isolate name	Species	ARG	Metal resistance
114-O-08	<i>A. nosocomialis</i>	<i>adeC, adeD, adeE, amvA, blaADC-130, cpxE</i>	<i>nreB</i>
199-O-12	<i>A. seifertii</i>	<i>adeE, amvA, blaADC-133, cpxE</i>	<i>nreB</i>
197-O-03	<i>A. nosocomialis</i>	<i>adeC, adeD, adeE, amvA, blaADC-130, cpxE</i>	<i>nreB</i>
197-O-04	<i>A. seifertii</i>	<i>adeE, amvA, blaADC-133, cpxE</i>	<i>nreB</i>
265-O-08	<i>A. seifertii</i>	<i>adeD, adeE, amvA, blaADC-335, cpxE</i>	<i>nreB</i>
251-O-10	<i>A. nosocomialis</i>	<i>abaF, adeC, adeD, adeE, amvA, blaADC-350, cpxE,</i>	<i>nreB</i>

Table F2. AMRFinderPlus result after setting a low threshold of 50% identity.

Name	Gene	Element name	Coverage of reference (%)	Identity to reference (%)	Class
083-O-01	<i>arsB</i>	arsenite efflux transporter membrane subunit ArsB	99.3	73.71	Arsenic
	<i>blaPDC</i>	PDC variant family class C beta-lactamase	94.71	58.24	Beta-lactam
	<i>cadR</i>	cadmium resistance transcriptional regulator CadR	100	76.19	Cadmium
	<i>chrR</i>	class I chromate reductase ChrR	97.31	71.82	Chromate
	<i>copA</i>	laccase-like oxidase CopA	100	72.41	Copper
	<i>copB</i>	copper-binding protein CopB	77.13	69.17	Copper
	<i>copC</i>	copper homeostasis periplasmic binding protein CopC	99.21	64	Copper
	<i>copR</i>	heavy metal response regulator transcription factor CopR	98.68	60.71	Copper
	<i>copR</i>	heavy metal response regulator transcription factor CopR	97.8	71.17	Copper
	<i>copR</i>	heavy metal response regulator transcription factor CopR	98.68	56.7	Copper
	<i>copR</i>	heavy metal response regulator transcription factor CopR	96.1	55.61	Copper
	<i>cueA</i>	copper resistance metal-translocating P1-type ATPase CueA	91.09	84.18	Copper
	<i>ssmE</i>	multidrug efflux SMR transporter SsmE	99.09	56.88	Efflux
	<i>mexE</i>	multidrug efflux RND transporter periplasmic adaptor subunit MexE	100	78.86	Efflux
	<i>mdsB</i>	multidrug efflux RND transporter permease subunit MdsB	99.24	63.3	Efflux
	<i>emhC</i>	efflux RND transporter outer membrane subunit EmhC	100	86.83	Efflux
	<i>emhB</i>	efflux RND transporter permease subunit EmhB	100	81.74	Efflux
	<i>emhA</i>	efflux RND transporter periplasmic adaptor subunit EmhA	100	80.52	Efflux
	<i>ttgR</i>	efflux transport transcriptional regulator TtgR	99.52	69.86	Efflux
	<i>emrB</i>	multidrug efflux MFS transporter permease subunit EmrB	95.64	61.78	Efflux
	<i>emrA</i>	multidrug efflux MFS transporter periplasmic adaptor subunit EmrA	94.66	58.33	Efflux
	<i>mdsB</i>	multidrug efflux RND transporter permease subunit MdsB	98.86	70.47	Efflux
	<i>fos</i>	FosA family fosfomycin resistance glutathione transferase	99.26	67.41	Fosfomycin
	<i>iroN</i>	siderophore salmochelin receptor IroN	95.72	53.49	NA
	<i>dpsA</i>	non-specific DNA-binding protein DpsA	97.53	74.68	NA
	<i>cnrT</i>	nickel diffusion facilitator CnrT	97.72	60.87	Nickel
	<i>tmexD</i>	multidrug efflux RND transporter permease subunit TMexD	98.75	61.82	Tetracycline
	<i>dfrA3</i>	trimethoprim-resistant dihydrofolate reductase DfrA3	93.83	50.31	Trimethoprim
173-C-08	<i>arsN1</i>	arsinothricin resistance N-acetyltransferase ArsN1 family B	83.87	64.74	Arsenic
	<i>arsR</i>	As(III)-sensing metalloregulatory transcriptional repressor ArsR	92.31	50.93	Arsenic
	<i>arsB</i>	arsenite efflux transporter membrane subunit ArsB	99.53	75.41	Arsenic
	<i>arsH</i>	arsenic resistance NADPH-dependent reductase ArsH	99.57	68.51	Arsenic
	<i>arsR</i>	As(III)-sensing metalloregulatory transcriptional repressor ArsR	92.31	50.93	Arsenic
	<i>arsB</i>	arsenite efflux transporter membrane subunit ArsB	99.3	73.71	Arsenic
	<i>blaPDC</i>	PDC variant family class C beta-lactamase	94.71	58.24	Beta-lactam
	<i>cadR</i>	cadmium resistance transcriptional regulator CadR	100	76.19	Cadmium
	<i>chrR</i>	class I chromate reductase ChrR	97.31	71.82	Chromate
	<i>copR</i>	heavy metal response regulator transcription factor CopR	96.1	55.61	Copper
	<i>cueA</i>	copper resistance metal-translocating P1-type ATPase CueA	91.09	84.18	Copper
	<i>copC</i>	copper homeostasis periplasmic binding protein CopC	98.41	76.61	Copper

<i>copS</i>	copper resistance membrane spanning protein CopS	99.35	87.36	Copper
<i>copR</i>	heavy metal response regulator transcription factor CopR	97.84	95.58	Copper
<i>copA</i>	laccase-like oxidase CopA	100	73.71	Copper
<i>copB</i>	copper-binding protein CopB	96.34	66.77	Copper
<i>copA</i>	laccase-like oxidase CopA	100	72.41	Copper
<i>copB</i>	copper-binding protein CopB	77.13	69.17	Copper
<i>copC</i>	copper homeostasis periplasmic binding protein CopC	99.21	64	Copper
<i>copR</i>	heavy metal response regulator transcription factor CopR	98.68	60.71	Copper
<i>copR</i>	heavy metal response regulator transcription factor CopR	98.68	56.7	Copper
<i>copR</i>	heavy metal response regulator transcription factor CopR	97.8	71.17	Copper
<i>silA</i>	Cu(+)/Ag(+) efflux RND transporter permease subunit SilA	99.05	67.24	Copper/Silver
<i>ssmE</i>	multidrug efflux SMR transporter SsmE	99.09	56.88	Efflux
<i>mdsB</i>	multidrug efflux RND transporter permease subunit MdsB	99.24	63.3	Efflux
<i>mexE</i>	multidrug efflux RND transporter periplasmic adaptor subunit MexE	100	79.1	Efflux
<i>emrA</i>	multidrug efflux MFS transporter periplasmic adaptor subunit EmrA	94.66	58.33	Efflux
<i>emrB</i>	multidrug efflux MFS transporter permease subunit EmrB	95.64	61.78	Efflux
<i>emhC</i>	efflux RND transporter outer membrane subunit EmhC	100	86.83	Efflux
<i>emhB</i>	efflux RND transporter permease subunit EmhB	100	81.74	Efflux
<i>emhA</i>	efflux RND transporter periplasmic adaptor subunit EmhA	100	80.52	Efflux
<i>ttgR</i>	efflux transport transcriptional regulator TtgR	99.52	69.86	Efflux
<i>mdsB</i>	multidrug efflux RND transporter permease subunit MdsB	98.86	70.47	Efflux
<i>fos</i>	FosA family fosfomycin resistance glutathione transferase	99.26	66.67	Fosfomycin
<i>golS</i>	Au(I) sensor transcriptional regulator GolS	81.82	56.35	Gold
<i>merR</i>	mercury resistance transcriptional regulator MerR	95.14	77.37	Mercury
<i>merT</i>	mercuric transport protein MerT	100	85.34	Mercury
<i>merP</i>	mercury resistance system periplasmic binding protein MerP	98.9	74.73	Mercury
<i>merC</i>	organomercurial transporter MerC	98.58	73.38	Mercury
<i>merA</i>	mercury(II) reductase	100	75.62	Mercury
<i>merD</i>	mercury resistance co-regulator MerD	90.08	60.55	Mercury
<i>merE</i>	broad-spectrum mercury transporter MerE	76.92	66.67	Mercury
<i>iroN</i>	siderophore salmochelin receptor IroN	95.72	53.49	NA
<i>dpsA</i>	non-specific DNA-binding protein DpsA	97.53	74.68	NA
<i>cnrT</i>	nickel diffusion facilitator CnrT	97.72	60.87	Nickel
<i>silP</i>	Ag(+)-translocating P-type ATPase SilP	97.69	50.31	Silver
<i>tmexD</i>	multidrug efflux RND transporter permease subunit TMexD	98.75	61.82	Tetracycline

Appendix G: Supplementary data - Chapter 6

Table G1. Summary of mercury resistance genes located on plasmids in *Aeromonas spp.*

Isolate	Species	Plasmid	Resistance genes	Plasmid features	Predicted mobility
093-C-06	<i>A. salmonicida</i>	AA364	<i>merD, merE, merF, merP, merR, merT</i>	Replicon cluster 1169	Non-mobilizable
217-C-16	<i>A. salmonicida</i>	AA364	<i>merD, merE, merF, merP, merR, merT</i>		Non-mobilizable
135-C-13	<i>A. salmonicida</i>	AA583	<i>merD, merE, merP, merR, merT</i>		Non-mobilizable
170-C-20	<i>A. salmonicida</i>	AC431	<i>merP, merR, merT</i>		Non-mobilizable
223-C-13	<i>A. salmonicida</i>	AC431	<i>merP, merR, merT</i>		Non-mobilizable
262-C-10	<i>A. salmonicida</i>	AC431	<i>merP, merR, merT</i>		Non-mobilizable
275-C-07	<i>A. salmonicida</i>	AC431	<i>merP, merR, merT</i>		Non-mobilizable
014-C-01	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfT</i>	Non-mobilizable
040-C-05	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfT</i>	Non-mobilizable
094-C-17	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfF</i>	Non-mobilizable
170-C-22	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfT</i>	Non-mobilizable
200-C-15	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfF</i>	Non-mobilizable
221-C-11	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfT</i>	Non-mobilizable
232-C-14	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfT</i>	Non-mobilizable
298-C-12	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfF</i>	Non-mobilizable
312-C-05	<i>A. salmonicida</i>	AC542	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfT</i>	Non-mobilizable
050-C-07	<i>A. salmonicida</i>	AE564	<i>merR, merT</i>	replicon cluster 71, <i>mpfF</i>	Non-mobilizable
069-C-02	<i>A. salmonicida</i>	AE564	<i>merD, merE, merF, merP, merR, merT</i>	replicon cluster 71, <i>mobH, mpfF</i>	Conjugative
092-C-07	<i>A. salmonicida</i>	AE564	<i>merD, merE, merF, merP, merR, merT</i>	replicon cluster 71, <i>mobH, mpfF</i>	Conjugative
103-C-05	<i>A. salmonicida</i>	AE564	<i>merD, merE, merF, merP, merR, merT</i>	replicon cluster 71, <i>mobH, mpfF</i>	Conjugative
137-C-06	<i>A. salmonicida</i>	AE564	<i>merA, merD, merE, merR, merT</i>	replicon cluster 71, <i>mobH mpfF</i>	Conjugative
206-C-18	<i>A. salmonicida</i>	AE564	<i>merD, merE, merF, merP, merR, merT</i>	<i>mpfF</i>	Non-mobilizable
214-C-18	<i>A. salmonicida</i>	AE564	<i>merA, merD, merE, merR, merT</i>	replicon cluster 71, <i>mobH, mpfF</i>	Conjugative
252-C-10	<i>A. salmonicida</i>	AE564	<i>merA, merD, merE</i>	replicon cluster 71, <i>mpfF</i>	Non-mobilizable
311-C-09	<i>A. salmonicida</i>	AE564	<i>merA, merD, merE, merF, merP, merR, merT</i>	<i>mobH, mpfF</i>	Conjugative
026-C-01	<i>A. salmonicida</i>	AE565	<i>merD, merE, merF, merP, merR, merT</i>	<i>mobH, mpfF</i>	Conjugative

008-C-04	<i>A. salmonicida</i>	AE974	<i>merD, merE, merF, merP, merR, merT</i>		Non-mobilizable
040-C-02	<i>A. salmonicida</i>	AE974	<i>merD, merE, merF, merP, merR, merT</i>		Non-mobilizable
052-C-01	<i>A. salmonicida</i>	AE974	<i>merD, merE, merF, merP, merR, merT</i>		Non-mobilizable
060-C-01	<i>A. salmonicida</i>	AE974	<i>merD, merE, merF, merP, merR, merT</i>		Non-mobilizable
003-C-01	<i>A. salmonicida</i>	Novel	<i>merA, merD, merR, merT</i>	<i>incP, mobP, mpfT</i>	Conjugative

Table G2. List of MGEs detected in *Aeromonas* spp. that are depicted in the Figure 15 heatmap.

MGE	MGE identifier
Plasmids	Novel, AC373, AC542, AC286, AE564, AE974, AA113, AA622, AE766, AC277, AC266, AB229, AC279, AE565, AA857, AC247, AA583, AC218, AB961, AE769, AC079, AA364, AC219, AC821, AB981, AD559, AD558, AC507, AC280, AC216, AC260, AE774, AE232, AC823, AC275, AC145, AC431, AA591, AB983, AC214, AC217
Transposable elements	ISGpr3, ISAhyl, ISKpn26, ISAs6, ISAs4, ISApu2, ISAs27, ISAs31, ISAs3, ISKpn3, ISKpn31, ISAs24, ISKpn9, ISAs18, ISAeme6, ISAeme15, ISShma11, ISAs19, ISAhyl2, ISAs29, ISAs21, ISApul, ISAs32, ISPa31, ISAVE3, ISAs30, ISAeme21, ISAs28, ISAs17, ISAhyl3, ISAeca5, TnAs1, ISAeme12, ISAs26, ISAs16, ISAeme17, ISUnCu16, ISAs2, ISVapa4, ISPa60, ISPa38, ISAs22, ISAeme1, ISShes9, ISAs25, ISAeme10, ISAVE2, ISKpn25, ISKpn40, ISAeme13, ISShes10, ISPsmel, ISEae1, ISPst2, ISAs9, ISPpu12, ISArsp6, ISAs17, IS1133

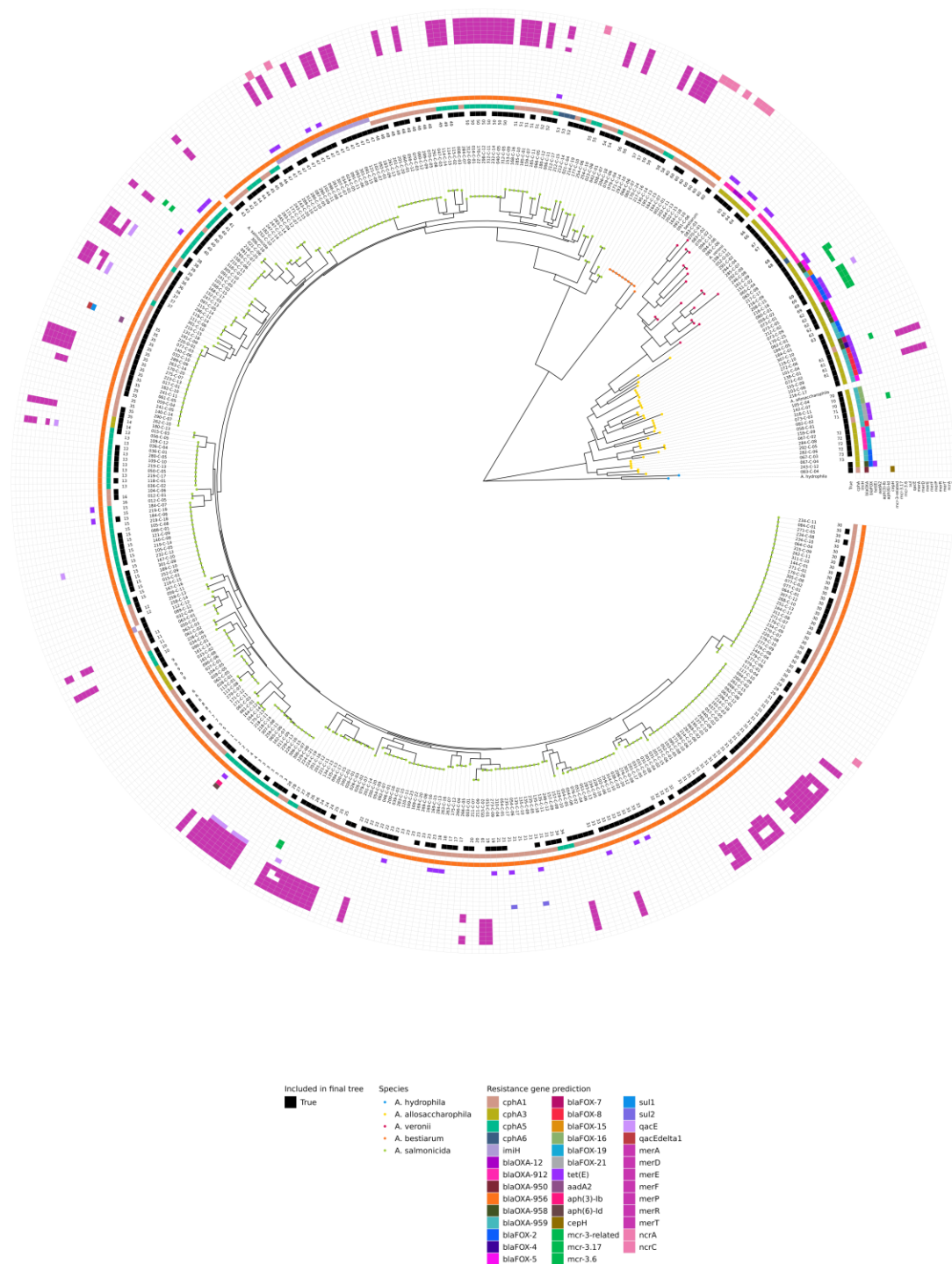


Figure G1. Midpoint-rooted maximum likelihood tree of the initial 407 *Aeromonas* isolate sequences used to determine unique isolates. Numbers adjacent to sample labels are cluster assignments. Reference sequences are labelled as their species identity.