Antimicrobial stewardship in British Columbia farmed finfish: linking antimicrobial use and resistance in the context of Yellow Mouth disease

by

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ABSTRACT

Antimicrobial resistance (AMR) and antimicrobial use (AMU) are important management tools for diseases such as yellow mouth (YM). There are concerns about the potential impacts of AMR on the environment, fish stock, and the potential for transmission to humans. As such, AMR in the marine environment poses a unique One Health threat that requires the attention of researchers in public health, veterinary medicine, and environmental science. The close relationship between industry and multiple levels of government in British Columbia (BC) aquaculture necessitates collaboration between these entities to find a meaningful and lasting solution. The majority of AMU in BC is attributed to YM, caused by the bacterium *Tenacibaculum maritimum*. Understandings of this bacterium, pathogenesis and risk factors, have only recently being explored. It is vital to evaluate this important disease in the context of the unique marine environment to identify strategies for reducing AMU.

The objectives of this thesis were to: 1) describe the antimicrobial susceptibilities of a historical provincial-level collection of bacterial isolates from farmed BC salmonids; 2) evaluate the relationship between AMR and AMU in BC finfish aquaculture using retrospective, province-level surveillance data provided by the BC Government; 3) assess the factors associated with YM outbreaks and requiring antimicrobial treatment using industry data for farmed Atlantic salmon in BC; and 4) to assess the practicality of using Cox proportional hazards models for this purpose. To address objective 1, this thesis presents historical AMR characteristics for the BC finfish industry, prevalence, and trends over time using AMR data from the BC Animal Health Centre (AHC) for the years 2007-2018 (Chapter 2). Chapter 3 (objective 2) presents the analysis of historical, provincial-level AMR and AMU data using logistic regression models. The

outcome variables for our regression models were oxytetracycline, florfenicol, and grouped potentiated sulfonamide resistance.

Objectives 3 and 4 were addressed by applying survival analysis models to industry-provided data for BC Atlantic salmon, consisting of daily environmental and biological variables such as temperature and fish density, for the first 120 days after pen placement in the ocean. Daily measurements evaluation of the effects of different variables on the risk (hazard) of breaking out with YM over time (Chapter 4).

Of the 1,237 unique bacterial isolates isolated between 2007-2018 from the BC AHC (Chapter 2), most (n=1,042) were from Atlantic salmon, with 69 unique bacterial species, dominated by *Aeromonas salmonicida* (n=174), *Aliivibrio wodanis* (n=84), and *Yersinia ruckeri* (n=79). Resistance to all tested antimicrobials was detected, albeit at low levels. Sparse distribution of resistance data prevented assessment of chronological trends, however, there was concerning multi-class drug resistance in some isolates. Logistic regression analysis (Chapter 3) identified that AMR to one drug was associated with resistance to any one other drug. We did not find any significant association between resistance and the use of any given antimicrobial except for sulfonamide use in the sulfonamide resistance model.

The survival analysis (Chapter 4) identified many environmental, biological, and managerial factors that are potentially associated with AMU for YM outbreaks. Of the 321 pen placements at sea, 292 had AMU for YM within the first 120 days. The average time until treatment was 30 days. Ongoing challenges with violation of Cox proportional hazards model assumptions forced the use of a simplified model including only the time-varying covariates,

10m depth temperature and salinity using restricted cubic splines, but these proved to be a challenge, with model syntax and software precluding interpretation.

The results of this thesis provide important baseline data and contribute to the increasingly pertinent body of literature on AMR and AMU data in the unique marine aquaculture environment. Despite the relatively low level of AMR, the association between resistance to different antimicrobials highlights the potential for co-selection for AMR and multidrug resistance. These results should be considered in the context of potential threats to One Health and public health that require further study. We present evidence that environmental factors are important risk factors for the development of YM, a leading cause of AMU in BC, and encourage and guide future research on aquaculture-based survival analysis. The results of this thesis have both scientific and industry applications that we hope will be utilized by both entities during this transformative time in BC aquaculture.

PREFACE

This thesis represents original work completed by Etienne de Jongh under the supervision of Dr. Simon Otto and thesis committee members Dr. Patrick Hanington and collaborator Dr. Carl Uhland. The conceptualization and design of this thesis was conducted by Etienne de Jongh under the supervision of Dr. Simon Otto and the supervisory committee. This thesis is based on data provided directly from the Government of British Columbia and an industry partner under a confidentiality agreement signed in 2021. No parts of this thesis have been previously published. Chapter 2 contains revised data that have been updated from a previously submitted confidential, internal report for the British Columbia Ministry of Agriculture and Fisheries written by Etienne de Jongh, Dr. Simon Otto, and others. This research was funded by support from the industry partner, the Federal Student Work Experience Program through the Public Health Agency of Canada, a contract with the British Columbia Ministry of Agriculture through the Canadian Agricultural Partnerships program (GSACP19-305), a grant from the Alberta Ministry of Technology and Innovation - the Major Innovation Fund Program for the AMR – One Health Consortium, through the HEAT-AMR (Human-Environment-Animal Transdisciplinary AMR) Research Group, the Harry Glenn de Mille Award in Veterinary Public Health from the University of Calgary Faculty of Veterinary Medicine, Calgary, AB, Canada, and the Public Health Innovation Scholarship from the University of Alberta School of Public Health, Edmonton, AB, Canada.

Etienne de Jongh was responsible for the data collection, cleaning, sorting, analysis, preliminary drafting, and revisions for all chapters of this thesis under the supervision of Dr. Simon Otto and committee members. Dr. Simon Otto and the supervisory committee were responsible for substantial revisions and critical review of this thesis. Dr. Carl Uhland

contributed specifically to the revision of Chapters 2 and 3 and provided an important industry perspective. Collection, and cleaning of data for Chapter 4 was accomplished through a joint effort of multiple HEAT-AMR lab members including Dr. Simon Otto, Etienne de Jongh, Rebecca Wassmuth, and Kelsey Robertson. Rebecca Wassmuth made substantial contributions to the creation, merging, and cleaning of the industry data that was utilized in Chapter 4 of this thesis.

DEDICATION

"It's been my enormous good fortune to work with a succession of friends with whom collaboration has been both very enjoyable and from my point of view extremely fruitful. But [it's] all a considerable pain to write."

- Sir David Cox, 1993

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LIST OF ABBREVIATIONS

AHC – Animal Health Centre

AMD – Antimicrobial Drug

AMU – Antimicrobial Use

AMR – Antimicrobial Resistance

ARG – Antimicrobial Resistance Gene

BC – British Columbia

EU – European Union

FLOR – Florfenicol

HGT – Horizontal Gene Transfer

MAF – Ministry of Agriculture and Fisheries

OXY – Oxytetracycline

PCU – Population Correction Unit

SMOR – Sulfadiazine + Ormetoprim

SXT – Trimethoprim + Sulfadiazine

CHAPTER 1 INTRODUCTION, INDUSTRY, LITERATURE REVIEW

1.1 Yellow mouth background

Yellow Mouth (YM), caused by the bacterium *Tenacibaculum maritimum*, is the primary cause for the vast majority of all antimicrobial treatment of finfish in British Columbia (BC) (Morrison & Saksida, 2013; Wade & Weber, 2020). Yellow Mouth is a contagious, opportunistic, and fatal disease that primarily affects young salmon soon after they are transferred from their freshwater rearing pens to their final saltwater homes (Avendaño-Herrera et al., 2006; Wynne et al., 2020). Presentation and diagnosis of YM is highly varied in the literature, ranging from numerous disseminate plaques and necrosis all over the body and fins to small singular focal lesions inside the mouth (Avendaño-Herrera et al., 2006; Frisch, Småge, Johansen, et al., 2018). Many articles restrict YM to a disease process resulting in plaques in the oral cavity, while plaques elsewhere on the body are classified into similar disease entities, such as tenacibaculosis, flexibacteriosis, mouthrot, eroded mouth syndrome, black patch necrosis, ulcerative stomatitis, and bacterial stomatitis (Avendaño-Herrera et al., 2006; Wade & Weber, 2020); this clinical distinction and nomenclature standard is not preserved in all articles. We have opted to stick with “Yellow Mouth” in this study as it conforms to the most recent industry standards. In many cases of YM, an increase in oral plaques may precede a high mortality event, with little to no clinic signs in the deceased fish (Fisheries and Oceans Canada, 2020; Nowlan et al., 2020; Wade & Weber, 2020). For the purposes of this project, we will utilize the case definition provided by the Department of Fisheries and Oceans Canada, Canadian Science Advisory Secretariat, which includes gross yellow plaques in the mouth, gill rakers and/or palate, and assume concurrent increases in mortality—if present—are also due to YM (Wade & Weber, 2020). Attributing all mortalities in a pen that occur during the presence of YM to YM is

consistent with both industry and literature methodology (Nowlan et al., 2021; Wade & Weber, 2020).

1.2 YM Bacteriology

Tenacibaculum maritimum is a gram-negative rod belonging to the family Flavobacterium. The species is highly diverse and is commonly characterized into three subgroups with numerous strains and sequence types. Ubiquitously distributed in marine environments off the coast of British Columbia, Western Canadian *T. maritimum* forms a distinct subgroup consisting of *TmarCan1*, *TmarCan2*, and *T. maritimumT* sequence types (Frisch, Småge, Brevik Ø, et al., 2018). Yellow Mouth pathogenesis may involve concurrent *Tenacibaculum* infections with species such as *T. maritimum*, *Tenacibaculum dicentrachi*, and *Tenacibaculum. finnnmarkense* (Frisch, Småge, Vallestad, et al., 2018). Current evidence disputes the innate virulence of *T. maritimum*, although most sources generally agree that the bacterium acts in an opportunistically pathogenic matter (Fisheries and Oceans Canada, 2020; Nowlan et al., 2020; Wynne et al., 2020). Pathogenesis begins with strong adherence to the hydrophobic mucus present in the oral cavity and gills of young fish. This process is facilitated through the formation of a biofilm and virulence factors such as collagenase (Frisch, Småge, Johansen, et al., 2018; Mabrok et al., 2022; Nowlan et al., 2020). Establishment of *T. maritimum* usually begins in the gingival pockets of the teeth due to a particular trophism of the bacterium for collagen and calcium rich tissue (Frisch, Småge, Johansen, et al., 2018; Mabrok et al., 2022; Nowlan et al., 2020). From here, filamentous mats form which invade into the tissue and cause local ulcerative necrosis (Frisch, Småge, Johansen, et al., 2018; Mabrok et al., 2022). Initial oral infection may result in peridonatal and odontal disorders, including complete loss of teeth and causing hyporexia or anorexia (Frisch, Småge, Johansen, et al., 2018; Mabrok et al., 2022).

Lesions may also form on the gills and scales, but these lesions may only be present in experimental models, where high density and space constraints lead to physical seeding of bacterial aggregates (Avendaño-Herrera et al., 2006; Powell et al., 2004). Invasion from the oral cavity into vasculature (typically the vascular tooth pulp) leads to systemic colonization of the bacteria (Frisch, Småge, Johansen, et al., 2018; Nowlan et al., 2020). Notably, plaques usually remain isolated to the oral cavity and auxiliary infected tissues, although positive for the bacterium, do not form gross lesions or result in additional clinical signs (Frisch, Småge, Johansen, et al., 2018). The mechanism of mortality for YM is unknown, but progression may be very rapid and result in mass mortality events (Nowlan et al., 2020; Wynne et al., 2020).

1.3 **YM Epidemiology**

Yellowmouth is common in a wide variety of fish (Avendaño-Herrera et al., 2006; Chen et al., 1995). In salmonids, the disease first appears between days 100-120 post hatch, about 2-3 weeks after smoltification, and about a week after entry into saltwater pens (Avendaño-Herrera et al., 2006; Hewison & Ness, 2015; Wade & Weber, 2020). Incidence of the disease almost entirely disappears by the time the smolts reach about 500g in body size, although previously infected pens can continue to have recurrent outbreaks (Avendaño-Herrera et al., 2006; Escribano et al., 2020; Wade & Weber, 2020). Outbreaks have been linked to a wide variety of both host and environmental factors, although the distinction and interaction between these factors is unclear in the marine environment (Mabrok et al., 2022; Sajid et al., 2024). On the host side, YM has been linked to ages between 100-120 days, body size under 500g, smoltification, and stress (Avendaño-Herrera et al., 2006; Escribano et al., 2020; van Gelderen et al., 2011). These occur around the same time that smolts are transitioned to the marine environment, making it difficult to determine causal relationships. Smoltification and the process of

transitioning to salt water can be incredibly stressful to young fish. Handling, transport, rapid growth, increased crowding, and hormonal changes associated with smoltification all contribute to stress during this period (Escribano et al., 2020; van Gelderen et al., 2011; Wynne et al., 2020).

Environmental factors are frequently linked to YM breakouts, including salinity, temperature, oxygen, washout events, algae blooms, organic pollution and runoffs, and many other variables (Mabrok et al., 2022; Nowlan et al., 2021; Watts et al., 2017). High water temperatures have been found to be conducive to *T. maritimum* breakouts, although these parameters seem to fluctuate depending on the host species and geographic location (Avendaño-Herrera et al., 2006; Brosnahan et al., 2019; Mabrok et al., 2022; Yamamoto et al., 2010). Algae blooms and jellyfish infestations are frequently associated with YM, but it is hard to separate these events from other environmental factors, such as a raise in temperature or a drop in oxygen (Nowlan et al., 2021; Sajid et al., 2024). Stress due to changes in water conditions from storms, washout events, or other adverse weather events may also increase host susceptibility to *T. maritimum*. To this end, the process of smolitification and marine transition is a time of extreme fluctuation. Combined with stress, handling, injuries, and great physiologic and metabolic demands, it is difficult to separate any one of these variables and analyze their relationship with YM mortality in isolation (Escribano et al., 2020; Nowlan et al., 2021; Sajid et al., 2024; van Gelderen et al., 2011). This difficulty is compounded by the uncertainties surrounding *T. maritimum* pathogenesis, and the theory that only concurrent infections with multiple *Tenacibaculum* species result in clinic signs. For example, *T. maritimum* and *T. finnmarkense* have preferred temperature ranges of 15-34ºC and 2-20ºC and pH ranges of 5.9-8.6 and 4-9 respectively (Mabrok et al., 2022)

Despite these known associations between host and environmental factors and YM, the complex marine environment makes proving a casual pathway difficult. For example, a recent article by Wynne et al. (2020) hypothesized that dysbiosis in young smolts may be linked to the emergence of YM, but they could not elucidate whether dysbiosis itself is a necessary cause or whether it occurs secondarily to environmental stressors such as salinity changes, and itself lies outside the causal pathway. Furthermore, studies have found that an increase in organic matter, due to water disturbances after storms, soil runoff due to extreme rainfall events, or pollution from terrestrial agricultural systems or sewage have all been linked to increases in marine bacterial communities. Therefore, any factor that causes water disturbances, such as boat traffic or storms, or increases organic matter, such as uneaten aquaculture feed, may alter the local microbiome.

The process of YM transmission is unclear, with many factors influencing its appearance, spread, and virulence in populations (Wade & Weber, 2020; Wynne et al., 2020). Environmental transmission of YM may be augmented by environmental conditions and facilitated by vectors such as (jellyfish). Further studies propose that *T. maritimum* is ubiquitously present in the environment, and infection and transmission are related on host factors that alter susceptibility instead of bacteria factors that alter virulence (Fisheries and Oceans Canada, 2020; Wynne et al., 2020). Importantly, *T. maritimum* does not contain any plasmids, limiting its ability to contribute and source from the environmental resistome and benefit from plasmid-mediated horizontal gene transfer (HGT) (Nowlan et al., 2023; Pérez-Pascual et al., 2017).

1.4 **YM Industry impacts**

Despite its devastating impact on the aquaculture industry (Wade & Weber, 2020), YM research is still quite new. Only as recently as 2018 was *T. maritimum* formally confirmed as the

etiological agent behind YM (Frisch, Småge, Vallestad, et al., 2018). Pressure from infectious diseases such as YM is a top concern for the future growth of the finfish aquaculture industry, where antimicrobial use (AMU) remains an important tool for bacterial disease management (Love et al., 2020; Lulijwa et al., 2020). A common conception in both scientific literature and the industry is that AMU is closely related to, and perhaps drives antimicrobial resistance (AMR) (Tuševljak et al., 2013). The high proportion of AMU attributable to YM raises concerns about the potential for increased selection of AMR in aquaculture production in BC.

Yellowmouth is present globally in aquaculture settings but is particularly impactful along the coast of western Canada, encompassing BC and Washington State (Frisch, Småge, Vallestad, et al., 2018; Wade & Weber, 2020). The cost of YM outbreaks for a single aquaculture company in BC can cost millions of dollars (Cermaq, 2022; Hewison & Ness, 2015). The mortality rate of YM is around 15-20% but can reach as high as 40% (Wynne et al., 2020). Direct die-off and productivity losses necessitate two to three annual series of in-feed treatments per site with antimicrobials in feed, primarily with florfenicol (trade name Aquaflor) (Hewison & Ness, 2015). Antimicrobial treatment for YM includes agents such as enrofloxacin, amoxicillin, nitrofurantoin, florfenicol, oxytetracycline, and trimethoprim-sulfamethoxazole (Avendaño-Herrera et al., 2008; Jonah et al., 2024; Mabrok et al., 2022). There are four antimicrobials licensed for use in finfish in Canada: florfenicol, trimethoprim-sulfadiazine (sulfa-trimethoprim), ormetoprim-sulfadimethoxine (sulfa-ormetroprim), and oxytetracycline (Canadian Food Inspection Agency (CFIA), 2019). In Canada, florfenicol and trimethoprim-sulfadiazine are the most commonly used antimicrobials for YM, with florfenicol being the preferred choice due to the poor palatability of sulfonamides (Wade & Weber, 2020). Palatability is important for

treating YM, as hyporexia is common clinical sign and medication is usually delivered orally as medicated feed.

1.5 **Marine AMR**

Antimicrobial stewardship involves both limiting antimicrobial misuse and addressing the current prevalence and acceleration of AMR. The global growth of the finfish aquaculture industry, coupled with the requirement to use antimicrobials to control bacterial pathogens, creates selection pressure for development of AMR and further potential for environmental exposure to AMR organisms and genes from aquaculture operations (Watts et al., 2017). Therefore, there remains a direct need for surveillance and empirical evidence on the linkages between AMR and AMU in the marine environment (McCubbin et al., 2021).

Antimicrobial resistance has been previously identified near aquaculture facilities that utilize antimicrobials (Avendaño-Herrera et al., 2008; Du et al., 2019; Eckstrand et al., 2024; Miranda & Rojas, 2007; Ojasanya et al., 2022; Schar et al., 2021; Shah et al., 2014). Within Canada, studies have shown common targets for antimicrobial treatment, such as *Aeromonas salmonicida*, to have varying levels of resistance to sulfa-ormetroprim, erythromycin, florfenicol, and oxytetracycline (Hawkins et al., 1997; McIntosh et al., 2008; Ojasanya et al., 2022; Sheppard et al., 1994). A recent comprehensive retrospective analysis of AMR in Atlantic salmon bacteria showed a substantial and diverse prevalence of resistance (Ojasanya et al., 2022). The study investigated 12 bacterial species from 18,776 salmonid samples and found high levels of resistance to florfenicol, oxytetracycline, ormetoprim-sulfadimethoxine, and trimethoprim-sulfamethoxazole (Ojasanya et al., 2022). These results were not reported alongside AMU data. Likewise, a recent study on the west coast found antimicrobial resistance genes (ARGs) to tetracyclines and vancomycin in Atlantic salmon in BC (Nowlan et al., 2021). Although studies

frequently attribute AMR surrounding aquaculture facilities to AMU, directives highlighting the necessity of more research on the link, and criticisms of current AMU-AMR evaluations exist (Brunton et al., 2019; Fraser et al., 2004; Larsson et al., 2018; Mardones et al., 2018; Miranda et al., 2013; Miranda et al., 2018; Preena et al., 2020; Watts et al., 2017).

Previous studies have supported the hypothesis that a diverse amount of AMR genes (ARGs) exist in fish farms (González-Gaya et al., 2022; Miranda et al., 2013). This collection of ARGs within microbial communities in the environment is known as the resistome. Although the resistome has naturally evolved over billions of years, AMU has accelerated and altered its growth and diversity. González-Gaya et al. (2022) showed that residual antimicrobials and metabolites from medicated aquaculture feeds can contribute to the benthic resistome near fish pens. Bacteria can contribute to and utilize resistance genes within the resistome, which facilitates the spread of current resistance and development of multi-drug resistance (González-Gaya et al., 2022). Additionally, the ARGs are able to spread independently of bacterial populations can persist as a sort of "resistance reservoir" long after AMU is eliminated (Domínguez et al., 2019). The marine resistome is of particular concern due to the dynamic and intimate relationship of marine animals and the marine environment.

Lozano-Munoz et al., (2021) theorize that AMU does not directly select for ARGs in the environment, but instead therapeutic AMU in fish pens drive AMR within the salmon gut microbiome, and that these commensal bacteria, when released through fecal material, may transfer their ARGs via horizontal gene transfer (HGT) to environmental bacteria. This pathway is supported by Gao et al 2012, who demonstrated that resistant intestinal bacteria could be isolated from sediments, suggesting host-to-environment transfer of ARGs. Furthermore, Rico et al., 2017 conducted a study that compared the resistance development risk for 12 different

antimicrobials used in terrestrial catfish systems in Vietnam. Although these aquaculture systems are different from the ones used in BC, it is interesting to note that all antimicrobials tested had a very high risk of resistance development (100% for all drugs isolated from sediments). Lastly, Tuševljak et al., 2013 described and summarized industry opinions on AMU and AMR in aquaculture and provided a direct drug-by-drug comparison of these results, although no empirical evaluation of the AMR-AMU link was provided.

The ability of florfenicol and other antimicrobial drug residues to retain and apply selection pressure for ARGs, and the known leakage of antimicrobial wastes from open concept pens into sediments and surrounding water, pose a potential EcoHealth risk (González-Gaya et al., 2022). Numerous alterations to the benthic environments that surround aquaculture pens in the Mediterranean have been described, such as the dynamics and persistence of florfenicol, oxytetracycline, and one other antimicrobial (González-Gaya et al., 2022). Oxytetracycline was shown to persist both within sediments as well as local invertebrates for two weeks after pen treatment. Other studies have shown conservation of ARGs in the benthic resistome for years after drug application (Domínguez et al., 2019). Moreover, the medicated feed pellets often used for therapeutic treatment can themselves alter the environment if not consumed by the fish. Pathak et al. (1993) described alterations in microbial communities and the prevalence of ARGs due to an increase in suspended organic matter. These alterations should be considered when assessing antimicrobial pharmacodynamics and AMR in the marine environment.

Reducing AMU in salmonid production in BC could have One Health benefits, such as limiting the potential risk of transfer of drug residues and resistant bacteria into the wild environment, migrating salmon populations, and our food chain (McCubbin et al., 2021). This attractive possibility has caused coastal BC salmon farms to place YM at the top of its priority

list (Hewison & Ness, 2015; Sapin, 2021). Reducing AMU not only retains financial benefits, it contributes immensely to the social license of BC Aquaculture (Henriksson et al., 2018). Concerns regarding the transfer of AMR through the food chain, and transfer of ARGs to human pathogens, have resulted in calls to reduce aquaculture-based AMU (Brunton et al., 2019; Cabello et al., 2013; Ochs et al., 2021; Reverter et al., 2020; Watts et al., 2017). These issues are increasingly pertinent as aquaculture licensing in BC is re-evaluated (Fisheries and Oceans Canada, 2024).

1.6 **Research questions and objectives**

Concurrent increases in consumer and social demand for a reduction in AMU, and industry need to control and manage infectious diseases such as YM, are contrasted with a lack of scientific literature on the British Columbian aquaculture environment. The main goal of this project is to provide data to support antimicrobial stewardship in the western Canadian finfish aquaculture by identifying environmental, biological, and managerial factors associated with YM which leads to AMU, and evaluating the role of this use in the context of AMR.

1.6.1 **Research Question 1:**

What are the recent long-term trends and characteristics of antimicrobial susceptibility to licensed aquaculture antimicrobials in marine salmon farms in BC, Canada?

1.6.2 **Research Question 2:**

What are the factors associated with AMR to commonly used aquaculture antimicrobials in marine salmon farms in BC, Canada?

1.6.3 **Research Question 3:**

What are the pen-level factors contributing to YM breakouts over time in BC salmon aquaculture sites, and can we use Cox proportional hazards modeling for this purpose?

1.6.4 **Research objectives:**

1) Describe the antimicrobial susceptibilities of a historical collection of bacterial isolates from farmed salmonids in BC, that were submitted to the provincial diagnostic laboratory (Chapter 2).
2) Evaluate the relationship between AMR and AMU in BC finfish aquaculture using retrospective, province-level surveillance data provided by the Government of BC (Chapter 3)
3) Assess the factors associated with pen outbreaks with YM and requiring antimicrobial treatment using historical industry data for farmed Atlantic salmon in British Columbia, Canada (Chapter 4).
4) Assess the practicality of using Cox proportional hazards models for identifying factors associated with pen outbreaks and AMU for YM (Chapter 4).

CHAPTER 2 ANTIMICROBIAL RESISTANCE IN THE BRITISH COLUMBIA FINFISH AQUACULTURE INDUSTRY (2007-2018)

2.1 Abstract

Antimicrobial use (AMU) in finfish aquaculture production raises concerns about the link between AMU and the development of antimicrobial resistance (AMR) in bacteria found in aquatic organisms and potential transmission to humans and the environment. The global growth of the finfish aquaculture industry, coupled with the requirement to use medically important antimicrobials to control bacterial diseases, create selection pressure for the development of AMR. In addition, the potential for environmental exposure to AMR organisms and genes from aquaculture operations poses a unique One Health threat. The objective of this study was to report AMR trends in a historical collection of bacterial isolates from British Columbia (BC), Canada, finfish aquaculture.

Antimicrobial susceptibility data were obtained from the BC Ministry of Agriculture via submissions to the Animal Health Centre (AHC) for 2007-2018 for florfenicol (FLOR), oxytetracycline (OXY), trimethoprim-sulfadiazine (SXT), and triple-sulfa (TRI) composed of sulphamerazine, sulphathiazole, and sulfadiazine. There were 1,237 unique isolates from all finfish species (69 unique bacterial species), of which 1,042 were from Atlantic salmon. For all fish species, the most common bacterial species isolated were *Aeromonas salmonicida* (n = 174), *Aliivibrio wodanis* (n = 84), and *Yersinia ruckeri* (n = 79). Resistance was detected to most antimicrobials tested, but levels were generally low. Resistance to FLOR was only detected in *A. salmonicida*. Resistance to OXY and SXT varied in comparison to isolates from finfish in Atlantic Canada for a similar time-period. Low annual isolate numbers precluded genera-specific annual comparisons for all pathogens. The detection of isolates with multi-class resistance should be considered as a concern. These results provide important baseline AMR data for the

finfish aquaculture industry in BC, Canada. These will support future Canadian AMR surveillance in farmed aquaculture.

2.2 Introduction

The impacts of antimicrobial resistance (AMR) on human and animal health make it one of the top global health concerns (Watts et al., 2017; World Health Organization (WHO), 2020). Antimicrobial use (AMU) in finfish aquaculture production may select for AMR, raising concerns about the link between AMR genes in bacteria found in aquatic organisms and potential transmission to humans and the environment (González-Gaya et al., 2022; Heuer et al., 2009; Love et al., 2020; Lulijwa et al., 2020; Millanao et al., 2018; Weir et al., 2012).

Aquaculture, the farming of aquatic organisms such as salmon, algae, and oysters, is responsible for more than half of the world's seafood production, reaching 123 million tonnes in 2022 (Food and Agriculture Organization of the United Nations, 2024). This number falls to around 94 million tonnes when excluding plants such as algae (Food and Agriculture Organization of the United Nations, 2024). The production of animal aquaculture products between 2020 and 2022 grew by 7.6% and around 60% of this growth is attributed to finfish (Food and Agriculture Organization of the United Nations, 2024). Pressure from infectious diseases is a top concern for the future growth of the finfish aquaculture industry, where AMU remains an important tool for bacterial disease management (Love et al., 2020; Lulijwa et al., 2020). In Canada, salmon makes up the largest portion of finfish aquaculture production, totaling 123,184 tonnes in 2018, with British Columbia (BC) being the largest producer at 87,010 tonnes of salmon production in 2018 (Government of Canada, 2024b).

There are four antimicrobials licensed for use in finfish in Canada: florfenicol (FLOR), trimethoprim-sulfadiazine (sulfa-trimethoprim – SXT), ormetoprim-sulfadimethoxine (sulfa-ormetroprim), and oxytetracycline (OXY) (Canadian Food Inspection Agency (CFIA), 2019; Love et al., 2020; Lulijwa et al., 2020). Resistance to all of these drugs exists in bacterial isolates from farmed salmonids in Canada's east coast (Ojasanya et al., 2022).

Within Canada, older studies have shown common salmonid pathogen targets for antimicrobial treatment, such as *Aeromonas salmonicida, Yersinia ruckeri*, and *Vibrio anguillarum,* have varying levels of resistance to sulfa-ormetroprim, erythromycin, FLOR, and OXY (Hawkins et al., 1997; McIntosh et al., 2008; Sheppard et al., 1994). Additionally, previous studies have supported the hypothesis that a diverse amount of AMR genes already exist and persist in fish farms (Miranda et al., 2013). González-Gaya et al. (2022) showed that residual antimicrobials and metabolites from medicated aquaculture feeds can contribute to the benthic resistome near fish pens (González-Gaya et al., 2022). Moreover, a recent comprehensive retrospective analysis of AMR in bacteria isolated from Atlantic salmon raised on the east coast of Canada showed a variable and diverse level of resistance in *Y. ruckeri*, *Renibacterium salmoninarum*, and *A. salmonicida,* among others (Ojasanya et al., 2022). Despite this recent Canadian publication, there is a paucity of data examining AMR in the Canadian salmonid finfish industry, particularly for production on the Pacific coast of BC. The objective of this study was to describe the antimicrobial susceptibilities of a historical collection of bacterial isolates from farmed salmonids in BC that were submitted to the provincial diagnostic laboratory from 2007-2018.

2.3 **Methods**

Anonymized antimicrobial susceptibility data for bacterial isolates from farmed finfish species were provided by the BC Ministry of Agriculture Animal Health Centre (AHC). The data included antimicrobial susceptibilities for bacterial isolates from finfish for FLOR, OXY, SXT, and sulphonamide compounds (triple-sulfa: sulphamerazine, sulphathiazole, and sulfadiazine) for the years 2007-2018, as well as sulfa-ormetoprim for 2007-2009. Antimicrobial susceptibility testing was conducted using the disk diffusion method and according to recognized breakpoints, with specific details not provided due to the historical nature of the dataset.

The isolates came from different finfish species including Atlantic salmon, Pacific salmon (including Chinook, Coho, and Pink salmon), Rainbow trout, Sablefish, Tilapia, and White sturgeon. Rainbow trout were kept as a separate category because it was not possible to determine if they were from marine (often called Steelhead trout) or freshwater sources.

A large variety of organisms were isolated and tested for antimicrobial susceptibility from salmon submissions to the AHC. Susceptibility for isolates of the genera *Aeromonas, Vibrio, Aliivibrio, Yersinia*, *Photobacterium, Pseudoalteromonas, Pseudomonas, Psychrobacter*, and *Serratia* spp. were reported to provide a broad overview of antimicrobial susceptibility in commonly isolated finfish bacteria. Of these genera, the following species were reported separately: *A. salmonicida, Aeromonas sobria, Aliivibrio wodanis, V. anguillarum, Vibrio ordalii, Vibrio splendidus, Vibrio tapetis,* and *Y. ruckeri*. The BC AHC also highlighted species of interest for focus in addition to these listed bacteria: *Tenacibaculum maritimum*, *R. salmoninarum*, and *Piscirickettsia salmonis*. Descriptive statistics were prepared in Excel® (Microsoft Corporation, Redmond, WA).

2.4 **Results**

There were 1,237 unique finfish isolates tested for antimicrobial susceptibility at the AHC from 2007-2018 (Table 2.1, Supplementary Table A1.1). Of these, 84.2% (n=1,042) isolates came from Atlantic salmon, 7.7% (n=95) from Pacific salmon, 5.7% (n=71) from Rainbow trout, and 2.3% (n=29) from other species (Sablefish, Tilapia, and White sturgeon) (Supplementary Table A1.1). Susceptibility data were missing for two of the Atlantic salmon isolates (total with complete data n=1,040).

There were 44 bacterial genera (Table 2.1, Supplementary Table A1.1) and 68 bacterial species (data not shown) isolated from all farmed salmonids over the time period. The most common bacterial genera isolated from all farmed salmonids included: *Aeromonas* (n=246), *Aliivibrio* (n=195), *Photobacterium* (n=117), *Pseudoalteromonas* (n=46), *Pseudomonas* (n=78), *Psychrobacter* (n=46), *Serratia* (n=34), *Vibrio* (n=227), and *Yersinia* (n=82) (Table 2.1). In Atlantic salmon, there were 33 bacterial genera and 54 bacterial species isolated (Table 2.1, Supplementary Table A1.1). The most common genera isolated from Atlantic salmon included: *Aeromonas* (n=206), *Aliivibrio* (n=186), *Photobacterium* (n=108), *Pseudoalteromonas* (n=44), *Pseudomonas* (n=53), *Psychrobacter* (n=41), *Serratia* (n=32), *Vibrio* (n=172), and *Yersinia* (n=79). The most common genera isolated from Pacific salmon included: *Aeromonas* (n=15), *Aliivibrio* (n=8), *Photobacterium* (n=5), *Pseudomonas* (n=8), *Psychrobacter* (n=4), and *Vibrio* (n=45). The most common genera isolated from Rainbow trout included: *Aeromonas* (n=20), *Arthrobacter* (n=3), *Carnobacterium* (n=5), *Edwardsiella* (n=3), *Iodobacter* (n=4), *Lactococcus* (n=3), and *Pseudomonas* (n=12).

The most common bacterial species of interest isolated from all farmed salmonids (Table 2.2) included: *A. salmonicida* (n=174), *A. wodanis* (n=85), *V. splendidus* (n=40), *V. tapetis* (n=44), and *Y. ruckeri* (n=79). The most common bacterial species of interest isolated from

Atlantic salmon (Table 2.2) included *A. salmonicida* (n=159), *A. wodanis* (n=82), *V. splendidus* (n=35), *V. tapetis* (n=43), and *Y. ruckeri* (n=77). The most common bacterial species of interest isolated from Pacific salmon (Table 2.2) included *A. salmonicida* (n=11), *A. wodanis* (n=3), *V. anguillarum* (n=16), *and V. ordalii* (n=13). The most common species isolated from Rainbow trout (Table 2.2) included *A. salmonicida* (n=2), *Aeromonas bestiarum* (n=2), *Aeromonas caviae* (n=2), *A. sobria* (n=8), *Edwardsiella ictalurid* (n=2), *Lactococcus lactis* (n=2), *Pseudomonas fluorescens* (n=2), and *Y. ruckeri* (n=2). The other species of interest (*T. maritimum*, *R. salmoninarum*, and *P. salmonis*) were not isolated by the standard culture methods over the time period.

Table 2.3 presents the antimicrobial susceptibility results for bacterial species of interest for all finfish species. Most bacterial genera of interest had no resistance to FLOR, apart from *A. salmonicida* (18%; n=32/174), *A. wodanis* (1.2%; n=1/84), and isolates included in other species (11%; n=86/762). *A. salmonicida* had the highest prevalence of resistance to FLOR (18%; n=32/174) and SXT (23%; n=40/174) and had OXY resistance (22%; n=38/174) comparable to *A. sobria* (24%; n=5/21). Pan-susceptibility (66%; n=683/1,040) was the most common antimicrobial susceptibility profile for all bacterial isolates from Atlantic salmon (Table 2.4). This was followed by TRI resistance (17%; n=177/1,040) and resistance to all four drugs tested (3.8%; n=40/1,040). Another four isolates were resistant to FLOR-OXY-SXT and one to FLOR-OXY-TRI, meaning that 4.3% (n=45/1,040) were resistant to all three drug classes.

A. salmonicida isolates (n=159) from all farmed salmonids was the only bacterial species with high enough frequency and prevalence of AMR to warrant descriptive reporting of annual antimicrobial susceptibility profiles. Even with this, temporal results should be interpreted with caution as annual sample sizes were often low. Most *A. salmonicida* isolates (67%; n=107/159)

were pan-susceptible (Table 2.5). However, the next most common profile was resistance to all four drugs (20%; n=32/159). The annual prevalence estimates of AMR (Table 2.6) indicated that the most common resistance was to TRI, appearing in 8/12 of years, with levels ranging from 8.3% (n=1/12) to 47.5% (n=19/40). Resistance to FLOR appeared in 2010, 2012, and 2013 at levels ranging from 18.8-37.5% (n=6/32 to n=12/32). Interestingly, these years were the only ones to have OXY-resistant isolates, ranging from 18.8-45.0% (n=6/32 to n=18/40); in addition, 2018 isolates had 12.5% (n=2/16) OXY resistance. Higher levels of resistance to SXT and TRI also tended to occur in 2010, 2012, and 2013.

2.5 **Discussion**

Our study reports the antimicrobial susceptibilities of a historical collection of bacterial isolates from farmed salmonids in BC, Canada, that were submitted to the provincial diagnostic laboratory. Overall, the prevalence of AMR to Canadian-approved antimicrobials for bacterial species of concern was low, with only *A. salmonicida* having any resistance to tested antimicrobials that were more than a single isolate over the study period. Among the bacterial species of interest, isolates were dominated by *A. salmonicida*, particularly from Atlantic salmon, followed by *A. wodanis*, *A. sobria*, *Y, ruckeri*, and *Vibrio* species. These susceptibility results will support future Canadian AMR surveillance in farmed aquaculture by providing baseline data.

Although little work on AMR and aquaculture has been undertaken in Canada, three additional studies were identified. A recent AMR surveillance study of isolates from farmed salmonids in Canada's Atlantic region found some similar and different patterns of isolates and AMR (Ojasanya et al., 2022). The study investigated 26 genera of bacteria from 2291 samples from farmed salmonids over a similar time-period (2000-2021). Over 90% of the samples in the

study were gathered from Atlantic salmon. Of the 2,291 samples, 515 resulted in species-level identification, of which 336 were tested for AMR. The authors stratified *A. salmonicida* isolates into furunculosis-causing (typical) and non-furunculosis-causing (atypical) subspecies. In terms of cases, *Y. ruckerii* was the most common, followed by *A. salmonicida* (when grouping typical and atypical strains together), and *R. salmoniarum*. Comparatively, when considering isolates tested for susceptibility, the three most common pathogens were *Y. ruckeri*, *A. salmonicida* (grouped), and *Pseudomonas fluorescens*. This partly coincides with the results of our study where the top three pathogens by isolate were *A. salmonicida*, *A. wodanis*, and *Y. ruckerii*. Our study was limited to historical isolates from the BC AHC and the methods they used for bacterial isolation at the time. It is not known how this may have impacted the ability to detect species such as *R. salmoniarum* or *T. maritimum*, which were not detected in BC.

The Atlantic bacterial isolates were tested for susceptibility to FLOR, OXY, sulfa-ormetoprim, another version of SXT – trimethoprim-sulfamethoxazole, and enrofloxacin (Ojasanya et al., 2022). When considering isolates from all fish species, results varied for some isolates and were similar for others. In the Atlantic *A. salmonicida* isolates, resistance to FLOR was 11.7% (n=13/111) (atypical) and 27.6% (n = 8/29) (typical), roughly similar to 18% in our *A. salmonicida* isolates. Resistance to OXY in *A. salmonicida* in their study (95.5% (n= 106/111) atypical and 58.6% (n= 17/29) typical) was higher than what was detected (22%) in our study. Resistance to SXT was 12.6% (n= 14/111) (atypical) and 24.1% (n= 7/29) (typical) in *A. salmonicida*, compared to 23% in our study. Resistance to FLOR, OXY, and SXT was not detected or negligible in *Y. ruckeri* isolates from both studies.

In addition to this study, an older Canadian study included data on 17 isolates from finfish in Atlantic Canada (Nova Scotia and New Brunswick) from 2002-2004, reporting high

levels of resistance in *A. salmonicida* to FLOR (76%; n=13/17) and OXY (94%; n=16/17) (McIntosh et al., 2008). Another older source for finfish from Newfoundland found slightly higher levels of resistance to OXY (37%; n=42/113) and sulfa-ormetoprim (34.6%; n=36/104) in *A. salmonicida* isolates from 1990-1995 (Hawkins et al., 1997).

Published studies including AMR data from salmonid isolates in other countries reported almost exclusively on *A. salmonicida*, except for one Chilean study that tested *Piscirickettsia salmonis* (Saavedra et al., 2017), one Chilean study on a variety of finfish bacterial isolates (n=5,018) including *Aeromonas* spp., *Pseudomonas* spp., *Serratia* spp., *Shewanella* spp., and *Psychrobacter* spp., among others, two Norwegian studies that included *Moritella viscosa* (Coyne et al., 2004) and *V. anguillarum* (Myhr et al., 1991), and a recent whole genome sequencing (WGS) analysis from the USA further described below (Eckstrand et al., 2024). In the USA, a six-year historical study on wild Chinook salmon (n=806), Coho salmon (n=623), Atlantic salmon (n=301), and Steelhead trout (n=385) in Michigan isolated *A. salmonicida* from 234 samples (11%) and found moderate resistance to OXY (22% of isolates), which is the same level of resistance found in our study (22%; n=38/174) (Diamanka et al., 2013). Compared to BC, *A. salmonicida* isolates from farmed salmon in China (2012-2016) had higher resistance to FLOR (52%, n=31/60) and OXY (40%, n=24/60) (Du et al., 2019). A more recent Chilean study found that 47 were resistant to FLOR and 44 were resistant to OXY (Higuera-Llanten et al., 2018).

Resistance to FLOR was 0% in Spanish isolates from 2001-2004 (Ortega et al., 2006) and in Scottish isolates in 1993 (Inglis et al., 1993). A study from Spain on Atlantic salmon and brown trout (sample sizes for each species not available) found 90% of *A. salmonicida* isolates displayed resistance or intermediate susceptibility to OXY, while 0% of isolates displayed

resistance to FLOR (Ortega et al., 2006). They tested a further 9 antimicrobials including amoxicillin, doxycycline, erythromycin, nalidixic acid, cotrimoxazole, flumequine, chloramphenicol, enrofloxacin, and novobiocin, and found resistance to at least one of these antimicrobials in 56% (n=190/341) of isolates.

Our study identified that 4.3% of all bacterial isolates from Atlantic salmon were resistant to all three antimicrobial classes tested. While we only tested a small number of drug classes for susceptibility, this does create some cause for concern about treatment effectiveness given that these include the only drugs approved for use in finfish in Canada (Canadian Food Inspection Agency (CFIA), 2019). Resistance to additional antimicrobials that were not included in the BC panel (e.g., erythromycin, oxolinic acid, or flumequine) are common internationally, but could not be compared to our results in BC due to a lack of inclusion in the testing panel for our isolates (Inglis et al., 1993; Jacobs & Chenia, 2007; Ortega et al., 2006). The Atlantic Canada study identified that 2.5% of all isolates were resistant to all antimicrobials tested, but this is not directly comparable as this included five antimicrobials across four antimicrobial classes: FLOR, OXY, sulfa-ormetoprim, SXT, and enrofloxacin). Similarly, the study of Chilean isolates by Higuera-Llantén et al. (2018) found a high degree of cross-resistance between FLOR, OXY, and other antimicrobials (Higuera-Llanten et al., 2018). A Scotland study investigated multidrug resistance in isolates already resistant to OXY and found that all isolates were resistant to at least one alternative antimicrobial, 63% to three or more antimicrobials, and 95% of OXY-resistant isolates were also resistant to trimethoprim (Inglis et al., 1993). The WGS study by Eckstrand et al. (2024) on 61 isolates—including *Aeromonas* spp., *Flavobacterium* spp., *Edwardsiella* spp., *Yersinia* spp., *Vibrio* spp., *Shewanella* spp., *Photobacterium* spp., *Pseudomonas* spp., *Acinetobacter* spp., and *Streptococcus* spp. sourced from a variety of saltwater, freshwater, and

ornamental fish from across the USA—found five *Edwardsiella* isolates with ARGs to FLOR, OXY, and sulfa-ormetoprim located on a single plasmid (Eckstrand et al., 2024). Due to the high degree of potential for horizontal gene transfer in marine settings, the prevalence of multi-drug resistance and rate of ARG dissemination in marine organisms may be high (Lupo et al., 2012). Genetic and molecular testing of future BC isolates is an important area of future research to investigate the potential AMR genes responsible for underlying phenotypic resistance, and to look for horizontal genetic elements potentially responsible for co-selection of AMR to these approved and used antimicrobials in finfish.

Eckstrand et al. (2024) showed that WGS was well suited for detection of resistance in the marine environment due to its ability to simultaneously analyze numerous ARGs that often coexist in fish. The authors also demonstrate that WGS is superior for isolate detection to other analytic methods, such as MALDI-TOF MS, due to better specificity (Eckstrand et al., 2024). Indeed, WGS is becoming increasingly popular as a method of identifying AMR genes in bacterial isolate from aquaculture samples. For example, a genomic analysis of *Tenacibaculum* spp. from farmed Atlantic salmon in BC in 2017-2022 by Nowlan et al. (2023) found acquired ARGs for OXY, while in Chile, Suarez et al. (2021) found ARGs in *Mycobacterium* spp. from farmed Atlantic and Coho salmon for beta-lactams, tetracyclines, gentamycin, macrolides, and rifampin (Nowlan et al., 2023; Suarez et al., 2021). Similarly, Dominguez et al. (2019) found multiple transferable ARGs against sulfonamides and trimethoprim in *Pseudomonas* spp. isolates against a backdrop of high multidrug resistance to FLOR, erythromycin, furazolidone, and amoxicillin (Domínguez et al., 2019). In Turkey, Saticioglu et al., (2021) found ARGs for OXY, FLOR, and sulfamethoxazole-trimethoprim in *Chryseobacterium* (a member of the family

Flavobacteriaceae) isolates (Saticioglu et al., 2021). These studies reinforce the future need to utilize WGS methods for AMR surveillance in farmed salmonid sampling in BC.

This study on historical data regarding resistance in BC finfish aquaculture had some limitations. We were reliant on historical antimicrobial susceptibility data from the AHC with no control over bacterial isolation, speciation, and antimicrobial susceptibility testing methods. Antimicrobial susceptibility testing was completed exclusively using disk diffusion and subsequent minimum inhibitory concentration data were not available for these isolates. We also did not have access to isolates for further investigation by WGS. Regardless, given the paucity of historical data for farmed salmonids in BC, these data still represent an important baseline for future surveillance. There is also potential for changes in Canadian finfish surveillance policy to have impacted annual levels of AMR in this dataset. The federal Department of Fisheries and Oceans Canada changed their surveillance regulations in 2015 for BC finfish (Government of Canada, 2024a). Finfish submissions prior to this point were voluntary, while submissions after 2015 were mandated through the new auditing program (Government of Canada, 2024a). This may have influenced annual resistance levels. However, given the relatively low numbers of isolates by pathogen species over time, it was not possible to formally assess the relative annual impact on AMR. Farm-specific AMU data directly linked to isolate submissions were also not available, precluding the ability to assess direct selection pressures at the farm level. However, the low levels of resistance to some commonly used antimicrobials, such as FLOR, create interesting questions about any links between its use and AMR. This is an important area for future research.

2.6 **Conclusions**

This report of BC data represents the first report of antimicrobial susceptibility of a historical collection of bacterial isolates from farmed salmonids in BC, Canada, from a diagnostic laboratory. The types of organisms isolated from BC finfish submissions to the BC Ministry of Agriculture were varied and numerous, making temporal interpretation of the data at the bacterial species level challenging. Overall, resistance was detected to all antimicrobials tested, but mostly at low levels. These data provide an important platform for future surveillance by providing a baseline of long-term resistance trends in salmonids along the BC Coast.

Table 2.1. Bacterial genera isolated from all farmed salmonid species in British Columbia by the Animal Health Centre from 2007-2018 and tested for antimicrobial susceptibility

Bacterial genera	Total isolates	Atlantic salmon	Pacific salmon	Rainbow trout	Other species*
		All numbers represent n (%), (LCL, UCL)			
Aeromonas	246	206 (83.7) (78.7, 88.8)	15 (6.1) (0, 18.2)	20 (8.1) (0, 20.1)	5 (2.0) (0, 14.4)
Vibrio	227	172 (75.8) (69.4, 82.2)	45 (19.8) (8.2, 31.5)	0 (0)	10 (4.4) (0, 17.1)
Aliivibrio	195	186 (95.4) (92.4, 98.4)	8 (4.1) (0, 17.8)	0 (0)	1 (0.5) (0, 14.5)
Photobacterium	117	108 (92.3) (87.3, 97.3)	5 (4.3) (0, 22.0)	0 (0)	4 (3.4) (0, 21.2)
Yersinia	82	79 (96.3) (92.2, 100)	1 (1.2) (0, 22.7)	2 (2.4) (0, 23.8)	0 (0)
Pseudomonas	78	53 (67.9) (55.4, 80.5)	8 (10.3) (0, 31.3)	12 (15.4) (0, 35.8)	5 (6.4) (0, 27.9)
Pseudoalteromonas	46	44 (95.7) (89.6, 100)	2 (4.3) (0, 32.6)	0 (0)	0 (0)
Psychrobacter	46	41 (89.1) (79.6, 98.7)	4 (8.7) (0, 36.3)	0 (0)	1 (2.2) (0, 30.8)
Serratia	34	32 (94.1) (86.0, 100)	1 (2.9) (0, 36.1)	1 (2.9) (0, 36.1)	0 (0)
Subtotal	1,071	921 (86.0) (83.8, 88.2)	89 (8.3) (2.6, 14.0)	35 (3.3) (0, 9.2)	26 (2.4) (0, 8.3)
Other 35 unique genera**	138	99 (71.7) (62.9, 80.6)	6 (4.3) (0, 20.7)	30 (21.7) (7.0, 36.5)	3 (2.2) (0, 18.7)
Overall Total	1,209	1020 (84.4) (82.1, 86.6)	95 (7.9) (2.4, 13.3)	65 (5.4) (0, 10.9)	29 (2.4) (0, 8.0)

n = number. LCL – lower 95% confidence limit. UCL – upper 95% confidence limit.

* Other species: Sablefish, Tilapia, and White Sturgeon. ** Other unique genera excludes 28 isolates unidentified; a complete list of genera is reported in Appendix 1, Table A1.1.

Table 2.2. Bacterial species of interest* isolated (n=473) from all farmed salmonid species in British Columbia by the Animal Health Centre from 2007-2018 and tested for antimicrobial susceptibility.

Bacterial species	Total isolates	Atlantic salmon	Pacific salmon	Rainbow trout	Other species**
		All numbers represent n (%), (LCL, UCL)			
Aeromonas salmonicida	174	159 (91.4) (87.0, 95.7)	11 (6.3) (0, 20.7)	2 (1.1) (0, 15.9)	2 (1.1) (0, 15.9)
Aliivibrio wodanis	84	82 (96.5) (92.5, 100)	3 (3.5) (0, 24.4)	0 (0)	0 (0)
Yersinia ruckeri	79	77 (97.5) (94.0, 100)	0 (0)	2 (2.5) (0, 24.3)	0 (0)
Vibrio tapetis	43	43 (97.7) (93.3, 100)	1 (2.3) (0, 31.5)	0 (0)	0 (0)
Vibrio splendidus	40	35 (87.5) (76.5, 98.5)	2 (5.0) (0, 35.2)	0 (0)	3 (7.5) (0, 37.3)
Aeromonas sobria	21	11 (52.4) (22.9, 81.9)	2 (9.5) (0, 50.2)	8 (38.1) (4.4, 71.7)	0 (0)
Vibrio anguillarum	19	1 (5.3) (0, 49.0)	16 (84.2) (66.3, 100)	0 (0)	2 (10.5) (0, 53.1)
Vibrio ordalii	13	0 (0)	13 (100)	0 (0)	0 (0)
Total	473	408 (86.3) (82.5, 89.3)	48 (10.1) (1.6, 18.6)	12 (2.5) (0, 11.4)	7 (1.5) (0, 10.4)

n = number. LCL – lower 95% confidence and UCL – upper 95% confidence limit for the percentage.

* The following species (*Aeromonas salmonicida*, *Aeromonas sobria*, *Vibrio anguillarum*, *Vibrio ordalii*, and *Yersinia ruckeri*) were identified *a priori* as being of interest for analysis and reporting by the British Columbia Ministry of Agriculture and Food. The additional species included had isolates greater than 30 for the study period.

** Other species: Sablefish, Tilapia, and White Sturgeon.

Table 2.3. Antimicrobial susceptibility results for isolates of the bacterial species of interest (n=473) from all farmed salmonid species in British Columbia by the Animal Health Centre from 2007-2018

Bacterial species*	n	FLOR		OXY		SXT		TRI	
		S	R	S	R	S	R	S	R
		All numbers represent n (%), (LCL, UCL)							
Aeromonas salmonicida	174	142 (81.6) (75.2, 88)	32 (18.4) (5.0, 31.8)	136 (78.2) (71.2, 85.1)	38 (21.8) (8.7, 35)	134 (77.0) (69.9, 84.1)	40 (23.0) (9.9, 36.0)	119 (68.4) (60.0, 76.7)	55 (31.6) (19.3, 43.9)
Aliivibrio wodanis	84	83 (98.8) (96.5, 100)	1 (1.2) (0, 22.4)	84 (100)	0 (0)	82 (97.6) (94.3, 100)	2 (2.4) (0, 23.5)	75 (89.3) (82.3, 96.3)	9 (10.7) (0, 30.9)
Yersinia ruckeri	79	79 (100)	0 (0)	78 (98.7) (96.3, 100)	1 (1.3) (0, 23.2)	78 (98.7) (96.3, 100)	1 (1.3) (0, 23.2)	51 (64.6) (51.4, 77.7)	28 (35.4) (17.7, 53.2)
Vibrio tapetis	43	43 (100)	0 (0)	43 (100)	0 (0)	43 (100)	0 (0)	38 (88.4) (78.2, 98.6)	5 (11.6) (0, 39.7)
Vibrio splendidus	40	40 (100)	0 (0)	39 (97.5) (92.6, 100)	1 (2.5) (0, 33.1)	38 (95.0) (88.1, 100)	2 (5.0) (0, 35.2)	34 (85.0) (73.0, 97.0)	6 (15.0) (0, 43.6)
Aeromonas sobria	21	21 (100)	0 (0)	16 (76.2) (55.3, 97.1)	5 (23.8) (0, 61.1)	21 (100)	0 (0)	10 (47.6) (16.7, 78.6)	11 (52.4) (22.9, 81.9)
Vibrio anguillarum	19	19 (100)	0 (0)	19 (100)	0 (0)	18 (94.7) (84.4, 100)	1 (5.3) (0, 49.0)	8 (42.1) (7.9, 76.3)	11 (57.9) (28.7, 87.1)
Vibrio ordalii	13	13 (100)	0 (0)	13 (100)	0 (0)	13 (100)	0 (0)	8 (61.5) (27.8, 95.3)	5 (38.5) (0, 81.1)
Total	473	440 (93.0) (90.6, 95.4)	33 (7.0) (0, 15.7)	428 (90.5) (87.7, 93.3)	45 (9.5) (0.9, 18.1)	427 (90.3) (87.5, 93.1)	46 (9.7) (1.2, 18.3)	343 (72.5) (67.8, 77.2)	130 (27.5) (19.8, 35.2)
All other species**	762	676 (88.7) (86.3, 91.1)	86 (11.3) (4.6, 18)	682 (89.5) (87.2, 91.8)	80 (10.5) (3.8, 17.2)	674 (88.5) (86.0, 90.9)	88 (11.5) (4.9, 18.2)	538 (70.6) (66.8, 74.5)	224 (29.4) (23.4, 35.4)
Total**	1,235	1116 (90.4)	119 (9.6)	1110 (89.9)	125 (10.1)	1101 (89.1)	134 (10.9)	881 (71.3)	354 (28.7)

	(88.6, 92.1)	(4.3, 14.9)	(88.1, 91.7)	(4.8, 15.4)	(87.3, 91.0)	(5.6, 16.1)	(68.4, 74.3)	(24.0, 33.4)

n = number of isolates.

S = susceptible. R = resistant. LCL = lower 95% confidence limit and UCL = upper 95% confidence limit for the percentage.

FLOR = florfenicol. OXY = oxytetracycline. SXT = trimethoprim-sulfadiazine. TRI = sulphamerazine, sulphathiazole, and sulfadiazine.

* The following species (*Aeromonas salmonicida*, *Aeromonas sobria*, *Vibrio anguillarum*, *Vibrio ordalii*, and *Yersinia ruckeri*) were identified *a priori* as being of interest for individual analysis and reporting by the British Columbia Ministry of Agriculture and Food. The additional species reported had 30 or more isolates for the study period. The remaining species isolates were reported together.

** Value included "*Bacteria*" genera group and "spp." species group.

Table 2.4. Unique antimicrobial susceptibility profiles for all bacterial isolates (n=1,040) from farmed Atlantic salmon by the British Columbia Animal Health Centre from 2007-2018.

Resistance pattern	N
Pan-susceptible	683
TRI	177
FLOR-OXY-SXT-TRI	40
FLOR	25
OXY-TRI	20
SXT-TRI	19
OXY	16
OXY-SXT-TRI	14
SXT	13
FLOR-SXT	11
FLOR-SXT-TRI	7
FLOR-OXY	7
FLOR-OXY-SXT	4
FLOR-TRI	2
OXY-SXT	1
FLOR-OXY-TRI	1

FLOR = florfenicol. OXY = oxytetracycline. SXT = trimethoprim-sulfadiazine. TRI = sulphamerazine, sulphathiazole, and sulfadiazine.

Table 2.5. Unique antimicrobial susceptibility profiles for *Aeromonas salmonicida* isolates (n=159) from farmed Atlantic salmon by the British Columbia Animal Health Centre from 2007-2018.

Resistance pattern	N
Pan-susceptible	107
TRI	12
SXT	3
SXT-TRI	1
OXY-SXT-TRI	4
FLOR-OXY-SXT-TRI	32

FLOR = florfenicol. OXY = oxytetracycline. SXT = trimethoprim-sulfadiazine. TRI = sulphamerazine, sulphathiazole, and sulfadiazine.

Table 2.6. Annual antimicrobial susceptibility results from *Aeromonas salmonicida* isolates (n=174) from all farmed salmonid species in British Columbia by the Animal Health Centre from 2007-2018.

Year	FLOR		OXY		SXT		Triple sulfa	
	S	R	S	R	S	R	S	R
	All numbers represent n (%), (LCL, UCL)							
2007	3 (100)	0 (0)	3 (100)	0 (0)	2 (66.7) (1.3, 100)	1 (33.3) (0, 100)	2 (66.7) (1.3, 100)	1 (33.3) (0, 100)
2008	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
2009	12 (100)	0 (0)	12 (100)	0 (0)	12 (100)	0 (0)	11 (91.7) (75.3, 100)	1 (8.3) (0, 62.5)
2010	26 (81.3) (66.2, 96.3)	6 (18.8) (0, 50)	26 (81.3) (66.2, 96.3)	6 (18.8) (0, 50.0)	26 (81.3) (66.2, 96.3)	6 (18.8) (0, 50)	25 (78.1) (61.9, 94.3)	7 (21.9) (0, 52.5)
2011	13 (100)	0 (0)	13 (100)	0 (0)	13 (100)	0 (0)	10 (76.9) (50.8, 100)	3 (23.1) (0 70.8)
2012	26 (65.0) (46.7, 83.3)	14 (35.0) (10.0, 60.0)	22 (55.0) (34.2, 75.8)	18 (45.0) (22, 68)	22 (55.0) (34.2, 75.8)	18 (45.0) (22, 68)	21 (52.5) (31.1, 73.9)	19 (47.5) (25.0, 70.0)
2013	20 (62.5) (41.3, 83.7)	12 (37.5) (10.1, 64.9)	20 (62.5 (41.3, 83.7)	12 (37.5) (10.1, 64.9)	20 (62.5) (41.3, 83.7)	12 (37.5) (10.1, 64.9)	18 (56.3) (33.3, 79.2)	14 (43.8) (17.8, 69.7)
2014	4 (100)	0 (0)	4 (100)	0 (0)	2 (50) (0, 100)	2 (50) (0, 100)	4 (100)	0 (0)
2015	4 (100)	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)	4 (100)	0 (0)
2016	5 (100)	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)	5 (100)	0 (0)

2017	13 (100)	0 (0)	13 (100)	0 (0)	12 (92.3) (77.2, 100)	1 (7.7) (0, 59.9)	8 (61.5) (27.8, 95.3)	5 (38.5) (0, 81.1)
2018	16 (100)	0 (0)	14 (87.5) (70.2, 100)	2 (12.5) (0, 58.3)	16 (100)	0 (0)	11 (68.8) (41.4, 96.1)	5 (31.3) (0, 71.9)

n = number of isolates.

S = susceptible. R = resistant. LCL = lower 95% confidence limit. UCL = upper 95% confidence limit.

FLOR = florfenicol. OXY = oxytetracycline. SXT = trimethoprim-sulfadiazine. TRI = sulphamerazine, sulphathiazole, and sulfadiazine.

CHAPTER 3 **Antimicrobial Use and Antimicrobial Resistance in the British Columbia Finfish Aquaculture Industry (2007-2018)**

3.1 **Abstract**

Antimicrobial use (AMU) is an important tool for bacterial disease management in finfish aquaculture. Despite the common use of antimicrobials, there remains a paucity of data investigating the linkages between aquatic AMU and the development of antimicrobial resistance (AMR) in finfish aquaculture production. The objective of this study was to evaluate the relationship between AMU and AMR in the British Columbia (BC) finfish aquaculture industry using historical surveillance data.

Antimicrobial susceptibility data were obtained from the BC Ministry of Agriculture and Food (BC MAF) via submissions to the Animal Health Centre (AHC) for 2007-2018. Antimicrobial use data were also provided by the Ministry of Agriculture from feed mill prescriptions for BC finfish through mandatory submissions for 2004-2018. Three multivariable logistic regression models were developed to determine significant associations with outcomes of resistance to trimethoprim sulfadiazine (SXT), oxytetracycline (OXY), and florfenicol (FLOR) using 1,040 bacterial species isolates from Atlantic salmon. All bacterial species were analyzed together using a random intercept for bacterial species.

An important predictor for resistance to any one drug as an outcome was resistance to the other antimicrobials as model variables. Resistance to SXT, FLOR, and/or OXY were all significantly associated with each resistance outcome in their respective models. Only the SXT resistance model was significantly associated with AMU, specifically potentiated sulfonamide use. Antimicrobial use was not significantly associated with AMR for any of the other resistance outcomes across these bacterial isolates from Atlantic salmon, but FLOR, OXY, and potentiated

sulfonamide use were confounding variables in the SXT and OXY resistance models. The results of this study contribute to the rapidly growing and increasingly pertinent body of literature on AMU and AMR in the unique marine aquaculture environment. Future research at the farm level linking pen-specific AMU to AMR outcomes will provide more understanding of selection pressure for AMR at the local level.

3.2 Introduction

With approximately 75 farms located in remote coastal areas of British Columbia (BC), Canada, salmon aquaculture is the province's largest agricultural export (Government of British Columbia, 2020). Atlantic salmon (*Salmo salar*) and Chinook salmon (*Oncorhynchus tshawytscha*) are the most commonly farmed finfish as they feed well on pellets and are able to grow quickly within the netted enclosures (Food and Agriculture Organization of the United Nations, 2020; Government of Canada, 2024b)

To control diseases in finfish aquaculture, producers and veterinarians utilize an array of management options that include vaccinations for prevention and in-feed antimicrobials in the face of outbreaks (Cabello et al., 2013; Fraser et al., 2004; Miranda et al., 2013; Smith, 2008; Sørum, 2005). There are upwards of 15 different antimicrobials commonly used in finfish aquaculture worldwide, with the most common active ingredients being oxytetracycline, florfenicol, and sulphadiazine (Lulijwa et al., 2020). However, in Canada, only four antimicrobials are licensed for use in finfish: florfenicol, trimethoprim-sulfadiazine (sulfa-trimethoprim), ormetoprim-sulfadimethoxine (sulfa-ormetroprim), and oxytetracycline (Canadian Food Inspection Agency (CFIA), 2019; Love et al., 2020; Lulijwa et al., 2020). All of these drugs are considered "medically important" by Health Canada, with the following categorization: florfenicol (category III), sulfadiazine-trimethoprim (II/III), sulfadimethoxine-

ormetoprim (III), and oxytetracycline (III) (Government of Canada, 2009). Resistance to these drugs exists in finfish pathogens. (González-Gaya et al., 2022; Heuer et al., 2009; Love et al., 2020; Miranda et al., 2013).

Previous studies have identified AMU as a risk factor for the emergence of AMR through selection pressure (González-Gaya et al., 2022; Love et al., 2020). Although there remains a need to empirically attribute AMR to local AMU in the context of aquaculture, there is no shortage of studies positing the existence of this link (Miranda et al., 2018; Miranda & Zemelman, 2002; Tuševljak et al., 2013). Attributions of AMR to AMU vary among authors, ranging from generalized assumptions to specific proposals that regional AMR profiles are a direct result of local AMU trends (Higuera-Llanten et al., 2018). Whether or not a clear causal relationship between AMU and AMR in the marine environment exists, it remains important to consider both AMR and AMU concurrently during surveillance efforts. Aquaculture in coastal BC provides a unique opportunity to observe such pooled data due to the relationship between government and industry in the area. The objective of this study was to evaluate the relationship between AMR and AMU in BC finfish aquaculture using retrospective, province-level surveillance data for AMR and AMU provided by the Government of BC.

3.3 **Methods**

Anonymized AMR data for farmed finfish species were provided by the BC Ministry of Agriculture and Food (MAF) Animal Health Centre (AHC). The data included antimicrobial susceptibilities for bacterial isolates from finfish for florfenicol (FLOR), oxytetracycline (OXY), trimethoprim-sulfadiazine (SXT), and a sulphonamide compound (triple-sulfa: sulphamerazine, sulphathiazole, and sulfadiazine) for the years 2007-2018, as described in Chapter 2. Isolates for

this study were limited to those from Atlantic salmon (*salmo salar*) from this isolate collection (1,040/1,237).

Antimicrobial use at the provincial level was estimated using the mg/PCU (population correction unit) metric. We collated annual weights (in mg) of antimicrobials administered through feed from prescription information, as well as annual slaughter production output by weight (kg) for the PCU as has been recommended and used for finfish due to lack of standard weights and reported fish numbers (European Medicines Agency, 2023; Grave et al., 2012; Narbonne et al., 2021). Antimicrobial use data were provided by the BC MAF from BC feed mills through mandatory submissions of antimicrobial prescriptions for 2004-2018 inclusive. These submissions were stratified by year, drug, and fish species (Atlantic or Pacific salmon or Rainbow trout). Antimicrobial use data were also limited to that from Atlantic salmon. The data included the following antimicrobial drugs: OXY, FLOR, SXT, sulfadimethoxine + ormetoprim (OMS), erythromycin, and lincomycin. We included use by drug for OXY, FLOR, SXT, and OMS to assess potential associates with different AMR outcomes. Erythromycin and lincomycin were included in the analysis for overall AMU, but susceptibility was not tested for these drugs.

Annual species-specific production (slaughter) weights were retrieved from the BC MAF via "Fast Stats" reports and "Seafood Industry Year in Review Reports" as well as Government of Canada aquaculture production values for BC (British Columbia Ministry of Agriculture, 2018; Government of British Columbia, 2018; Government of Canada, 2024b). Production, AMU, and AMR data for Pacific salmon (*Oncorhynchus tschawytscha*, *Oncorhynchus kisutch, Oncorhynchus keta*, *Oncorhynchus gorbusha*, and *Oncorhynchus nerka*), Sablefish or Blackcod (*Anoplopoma fimbria*), Arctic Char (*Salvelinus alpinus*), Tilapia (*Oreochromis niloticus*), and other marginal finfish species were excluded from the analysis. Annual AMU for the province

was collated on Excel® (Microsoft Corporation, Redmond, WA) as total AMU (mg) standardized by the PCU into mg/PCU values for each year of data.

Separate multilevel, multivariable logistic regression models were created to identify factors associated with each outcome of FLOR, OXY, and SXT resistance for the bacterial isolates of all species (n=1,040) from Atlantic salmon over the study period as there were not enough for each individual species isolates to facilitate separate analyses. Given that AMR is highly likely to cluster by bacterial species, it was included as a random intercept in all models. The models were built using manual backwards stepwise selection. Based on the available data, explanatory variables considered in each model were: resistance to the drugs that were not included as the outcome, AMU in mg/PCU (use of FLOR, OXY, SXT, SXT+OMS, and total AMU of all drugs), and year to control for temporal effects.

Variables were assessed for collinearity using Spearman and Pearson correlation coefficients. Decisions were made to include one of two variables in situations where high collinearity interfered with model convergence or caused problems with parameter estimation. Total AMU was often highly correlated with the individual drug use variables and interfered with model convergence and was excluded from consideration in final models. Likelihood ratio tests (LRTs) were used to determine variable inclusion in the models. Individual variables were screened and included for consideration in the multivariable model if the LRT $p \leq 0.20$. Continuous variables were checked for the linear relationships with the outcome by assessing quadratics (squared terms), quartile indicators, and lowess and linear trend plots. Where required, Akaike and Bayesian Information Criteria (AIC/BIC) were used to assist in final decisions on how to model variables (lower values indicating better fit). The threshold for inclusion in the final multivariable model was $p \leq 0.05$. Two-way interactions were tested for all

statistically significant variables in the final model and were included if they had LRT $p \leq 0.05$. All non-significant AMU variables were considered for confounding and were included if they changed the log odds of any coefficients of the final model by greater than 25%. All analyses were complete using STATA® BE (version 17.0, College Station, TX).

3.4 **Results**

The proportions of resistance to OXY, FLOR, and SXT, as well as the mg/PCU AMU values for OXY, FLOR, SXT, and potentiated sulfonamides are shown in Table 3.1. A comprehensive description of AMU is described elsewhere (Narbonne, 2021) and resistance trends can be found in Chapter 2.

All variables (FLOR resistance, OXY resistance, FLOR use quartiles, OXY use quadratic, SXT use quartiles, potentiated sulfonamide use quartiles, and year quadratics) were significantly associated with SXT resistance in the univariable models ($p<0.05$) (Table 3.2). For the final multivariable SXT model, FLOR resistance, OXY resistance, potentiated sulfonamide use quartile indicators, and an interaction between OXY resistance and potentiated sulfonamide use were significantly associated with SXT resistance (Table 3.3). Confounders in the model included the OXY use quadratic, FLOR use quartiles, and a year quadratic. Isolates with FLOR resistance had significantly higher odds of being associated with SXT resistance (OR 22.02) (Table 3.3). Linear combinations of the interaction between OXY and sulfonamide use were tested holding all other variables in the model constant (Table 3.4, 3.5, 3.6). Generally, isolates that were resistant to OXY and had potentiated sulfonamide use in Q2, Q3, or Q4 had significantly higher risk of being resistant to SXT than isolates not resistant to OXY (n=8/12 contrasts) (Table 3.4). Comparisons of OXY resistant isolates and different sulfonamide use quartiles to OXY resistant isolates revealed only one significant result (Table 3.5); comparison

of OXY-susceptible isolates to other susceptible isolates within sulfonamide use quartiles revealed no significant differences (Table 3.6).

Florfenicol resistance, SXT resistance, FLOR use quartiles, OXY use quadratic, SXT use quadratic, and year quadratic were all significantly associated with OXY resistance in the univariable models ($p<0.05$); the potentiated sulfonamide use quadratic ($p=0.10$) was also considered for the final multivariable model (Table 3.7). Only two variables were significantly associated with OXY resistance in the final, multivariable model: FLOR resistance, and SXT resistance (Table 3.8); there were no confounding variables or interactions (data not shown). Isolates that were resistant to FLOR had 28.6 times higher odds of being resistant to OXY compared to FLOR-susceptible isolates. Isolates that were resistant to SXT had 10.56 times higher odds of being resistant to OXY resistance when compared to FLOR-susceptible isolates.

Oxytetracycline resistance, SXT resistance, FLOR use quartiles, and SXT use quartiles were all significantly associated with OXY resistance in the univariable models for FLOR resistance ($p<0.05$); the potentiated sulfonamide use quadratic and year quadratic were also considered ($p<0.20$) (Table 3.9). The final model for FLOR resistance included resistance to SXT resistance to OXY, year, and an interaction between OXY resistance and the year quadratic (Table 3.10), as well as a quadratic for potentiated sulfonamide use, and quartiles for both OXY and FLOR use as confounders. Isolates that were resistant to SXT had 37.28 times higher odds of being resistant to FLOR resistance compared to SXT-susceptible. The predicted probability of FLOR resistance based on the interaction of OXY resistance and year (Figure 3.1) showed that OXY-resistant isolates had higher odds of being FLOR-resistant compared to OXY-susceptible isolates, and that this resistance was greatest between 2011-2014, peaking in 2012.

3.5 **Discussion**

Our study provides the first long-term analysis of the relationship between AMR of a group of bacterial isolates and AMU in BC finfish aquaculture using retrospective, province-level data from BC provincial surveillance. Our results indicate that AMR in finfish pathogen isolates from the provincial diagnostic laboratory were generally not associated with AMU of drugs for which antimicrobial susceptibility was tested at the provincial level. The most common predictor associated with AMR outcomes was resistance to another drug in that isolate.

Although other studies have attributed AMR in a region to local AMU by aquaculture (Higuera-Llanten et al., 2018; Tuševljak et al., 2013), the link between AMR and AMU in the unique aquaculture environment is not yet well supported empirically, and multiple authors state the need for future research in this area (Brunton et al., 2019; Fraser et al., 2004; Larsson et al., 2018; Mardones et al., 2018; Miranda et al., 2013; Miranda et al., 2018; Preena et al., 2020). Watts et al. (2017) addressed the fact that most studies evaluating AMR in aquaculture do not assess or determine the direct, actual association between AMU and the prevalence of AMR. The authors did posit, however, that it is highly likely AMU drives AMR, as it does in terrestrial systems (Vanderhaeghen & Dewulf, 2017), and that environmental residues certainly apply selection pressure for AMR genes (ARGs) and accelerate horizontal gene transfer.

One of the reasons for the scarcity of studies on the links between AMU and AMR in farmed salmonids is due to a universal lack of data (Caputo et al., 2023; Fraser et al., 2004; Watts et al., 2017). Even when AMU data are available, they are often not reported alongside or linked directly to AMR at the isolate, clinical specimen, or farm level. For example, a recent Canadian East-Coast study that reported AMR in diagnostic submissions from farmed salmon did not assess linkages to AMU (Ojasanya et al., 2022). Alternatively, both Morrison and

Saksida (2013) and Jonah et al. (2024) reported regional AMU data for BC salmonids, but did not compare this data with local AMR.

Even when both AMR and AMU data are available and reported, authors such as Smith (2008) criticize the overall validity of assessing AMR based on current analytical methods. As of 2020, there exists no objective, standardized, or validated clinical breakpoints for bacterial species and antimicrobials within the marine environment. For example, Smith (2006) described various breakpoints used in different laboratories for classifying marine bacteria as susceptible or resistant to OXY based on disk diffusion assays, and noted a zone of inhibition variance of 24mm between labs. As a result, comparisons of AMR prevalence between studies needs to be conducted with caution and attention to these differences. Additional limitations in AMR and AMU studies include the lack of accounting (or lack of data) for intrinsic resistance, the scarcity of comprehensive regional AMU data, and the presumption that terrestrial and marine pharmacodynamics are comparable in terms of residue half-life and the degree and reach of selection pressure imposed by residues, among others (Smith, 2008; Watts et al., 2017).

Although the lack of statistical association between AMR for the drugs tested and AMU in our collection of isolates seems contradictory to commonly stated understandings of antimicrobial selection pressure, our findings agree with much of the existing literature in aquaculture. A Chilean study on farmed salmon from 2012 identified a high degree of phenotypic resistance to OXY, FLOR, and oxolinic acid in sediments near an aquaculture site using these AMDs, but found a similar composition of ARGs in distant populations with no appreciable AMU nearby (Buschmann et al., 2012). The findings seem to indicate that local AMU may not be the primary driver of local phenotypic resistance, and that resistance to one drug is highly associated with resistance to others. Another consideration is the lack of

understanding of the potential link, or lack thereof, between phenotypic and genotypic resistance that is common to more than aquaculture bacteria. The unpredictable nature of this link in marine fish bacteria has been questioned (Gao et al., 2023). Collectively, this suggests the need for more directed research in this area.

Another study found that the proportion of phenotypic AMR in bacterial isolates was higher in aquaculture site sediments than control site sediments, but noted that the difference was not large and the AMR profile was similar between the two sample sites (Miranda & Rojas, 2007). A separate study assessed the links between phenotypic AMR and AMU in a local aquaculture facility by sampling 17 sites upstream, immediately downstream, and far downstream of freshwater aquaculture facilities (Gordon et al., 2007). There was no correlation between the presence of the antimicrobial use and phenotypic AMR. A global review on drivers of AMU in aquaculture identified compensatory increases in antimicrobial treatment in response to AMR as a reason for growing AMU trends, providing some competing evidence against the narrative that AMU instead drives AMR (Henriksson et al., 2018).

There are studies that do not align with our findings. A study of sediment samples below, near, and far from marine salmon pens following treatment with OXY identified that the prevalence of OXY resistance was significantly higher in samples taken underneath pens compared to distant samples, accounting for background resistance rates (Kerry et al., 1996). However, there was no quantitative link, as higher AMU was not associated with higher levels of AMR, and the authors suspected another unknown factor may be responsible for the differences in AMR. They also reported that resistance was found beyond the detection of residues, affirming that resistance may spread independently of selection pressure, possibly through horizontal genetic transfer. Similarly, Tendencia and de la Peña (2001) provided strong evidence

of the link between AMR and AMU in the context of terrestrial, semi-closed shrimp systems, as well as co-selection of ARGs. Purely statistical associations between AMU and multidrug resistance in aquaculture species (including molluscs, ornamental fish, and some terrestrial systems) over 20 years in Asia have also been reported (Schar et al., 2021).

This raises the question about phenotypic resistance compared to evidence that AMU increases the selection pressure for development and maintenance of ARGs in the environment. Many studies highlight the danger of increasing the rate of horizontal genetic transfer with sub-therapeutic levels of antimicrobials, both at the site of AMU and distally (Gao et al., 2012; Kerry et al., 1996; Millanao et al., 2018; Shah et al., 2014; Smith, 2008; Tendencia & de la Peña, 2001; Watts et al., 2017; Zhu et al., 2017). Gao et al. (2012) and Kerry et al. (1996) both provide some of the strongest evidence supporting the selection pressure imposed by aquaculture AMU for ARGs in the local environment. These studies compared ARG ratios against various drugs in nearby bacteria and compared these ratios to the most prevalent antimicrobial used locally at aquaculture facilities. The authors found an increase in ARGs corresponding to the most locally pervasive antimicrobial drug. It is important to note that only one study (Kerry et al., 1996) compared the phenotypic resistance profile to background resistance from a distant site free of selective pressure, while the other (Gao et al., 2012) did not. Millanao et al. (2018) showed that AMU derived selection pressure affected microbe populations up to 8km distant to their site of use. Another study showed that ARGs conferring multi-drug resistance can persist for years after AMU is stopped (Domínguez et al., 2019).

Horizontal genetic transfer and an increase in the environmental resistome exacerbates the threat of co-selection and cross resistance in aquaculture, leading to the emergence of multi-drug resistance (González-Gaya et al., 2022; Higuera-Llanten et al., 2018; Inglis et al., 1993).

This is supported by the results of our study, where we identified a strong association between resistance of drugs from different classes in our isolates, as well as by the consensus of current literature. Studies have identified multiple ARGs to different classes of antimicrobials on the same mobile genetic element, facilitating the transfer of multidrug resistance from the selection pressure of just one antimicrobial (Domínguez et al., 2019; Eckstrand et al., 2024; Higuera-Llanten et al., 2018; Tendencia & de la Peña, 2001). Further studies have identified the risk for environmental elements, such as the metals used to construct marine fish pens, for providing enough selection pressure for maintenance and proliferation of these multi-drug resistance elements (Watts et al., 2017).

Our bacterial isolate data were skewed towards *A. salmonicida* (Chapter 2), which is not uncommon for aquaculture studies. Ojasanya et al., found *A. salmonicida* composed 16% (the second highest percentage) of their isolates. Although *A. salmonicida* is the most common species in our data, the findings of Gordon et al. (2007) appear to validate its use as an appropriate study organism for the link between AMR and AMU. Gordon et al. (2007) deliberately focused their AMR-AMU comparison on *Aeromonas* isolates compared to *Pseudomonas* due to low intrinsic resistance to FLOR and OXY. Furthermore, AMR studies on *A. salmonicida* are well documented in the literature; Sørum (2005) provided a comprehensive, chronological overview of resistance in *A. salmonicida* in aquaculture starting in 1959 (the first study on acquired resistance in a fish pathogen).

Our study was limited by the number of isolates of each bacterial species. While it would be ideal to have a larger isolate population, these data represent 11 years of data, with it remaining unlikely that more isolates per species will be available over time. The species-specific elements that likely impact AMR were accounted for by modeling bacterial species as a

random effect in our models, but specific species affects could not be assessed using this approach. It is also possible that assessing AMU at the level of the provincial population of farmed salmonids and AMR at the level of the provincial bacterial isolate collection also obscured direct linkages between AMR and AMU at the farm site, pen, or fish-level. Having AMU and AMR data linked at the fish production unit level over time would be an ideal way to assess this direct relationship. However, such data were lacking in provincial and national surveillance programs at the time of this study. This is an important area for future research.

The limitations regarding data inclusion and validation stem from our reliance of historical provided data for this study. For this reason, organisms of current interest to the aquaculture industry, such as *T. maritimum*, were not isolated. Another limitation is that this is a cross-sectional study. As a result, it is not possible to infer causation from statistical associations.

3.6 **Conclusions**

This analysis of historical finfish bacterial isolates provides an important first look at the linkage between AMR and AMU in marine aquaculture settings off the coast of BC using province-level data. We found no significant statistical association between use of an antimicrobial and resistance to that same antimicrobial other than sulfonamide use and SXT resistance. The most common association with each resistance outcome was resistance to another drug, highlighting the threat of horizontal gene transfer in the marine resistome. These results will guide future research and surveillance on bacterial species-specific AMU-AMR trends as well as more holistic analyses elucidating environment, managerial, or fish-related factors responsible for AMU and AMR in BC aquaculture.

Table 3.1 Summary statistics for the variables considered in the assessment of links between antimicrobial use (mg/PCU – population correction unit in kg) and antimicrobial resistance (AMR) in bacterial isolates from farmed Atlantic salmon in British Columbia, Canada.

Variable	Proportion (95% CI)		
OXY resistance	0.10 (0.08, 0.12)		
FLOR resistance	0.09 (0.08, 0.11)		
SXT resistance	0.10 (0.09, 0.12)		
Variable	Mean (95% CI)	Standard Deviation	Notes
OXY use (mg/PCU)	51.73 (49.76, 53.71)	32.48	Q1: <29.39 Q2: 29.39<44.60 Q3: 44.60<61.09 Q4: >61.09
FLOR use (mg/PCU)	19.15 (18.25, 20.05)	14.78	Q1: <7.86 Q2: 7.86<18.69 Q3: 18.69<29.03 Q4: >29.03
SXT use (mg/PCU)	2.51 (2.30, 2.71)	3.41	Q1: 0.00 Q2: 0.00<1.00 Q3: 1.00<4.47 Q4: >4.47
Potentiated sulfonamide use (mg/PCU)	2.88 (2.67, 3.08)	3.32	Q1: <1.05 Q2: 1.05<1.67 Q3: 1.67<4.77 Q4: >4.77

Table 3.2. Results of the multilevel, univariable logistic regression models for trimethoprim-sulfadiazine (SXT) resistance in bacterial isolates from Atlantic salmon from British Columbia. The models included a random intercept for bacterial species.

Variable		Odds Ratio (95% CI)	Wald *P* Value	LRT *P* Value
FLOR resistance				<0.01
	Susceptible	Referent	--	
	Resistant	67.14 (27.28, 165.25)	<0.01	
OXY resistance				<0.01
	Susceptible	Referent	--	
	Resistant	26.85 (13.89, 51.92)	<0.01	
FLOR use*			0.01	<0.01
	Quartile 1	Referent	--	
	Quartile 2	1.27 (0.76, 2.11)	0.36	
	Quartile 3	0.25 (0.06, 1.15)	0.08	
	Quartile 4	0.52 (0.28, 0.97)	0.04	
OXY use*			0.06	0.02
	OXY use	1.04 (1.00, 1.08)	0.04	
	$(OXY)^2$	1.00 (1.00, 1.00)	0.02	
SXT use*			0.04	0.03
	Quartile 1	Referent	--	
	Quartile 2	1.15 (0.49, 2.70)	0.75	
	Quartile 3	2.21 (1.18, 4.17)	0.01	
	Quartile 4	2.25 (1.16, 4.34)	0.02	

Potentiated sulfonamide use*			0.02	0.01
	Quartile 1	Referent	--	
	Quartile 2	2.74 (1.36, 5.49)	<0.01	
	Quartile 3	1.39 (0.71, 2.71)	0.34	
	Quartile 4	2.20 (1.13, 4.26)	0.02	
Year			0.02	0.01
	Year	0.88 (0.79, 0.97)	<0.01	
	$(Year)^2$	0.99, (0.96, 1.01)	0.38	

LRT – likelihood ratio test. CI - confidence interval. OXY – oxytetracycline. FLOR – florfenicol. *mg/PCU – antimicrobial use measured as total mg of antimicrobial administered by population correction unit – kg of annual slaughter mass.

Table 3.3. Final results of the multilevel, multivariable logistic regression model for trimethoprim-sulfadiazine (SXT) resistance in bacterial isolates from Atlantic salmon from British Columbia. The model included a random intercept for bacterial species.

Variable		Odds Ratio (95% CI)	Wald *P* Value	LRT *P* Value
FLOR resistance				<0.01
	Susceptible	Referent	--	
	Resistant	22.02 (7.53-64.42)	<0.01	
OXY resistance				<0.01
	Susceptible	Referent	--	
	Resistant	0.88 (0.16-4.69)	0.88	
Potentiated sulfonamide use*				<0.01
	Quartile 1	Referent	--	
	Quartile 2	0.80 (0.05-12.66)	0.87	
	Quartile 3	0.12 (0.00-3.57)	0.22	
	Quartile 4	8.14 (0.98-67.54)	0.92	
OXY resistance x potentiated sulfonamide use*				<0.01
	OXY S * Quartile 1	Referent	--	

	OXY R * Quartile 2	50.74 (4.91-524.05)	<0.01	
	OXY R * Quartile 3	29.12 (3.07-275.94)	<0.01	
	OXY R * Quartile 4	8.14 (0.98-67.54)	0.05	
FLOR use*				0.56**
	Quartile 1	Referent	--	
	Quartile 2	0.17 (0.01-4.56)	0.29	
	Quartile 3	4.42 (<0.01-7313.59)	0.69	
	Quartile 4	--	--	--
OXY use*				0.76**
	OXY use	1.08 (0.88-1.32)	0.48	
	$(OXY)^2$	0.9995 (0.998-1.001)	0.52	
Year				0.71**
	Year	0.79 (0.40-1.58)	0.51	
	$(Year)^2$	0.94 (0.81-1.09)	0.41	
Random effect – Isolate species		Estimate		LRT *P* Value
Species level SD (95% CI)		1.21 (0.67, 2.20)		<0.01

LRT – likelihood ratio test. CI - confidence interval. OXY – oxytetracycline. FLOR – florfenicol. *mg/PCU – antimicrobial use measured as total mg of antimicrobial administered by population correction unit – kg of annual slaughter mass. ** Included as confounders.

Table 3.4. Linear combination odds ratios and 95% confidence intervals (CI's) between potentiated sulfonamide use quartiles and isolates that were resistant and susceptible to oxytetracycline (OXY). Contrasts that had a significant likelihood ratio test p-value are left white while those that were not significant are shaded grey.

Resistance to OXY	Yes				
No	Potentiated sulfonamide use quartile	Q1	Q2	Q3	Q4
	Q1	0.88 (0.16-4.69)	35.50 (1.64-768.36)	3.00 (0.10-89.76)	8.52 (0.17-420.26)
	Q2	1.10 (0.05-24.45)	44.45 (8.94-221.09)	3.75 (0.37-37.98)	10.66 (1.39-81.95)
	Q3	7.46 (0.20-282.47)	302.22 (21.33-4282.68)	25.51 (5.16-126.00)	72.50 (4.37-1202.07)
	Q4	0.73 (0.01-36.08)	29.73 (4.26-207.61)	2.51 (0.24-26.17)	7.13 (1.92-26.53)

Table 3.5. Linear combination odds ratios and 95% confidence intervals (CI's) between potentiated sulfonamide use quartiles and isolates that were resistant to oxytetracycline (OXY). Contrasts that had a significant likelihood ratio test p-value are left white while those that were not significant are shaded grey. Repeated contrasts were not included (redacted cells).

Resistance to OXY	Yes				
Yes	Potentiated sulfonamide use quartile	Q1	Q2	Q3	Q4
	Q1		40.52 (1.36-1207.37)	3.42 (0.10-122.54)	9.72 (0.16-595.74)
	Q2			0.08 (0.01-1.08)	0.24 (0.03-2.25)
	Q3				2.84 (0.19-41.70)
	Q4				

Table 3.6. Linear combination odds ratios and 95% confidence intervals (CI's) between potentiated sulfonamide use quartiles and isolates that were susceptible to oxytetracycline (OXY). Contrasts that had a significant likelihood ratio test p-value are left white while those that were not significant are shaded grey. Repeated contrasts were not included (redacted cells).

Resistance to OXY	No				
No	Potentiated sulfonamide quartile	Q1	Q2	Q3	Q4
	Q1		0.80 (0.05-12.66)	0.12 (0.00-3.57)	1.19 (0.03-46.29)
	Q2			0.15 (0.01-1.57)	1.50 (0.28-8.10)
	Q3				10.17 (0.87-119.06)
	Q4				

Table 3.7. Results of the multilevel, univariable logistic regression models for oxytetracycline (OXY) resistance in bacterial isolates from Atlantic salmon from British Columbia. The models included a random intercept for bacterial species.

Variable		Odds Ratio (95% CI)	Wald *P* Value	LRT *P* Value
FLOR resistance				<0.01
	Susceptible	Referent	--	
	Resistant	117.56 (40.92, 337.74)	<0.01	
SXT resistance				<0.01
	Susceptible	Referent	--	
	Resistant	35.45 (17.14, 73.36)	<0.01	
FLOR use*			0.02	0.01
	Quartile 1	Referent	--	
	Quartile 2	1.47 (0.84, 2.57)	0.18	
	Quartile 3	0.15 (0.02, 1.32)	0.09	
	Quartile 4	0.55 (0.27, 1.11)	0.10	
OXY use*			0.07	0.01
	OXY use	1.05 (1.00, 1.10)	0.06	
	$(\text{OXY use})^2$	1.00 (1.00, 1.00)	0.03	
SXT use*			0.03	0.02
	SXT use	1.40 (1.08, 1.80)	0.01	
	$(\text{SXT use})^2$	0.96 (0.94, 0.99)	0.01	
Potentiated (p°) sulfonamide use*			0.13	0.10

	p° Sulfonamide use	1.29 (0.99, 1.69)	0.06	
	(p° Sulfonamide use)^2	0.97 (0.95, 1.00)	0.04	
Year			0.03	0.02
	Year	0.87 (0.78, 0.97)	0.01	
	(Year)^2	0.97 (0.94, 1.00)	0.03	

LRT – likelihood ratio test. CI - confidence interval. OXY – oxytetracycline. FLOR – florfenicol. *mg/PCU – antimicrobial use measured as total mg of antimicrobial administered by population correction unit – kg of annual slaughter mass.

Table 3.8. Final results of the multilevel, multivariable logistic regression model for oxytetracycline (OXY) resistance in bacterial isolates from Atlantic salmon from British Columbia. The model included a random intercept for bacterial species.

Variable		Odds Ratio (95% CI)	Wald *P* Value	LRT *P* Value
FLOR resistance				<0.01
	Susceptible	Referent	--	
	Resistant	28.60 (9.07-90.17)	<0.01	
SXT resistance				<0.01
	Susceptible	Referent	--	
	Resistant	10.56 (4.69-23.75)	<0.01	
Random effect – Isolate species		Estimate		LRT *P* Value
Species level SD (95% CI)		2.93 (1.89, 4.56)		<0.01

Table 3.9. Results of the multilevel, univariable logistic regression models for florfenicol (FLOR) resistance in bacterial isolates from Atlantic salmon from British Columbia. The models included a random intercept for bacterial species.

Variable		Odds Ratio (95% CI)	Wald *P* Value	LRT p-value
OXY resistance				<0.01
	Susceptible	Referent	--	
	Resistant	117.84 (40.59, 342.13)	<0.01	
SXT resistance				<0.01
	Susceptible	Referent	--	
	Resistant	108.21 (36.98, 316.65)	<0.01	
SXT use*			0.01	0.01
	Quartile 1	Referent	--	
	Quartile 2	0.74 (0.20, 2.76)	0.65	
	Quartile 3	3.46 (1.42, 8.44)	0.01	
	Quartile 4	1.50 (0.56, 4.02)	0.41	
Potentiated sulfonamide use*			0.21	0.16
	Sulfonamide use	1.19 (0.85, 1.66)	0.32	
	(Sulfonamide use)^2	0.98 (0.94, 1.01)	0.17	
FLOR use*			0.28	0.25**
	Quartile 1	Referent	--	
	Quartile 2	1.20 (0.61, 2.37)	0.59	
	Quartile 3	0.59 (0.13, 2.69)	0.49	
	Quartile 4	0.51 (0.21, 1.24)	0.14	
OXY use*			0.85	0.84**
	Quartile 1	Referent	--	
	Quartile 2	1.18 (0.55, 2.49)	0.67	
	Quartile 3	0.79 (0.34, 1.80)	0.57	
	Quartile 4	0.96 (0.39, 2.34)	0.92	

Year			0.12	0.10
	Year	0.91 (0.79 1.04)	0.16	
	$Year^2$	0.96 (0.93, 1.00)	0.04	

LRT – likelihood ratio test. CI - confidence interval. OXY – oxytetracycline. FLOR – florfenicol. *mg/PCU – antimicrobial use measured as total mg of antimicrobial administered by population correction unit – kg of annual slaughter mass. ** Included as confounders.

Table 3.10. Final results of the multilevel, multivariable logistic regression model for florfenicol (FLOR) resistance in bacterial isolates from Atlantic salmon from British Columbia. The model included a random intercept for bacterial species.

Variable		Odds Ratio (95% CI)	Wald *P* Value	LRT p-value
SXT resistance				<0.01
	Susceptible	Referent	--	
	Resistant	37.28 (8.26-168.34)	<0.01	
OXY resistance				<0.01
	Susceptible	Referent	--	
	Resistant	73.61 (9.91-546.53)	<0.01	
Year				<0.01
	Year	0.03 (<0.01-5.43)	0.18	
	$Year^2$	0.35 (0.10-1.20)	0.1	
OXY resistance x year				0.02
	OXY R x Year	0.64 (0.38-1.09)	0.1	
	OXY R x $Year^2$	0.85 (0.75-0.95)	<0.01	
FLOR use*				0.19**
	Quartile 1	Referent	--	
	Quartile 2	<0.01 (<0.01-23.28)	0.13	
	Quartile 3	<0.01 (<0.01-1.38)	0.06	
	Quartile 4	318816 (0.01-1.68*10e13)	0.16	
OXY use*				0.06**
	Quartile 1	Referent	--	
	Quartile 2	3438.30 (0.03-3.51*10e8)	0.17	
	Quartile 3	65709.36 (0.01-6.30*10e11)	0.18	
	Quartile 4	4.95*10e9 (0.13-1.93*10e20)	0.07	

Potentiated sulfonamide use*				0.17**
	Sulfonamide use	0.86 (0.06-13.12)	0.92	
	(Sulfonamide use)^2	1.09 (0.87-1.38)	0.44	
Random effect – Isolate species		Estimate		LRT *P* Value
Species level SD (95% CI)		4.00 (2.59, 6.19)		<0.01

LRT – likelihood ratio test. CI - confidence interval. OXY – oxytetracycline. FLOR – florfenicol. *mg/PCU – antimicrobial use measured as total mg of antimicrobial administered by population correction unit – kg of annual slaughter mass. ** Included as confounders.

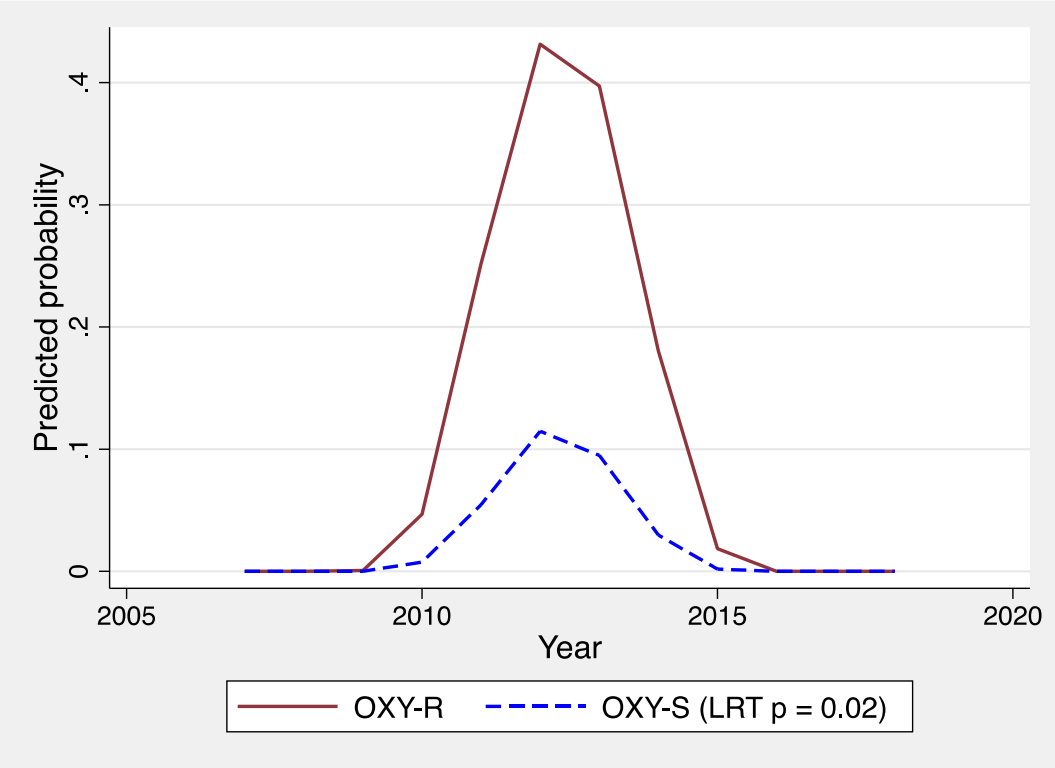

Figure 3.1. Predicted probability of SXT (trimethoprim sulfadiazine) resistance (SXT values set to resistant) from the final multivariable model that included an interaction between OXY (oxytetracycline) resistance and year quadratic when all other variables are set to median values.

CHAPTER 4 **Survival analysis to investigate risk factors for Yellow Mouth (*Tenacibaculum maritimum)* and antimicrobial use in finfish aquaculture in British Columbia**

4.1 Introduction

Yellow Mouth (YM) is a highly disruptive bacterial disease caused by one or more serotypes of the bacterium *Tenacibaculum maritimum* that frequently affects farmed salmon populations off the coast of British Columbia (BC), Canada (Wade & Weber, 2020). Mass mortality events can be caused by YM, and its effect on the aquaculture industry in BC has been estimated to cost upwards of $1.8 million in direct costs and $3.8 million in revenue loss every year (Cermaq, 2022)

Yellow Mouth is the primary reason for antimicrobial treatment of finfish in British Columbia (BC) (Wade & Weber, 2020). Antimicrobials remain an important tool for YM management. Florfenicol and potentiated sulfonamides are the most commonly used antimicrobials for the treatment of YM in BC (Wade & Weber, 2020). Antimicrobial use poses a risk of contaminating environmental sediments with active drugs, providing potential selection pressure for antimicrobial resistance genes, and the potential for increasing the rate of horizontal gene transfer in marine microbial communities. In addition to the financial cost, antimicrobial use also incurs a social cost to the aquaculture industry, as the potential for misuse and the threat of environmental pollution is frequently cited by opposition groups as a consequence of salmon aquaculture in BC (Rooney, 2023, 2024). These issues have become even more pertinent due to the recent decision of the Canadian Federal government on June 19, 2024 not to renew outstanding salmon farm licenses due to concerns raised by advocacy groups, and to ban open water salmon farming in BC entirely by June 30, 2029 (Fisheries and Oceans Canada, 2024).

The risk for YM emergence is driven by a range of environmental, biological, and managerial factors (Avendaño-Herrera et al., 2006; Mabrok et al., 2022; Nowlan et al., 2021; Santos et al., 2019; Yamamoto et al., 2010). Nowlan et al. (2021) discovered a significant relationship between salinity, oxygen, and temperature on YM associated mortalities and antimicrobial treatment while highlighting the complex interactions between these variables and biological factors such as water nutrient levels and plankton blooms. The strength and direction of this effect appeared to vary seasonally. Brosnahan et al. (2019) and Yamamoto et al. (2010) described a relationship between sea water temperatures and YM clinical signs and growth, respectively. (Santos et al., 2019) described a relationship between warm temperatures and high salinity and YM. The effect of environmental factors such as temperature on *Tenacibaculum maritimum* infection appears to vary by location and host (Mabrok et al., 2022). Time after entry of smolts into marine pens has previously been identified as an important risk marker for YM emergence, although it is confounded with age and body size of the young smolts (Avendaño-Herrera et al., 2006). This association is additionally confounded by the fact that the transfer process for smolts may introduce handling stress and skin abrasions, which are known to increase the risk of YM (van Gelderen et al., 2011.) There is a need for research evaluating the effect of extraneous environmental, managerial, and biological variables on YM incidence (Mabrok et al., 2022).

Survival analysis using Cox proportional hazards models is a pertinent way to model how variables may influence the probability of the first incidence of YM treatment with antimicrobials for marine pen placements of farmed salmon. The survival model, as presented by Cox (1972) can be described by the equation:

$$h(t, xi) = h_0(t)e^{\beta_1 xi}$$

In this model, h(t) is the risk (defined as the hazard) of a pen being treated with antimicrobials for the first time at time t, $h_0(t)$ is the baseline hazard, x is a range of independent variables that may be associated with the outcome, and β represents the coefficient(s) to be estimated for these variables. A hazard ratio greater than 1.0 represents a risk factor while a ratio lower than 1.0 is considered a protective factor.

Survival analysis allows us to model effects over time, as treatment of a pen with presumptive YM may be due to exposures that occurred days or weeks earlier and that change day to day. Additionally, YM occurrence is known to be time-dependent, with first incidence of the disease usually occurring within 120d post transition of smolts to marine pens (Avendaño-Herrera et al., 2006). The objectives of our study were to: 1) assess the factors associated with pen outbreaks with YM and requiring antimicrobial treatment using historical industry data for farmed Atlantic salmon in British Columbia, Canada; and 2) to assess the practicality of using Cox proportional hazards models for this purpose. Typically, in survival analysis, subjects are removed from the analysis after the first failure, whereas subjects that do not experience the event are considered right-censored. However, industry data reflect that consecutive antimicrobial treatments in a pen are often required due to reoccurrence of the same disease (YM) and not normally a new disease (Wade & Weber, 2020). Given the additional complexity of repeat-event survival analysis, models with re-entry conditions for multiple treatments for a given pen placement were not considered for this study. Our attempts to meet this objective, and the barriers to analysis we uncovered along the way, are detailed below.

4.2 Methods and Results

A Cox proportional hazards survival model was used to identify the factors associated with YM emergence, represented by the first antimicrobial treatment date for each pen placement in the first 120 days at sea. Data provided by a salmon production company included date and amount of antimicrobial use (AMU) along with environmental and management factors for BC pen placements from 2015-2021. A complete listing of variables for which data were provided and which we considered for screening is available in Appendix 2, Table A2.1.

Daily environmental variables for each pen for 120 days, as well as a listing of AMU dates and relevant information such as amount, brand, reason for prescription, were collated in Microsoft Excel© (version 16.8, Redmond, WA) and then merged using Microsoft Access© (version 2016 Redmond, WA). Pen placements were provided with their own unique identification number utilizing a pen identified as well as placement date. This allowed us to merge environmental data that is measured daily at the pen level with antimicrobial use data that is provided at the placement level. Variables were screened for importance of their association with total AMU for each pen placement measured as total mg of use divided by the sum of the fish count-weighted kg of biomass at each time of treatment using random forest decision tree regression models (data not shown). The following variables were considered for the survival analysis: 5m depth salinity (sal5), 10m depth salinity (sal10), 5m depth temperature (temp5), 10m depth temperature (temp10), days between 100-500g (dbwg) after being placed at sea, average weight in grams when transferred to marine pens (ingrams), biological feed conversion ratio (bfcr), the year pens were placed in the ocean (yearp), the week of the year when pens were placed in the ocean (weekp), production area (area), and production site (site). It is important to

note that pens are grouped together within sites, which are further grouped within areas. We chose not to model ingrams due to high correlation with bfcr and their redundant representation of growth rate, which would result in collinearity in models with subsequent impacts on model fit. Some variables were measured at the date of pen input or harvest and had fixed values for all observation dates (dbwg, bfcr, yearp, weekp), while others were measured daily and represented time-varying covariates (temp5, temp10, sal5, sal10) (Table A2.1.). Time-varying covariates differ from time-dependent coefficients in that the variable is measured and changes over the time leading up to failure or censoring and must be modeled with an interaction with time, even if the proportional hazards assumption is not violated by not doing so. Conversely, fixed variables must be assessed for proportional hazards over time, a key assumption for Cox proportional hazards models (Therneau & Grambsch, 2000) by also checking the significance of an interaction with time (i.e., time-dependent coefficients). The data were reorganized into a multiple-line-per-subject setup to facilitate analysis of the time-varying covariates.

Data were imported and analyzed in STATA® BE (version 17.0, College Station, TX). Cox proportional hazard models with cluster-robust standard errors for area (n=5) were used to identify factors associated with the first incidence of YM (florfenicol administration). The hierarchical structure of salmon pen placements within sites (n=17), which are within areas (n=5), was important to consider. After considering multiple methods to account for this clustering, including shared-frailty models, fixed effects, and cluster-robust standard errors, we opted to use a cluster-robust standard error for area. In the Cox proportional hazards model, cluster-robust standard errors utilize generalized estimating equations with an independent working correlation structure (Harrell, 2001). The model fit with area as a cluster-robust standard error was determined to have the best fit through visual analysis of smoothed

Schoenfeld residual plots over time. This was better than considering site for cluster-robust standard errors or a model with site as a shared frailty and area as a fixed effect. It also became evident that shared-frailty models can be very difficult to fit due to non-proportional hazards, particularly with lopsided data.

It is important when building multivariable Cox proportional hazards models that the underlying assumption of proportional hazards be assessed throughout (Harrell, 2001; Therneau & Grambsch, 2000). All variables were screened for proportional hazards visually using smoothed Schoenfeld residual plots over time and quantitatively by assessing global chi-squared test results of the same residuals (with a $p<0.05$ indicating that the assumption is violated). This proved to be our first obstacle, as very few of our fixed variables did not violate proportional hazards, requiring time-dependent coefficients, along with our time-varying covariates.

The time-varying covariates (temperature 10m and salinity 10m—those with daily measurement) and their interactions with time were modeled using restricted cubic splines to smooth the hazard function over time (Harrell, 2001). Restricted cubic splines were chosen over other polynomial transformations due to the extreme values present for each of the environmental variables and the increased stability of splines at these extremes. These splines were chosen over categorization/dichotomization to avoid the loss of data that comes with the latter. The numbers of spline knots were optimized based on assessment of the Schoenfeld residual plots and Akaike and Bayesian information criteria. Non-time varying covariates were assessed by modeling their interaction with time to determine if they had time-dependent coefficients (i.e., the interaction term LRT $p<0.05$) and through LRT tests of the variable when interacted with time.

Our data included 321 unique pen placements with 292 failures (Table 4.1). The overall mean survival time was 30.26 compared to the mean survival time for failure of 21.26. The base Kaplan-Meier survivor plot indicated that most failures occurred within the first 25-30 days after placement (Figure 1).

This screening of individual variables was complicated by the majority of models violating the proportional hazards assumption and subsequent poor Schoenfeld residual plots, even after including the time interactions for these time-dependent covariates (data not shown). Attempts to include and assess the complete list of model variables created ongoing problems with violations of the proportional hazards assumption. For example, the assumption was violated when trying to include variables such as week (Figure 4.2) and year (data not shown) in models that also included the restricted cubic splines for the time-varying covariates (temp10 and sal10). This was so extreme as to force the use of a simplified model that only included the environmental variables temp10 and sal10. In other collaborative work that used linear regression to model factors associated with total AMU for each pen placement as a continuous outcome (data not shown), we found that multivariable analysis was important to consider the collective effects of predictors, interactions, and confounding when analyzing AMU as a proxy for YM incidence.

We therefore attempted to construct our model using only temp10 and sal10, as both variables have been previously validated as important for YM incidence (Nowlan et al., 2021). Testing of proportional hazards indicated that a 5-knot spline for sal10 and a 3-knot spline for temp10 (Table 4.2) in a model including a cluster-robust standard error for area provided the best models (Table 4.3).

Selected Schoenfeld residual plots for these specific knots of these time-varying covariates (Figures 4.3 and 4.4) show that the proportional hazards assumptions are held (straight lines). This is in comparison to Figure 4.1 for the model that included week of placement with the temp10 and sal10 splines, where the proportional hazards assumptions are clearly violated.

4.3 Discussion and Key Learnings

These results from our study highlight the complexity of modeling YM incidence using first antimicrobial treatment as a proxy in the marine environment using survival analysis to identify risk factors that are both fixed and vary over time. While we had a rich dataset in terms of measured environmental and management variables linked with AMU data, continued violations of the main Cox proportional hazards model assumption precluded our ability to clearly assess variables in single or multivariable analysis. The attempt to simply include temperature and salinity as key variables, while possibly valid in terms of model assumptions, met substantial barriers in terms of model interpretation with the software that we used for analysis and the inability to replicate published script (see Chapter 5). The findings of this study highlight the difficulties associated with analyzing data that contain both time-varying covariates and time-dependent coefficients. We strongly recommend that future research utilizes R© (version 4.3.1, Vienna, Austria) to complete such difficult modelling, as there is far more guidance on syntax available for this methodology, and it appears to be the preferred tool of analysts working in the space.

Biostatistics consultations with two independent people with expertise in survival modeling strongly advised that 1) Cox proportional hazards models with time-varying covariates are complex, 2) managing violations of the proportional hazards assumption can be a barrier, and

3) it would be advantageous to consider using R© (version 4.3.1, Vienna, Austria). To manage the assumption violations, parametric survival models are often considered, which require underlying assumptions of the underlying survival function, which is not simple task (Harrell, 2001; Therneau & Grambsch, 2000). In terms of software, while more complicated to learn and program, R© (version 4.3.1, Vienna, Austria) provides the advantage of increased built-in modeling capacity for survival analysis, along with the ability to interpret the output of restricted cubic splines. After seeking guidance, we attempted to generate model predictions for the simplified model with temp10 and sal10 that included restricted cubic splines for both based on STATA® BE (version 17.0, College Station, TX) instructions (StataCorp, 2024) and a published study (Shepherd & Rebeiro, 2017). However, implementation of this script and methodology was not possible with our context and level of expertise. Our data structure and question were different than provided examples, making straight-across comparisons and use of provided scripts invalid.

Although the loss of most of our variables in our final model limits the external validity of our findings, it does confirm the potential importance of environmental parameters of interest. We found both temperature at 10m and salinity at 10m to be significantly associated with YM incidence. These variables have been previously validated as factors associated with YM incidence. (Nowlan et al., 2021). We also found that many of our variables were influenced by time. Industry expertise leads us to believe that time prior to 120d is strongly associated with YM incidence. It is important that future research considers this relationship in connection with environmental and biological variables instead of in isolation. As we were reliant on historical data, there remains an opportunity for future research to track pen outbreaks directly through surveillance studies. In collaborative work, we found that most of the variables included in our

study were associated with YM in related analysis that considered overall AMU by pen placement (data not shown). Future research could consider social analysis of managerial factors, such as operator definitions of YM or mortality benchmarks to imitate treatment.

4.4 **Conclusion:**

Our study contributes to the paucity of data available regarding the reasons and mitigations for YM AMU in farmed salmonids in BC. In addition, our study highlights the difficulty in statistical modeling a multifactorial disease such as YM and insists that future modeling incorporate as many factors involved with the marine environment as possible, accompanied with advanced capabilities of programs such as R© (version 4.3.1, Vienna, Austria) to manage these complicated models. Strategies to reduce incidence of this economically important disease are important for fish health and productivity, and we hope our database can provide a launchpad for future comprehensive analyses

Table 4.1. Summary survival characteristic for pen placements of farmed Atlantic salmon with first antimicrobial treatment used as failure point and as a proxy for an outbreak of Yellow Mouth caused by *Tenacibaculum maritimum* in British Columbia, Canada.

Category	Total	Per Subject			
		Mean	Min	Median	Max
Number of pen placements	321	--	--	--	--
Number of failures*	292	--	--	--	--
Number of days at risk prior to treatment**					
All Pens	9682	30.26	4	13	120
Pens that failed	6207	21.26	4	13	118

* Number of pens treated with antimicrobials within 120d of placement at sea.

** Number of days each pen was at risk prior to being treated.

Table 4.2. Knot locations for restricted cubic splines for salinity at 10m depth and temperature at 10m depth utilizing previously validated variable distribution percentiles.

Variable	Knot1	Knot 2	Knot 3	Knot 4	Knot 5
Temp10	8.45	10	12.9	--	--
Sal10	24.5	28	30	31.5	34

Table 4.3. Simplified survival model hazard ratio and 95% confidence interval (CI) estimates for pen placements of farmed Atlantic salmon in British Columbia, Canada where failure represents first antimicrobial treatment as a proxy for an outbreak of Yellow Mouth caused by *Tenacibaculum maritimum*, and utilizing time adjusted restricted cubic spline transformations of temperature at 10m depth and salinity at 10m depth (see Table 4.2 for spline details).

Variable		Hazard Ratio (95% CI)	Robust Standard Error	Wald *P* Value
Temp10 spline terms				
	Knot 1	1.44 (0.93, 2.22)	0.32	0.10
	Knot 2	0.62 (0.44, 0.85)	0.10	<0.01
Temp10 spline terms x time				
	(Knot 1) x time	1.00 (0.98, 1.03)	0.01	0.71
	(Knot 2) x time	0.99 (0.97, 1.02)	0.61	
Sal10 spline terms				
	Knot 1	0.75 (0.58, 0.98)	0.10	0.03
	Knot 2	3.42 (0.37, 31.29)	3.87	0.28

	Knot 3	0.00 (0.00, 3291.06)	0.01	0.39
	Knot 4	30771.80 (0.00, 5.00e18)	513750.60	0.54
Sal10 spline terms x time				
	(Knot 1) x time	1.01 (1.00, 1.01)	0.00	0.15
	(Knot 2) x time	0.97 (0.90, 1.05)	0.04	0.48
	(Knot 3) x time	1.33 (0.65, 2.70)	0.48	0.43
	(Knot 4) x time	0.42 (0.05, 3.41)	0.45	0.41

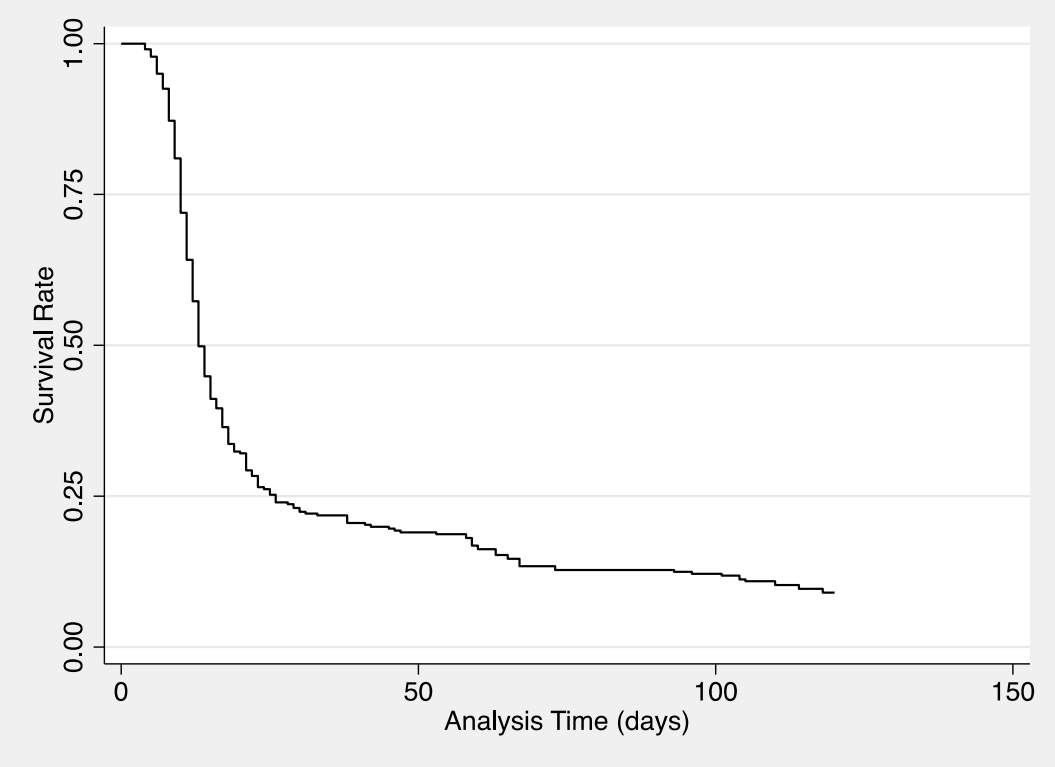

Figure 4.1. Estimated Kaplan-Meier survival curve for pen placements of farmed Atlantic salmon and time to first antimicrobial treatment as a proxy for an outbreak of Yellow Mouth caused by *Tenacibaculum maritimum* in British Columbia, Canada.

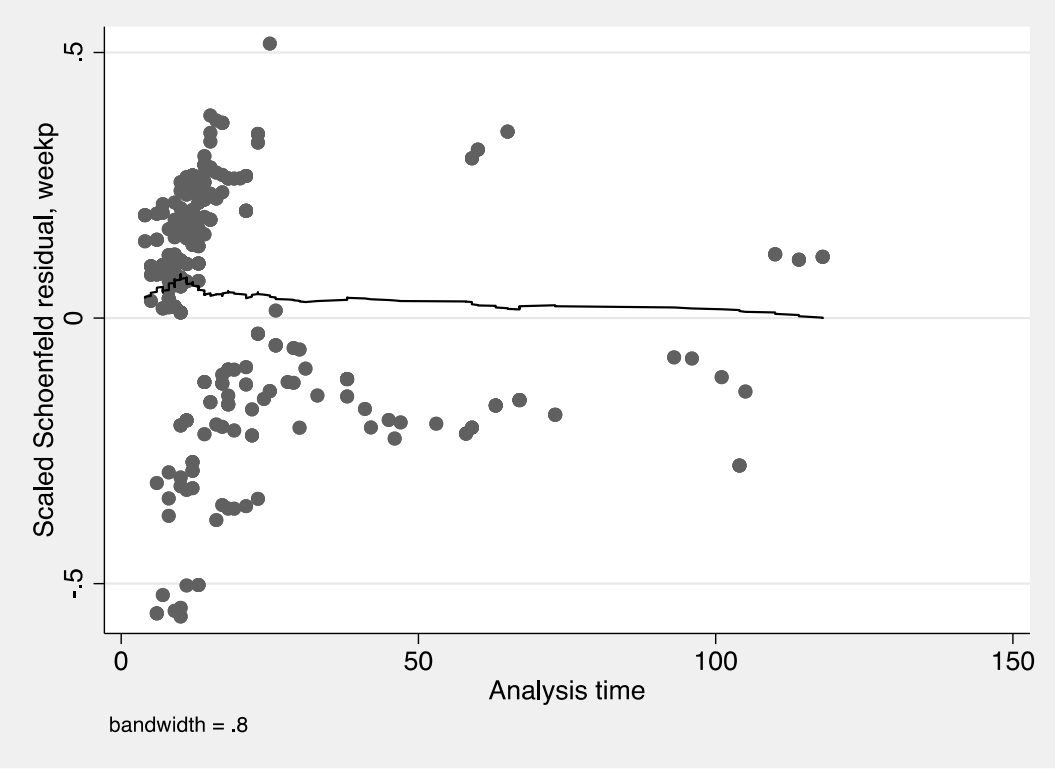

Figure 4.2. Schoenfeld residual plots over time (pen placement, days) for the week of pen placement interacting with time in a model that contained temp10 and sal10 to demonstrate unaccceptabile linearity and violation of proportional hazards.

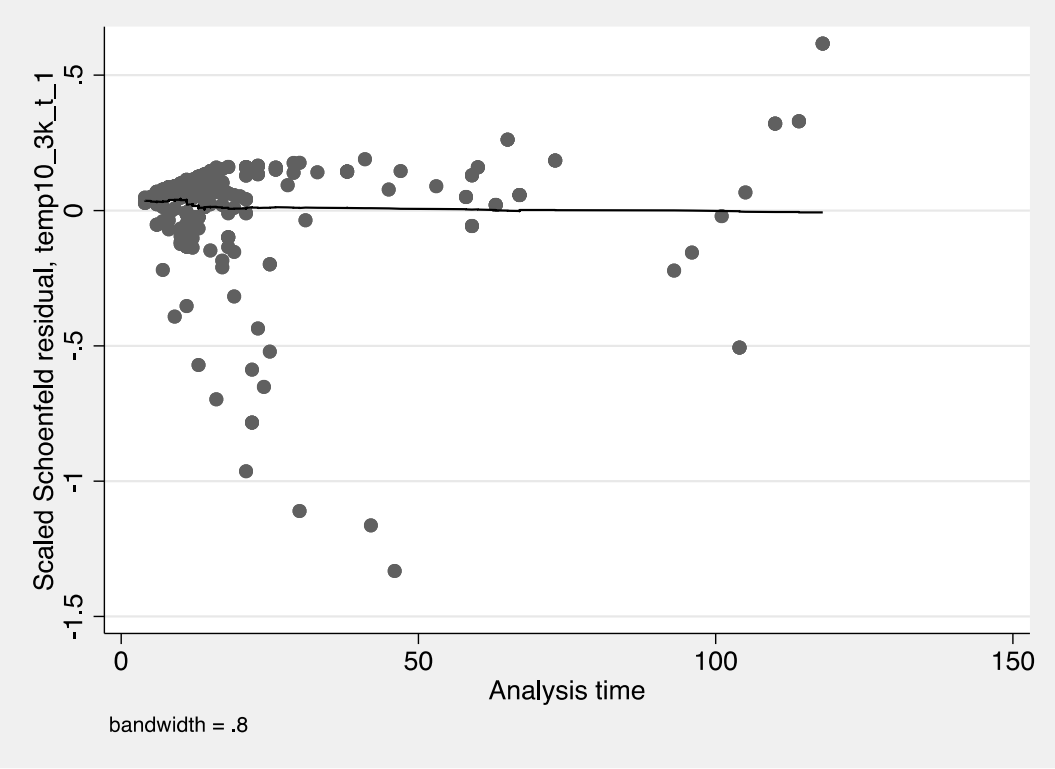

Figure 4.3. Schoenfeld residual plot over time (pen placement, days) for selected spline term (knot 1) of temperature at 10m depth interacted with time in a model that contained sal10 to demonstrate acceptable linearity and presence of proportional hazards.

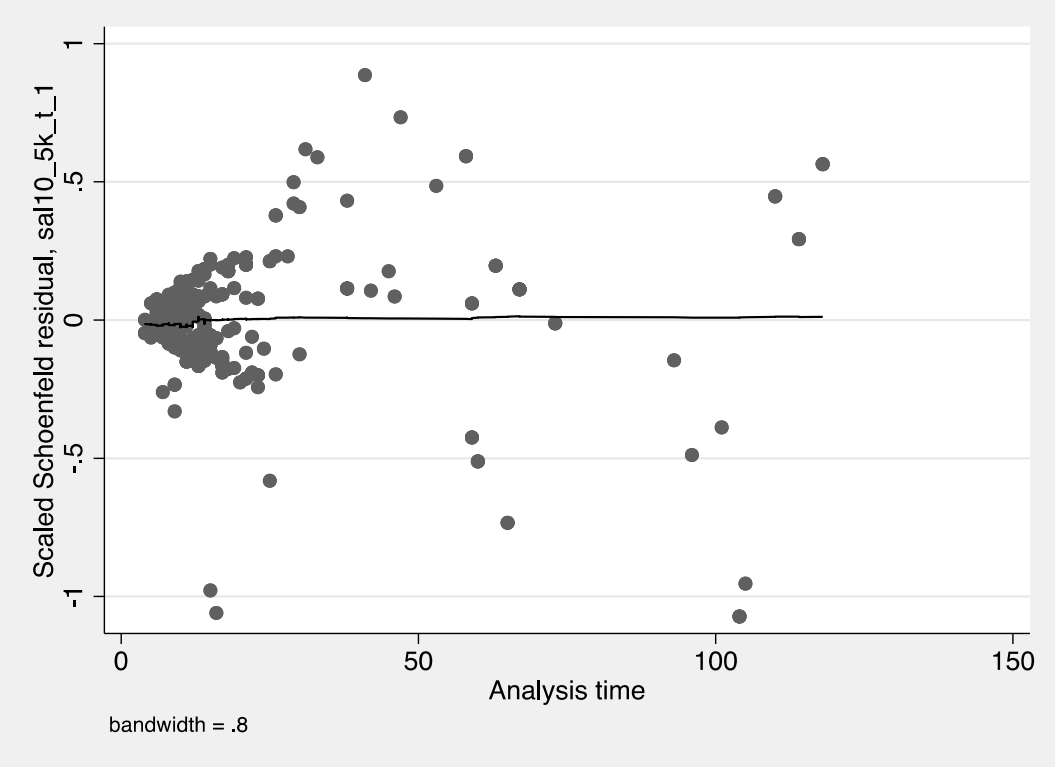

Figure 4.4. Schoenfeld residual plots over time (pen placement, days) for selected spline term (knot 1) of salinity at 10m depth interacted with time in a model that contained temp10 to demonstrate acceptable linearity and presence of proportional hazards.

CHAPTER 5 **CONCLUSION**

5.1 **Objectives and outcomes**

Antimicrobial use (AMU) in British Columbian finfish aquaculture, driven by yellow mouth (YM) outbreaks, and associated with antimicrobial resistance (AMR) in the marine environment, poses a unique One Health threat. Increased social demand to reduce antimicrobials, coupled with ever increasing yet insufficient production of salmon products are placing extreme pressure on finfish aquaculture production British Columbia (BC), Canada, partly in response to public pressure and decreasing social license for net-pen salmonid production (Fisheries and Oceans Canada, 2024; Food and Agriculture Organization of the United Nations, 2024). Although research surrounding marine AMR, AMU, and YM in Canada has begun to increase in recent years (Jonah et al., 2024; Nowlan et al., 2021; Ojasanya et al., 2022), there still exists a substantial gap in literature investigating and relating this deadly triad (Caputo et al., 2023; Fraser et al., 2004; Smith, 2008; Wade & Weber, 2020; Watts et al., 2017). Coupled with the dire consequences of these three factors, there have been numerous calls to action to address gaps in literature surrounding marine AMR, AMU, and YM, particularly off the coast of BC (Brunton et al., 2019; Fraser et al., 2004; Miranda et al., 2018; Preena et al., 2020; Smith, 2008; Wade & Weber, 2020).

The objectives of this thesis were to 1) describe the antimicrobial susceptibilities of a historical collection of bacterial isolates from farmed salmonids in BC, that were submitted to the provincial diagnostic laboratory; 2) evaluate the relationship between AMR and AMU in BC finfish aquaculture using retrospective, province-level surveillance data provided by the Government of BC; 3) assess the factors associated with pen outbreaks with YM and requiring antimicrobial treatment using historical industry data for farmed Atlantic salmon in British

Columbia, Canada; and 4) to assess the practicality of using Cox proportional hazards models for this purpose

We first explored current AMR characteristics, prevalence, and trends over time using historical AMR data from the BC Ministry of Agriculture and Fisheries (MAF) (Chapter 2). Next, we evaluated the potential for linkage between AMR and AMU using the historical, province-level AMR and AMU data using logistic regression models (Chapter 3). Lastly, we aimed to accomplish objectives 3 and 4 by applying survival analysis models to industry provided data consisting of daily environmental and biological variables such as water temperature and density of fish stock, among others for the first 120 days after pen placement in the ocean (Chapter 4). By analyzing these relationships, this thesis has provided evidence that marine AMR in aquaculture is diverse (Chapter 2), that phenotypic AMR of bacterial isolates from farmed Atlantic salmon was not linked to AMU at the provincial level, but it was linked to other resistance (Chapter 3), and that the relationship between YM and environmental, biological, and managerial factors is complex and multifaceted (Chapter 4). We have provided evidence for the interconnected nature of resistance in marine settings and speculated at the importance of the environmental resistome in marine AMR work. The results of our study questioned the validity of a direct causal pathway between increased AMU and AMR, but more farm-level analysis is required to substantiate this lack of a causal pathway. Our analysis was based on provincial-level phenotypic antimicrobial susceptibility and use data. The linkage between phenotypic resistance of drugs in different classes speaks strongly to the need for future work to consider genomic methods to further characterize the resistome (Domínguez et al., 2019; Eckstrand et al., 2024; Nowlan et al., 2021). The analysis of industry data to understand factors associated with AMU for YM highlighted the importance of collecting data on and analyzing

environmental and management factors, especially over time, to study the risk of marine infectious diseases such as YM. We have also demonstrated some of the statistical challenges in using survival analysis to understand these factors associate with first treatment incidence for YM, specifically the difficulties associated with modeling time-varying covariates in Cox proportional hazards models.

5.2 Statistical journey and outcomes

Throughout the course of this project, we utilized a variety of statistical methods to analyze the prevalence and drivers of AMR and AMU. In Chapter 2, we utilized Excel due to its ease-of-use ability to organize simple data, and graph creation features to visually present AMR trends between the years 2007-2018. In Chapter 3, we utilized multi-level logistic regression models with random intercepts to account for clustering at the level of bacterial species in STATA® BE (version 17.0, College Station, TX) to screen multiple variables simultaneously and uncover associations. In Chapter 4, we utilized Microsoft Access© (version 2016, Redmond, WA) and Microsoft Excel© (version 16.8, Redmond, WA) to manage and merge complex industry-provided datasets, R© (version 4.3.1, Vienna, Austria) for random forest decision tree analysis (conducted by collaborators), and STATA® BE (version 17.0, College Station, TX) for Cox proportional hazards models to analyze how different environmental, biological, and managerial variables influenced the risk (hazard) over time of a breakout of YM. In progressing through each of these steps we have learned important strengths and weaknesses of each software and database system and modeling strategy.

Throughout this process, we were frequently presented with the benefits of utilizing R© (version 4.3.1, Vienna, Austria) over STATA® BE (version 17.0, College Station, TX). We did

not expect to find such a dearth of knowledge on complex time-varying hazards modeling in STATA® BE (version 17.0, College Station, TX) , a program which is frequently utilized in teaching curriculums at the School of Public Health in Alberta and worldwide. While STATA® BE (version 17.0, College Station, TX) does possess tools for time-varying survival analysis, all of the support we sought strongly suggested that we would be better served by using R© (version 4.3.1, Vienna, Austria). The online resources we could find were not sufficient to allow for generation and implementation of functional STATA® BE (version 17.0, College Station, TX) script that could be adapted to our data and model to generate model outputs and predictions that we could trust and use to make meaningful interpretations of the research questions.

One of the greatest resources when trying to navigate between these two programs in our survival analysis was the paper presented by Shepherd and Rebeiro (2017). This study was unique among many of the survival models we reviewed in that the authors provided syntax for their statistical analysis in both R© (version 4.3.1, Vienna, Austria) and STATA® BE (version 17.0, College Station, TX) format. The ability to compare syntax across models greatly assisted us in our own analysis, as it may for many other prospective researchers; we therefore believe that the value of data translation across these programs cannot be understated. We strongly encourage future research to provide multi-platform syntax for their models to promote cooperation in the epidemiology community. Additionally, the normalization of syntax translation services would be a powerful contribution to the education and growth of new epidemiologists and biostatisticians.

During our statistical journey, one of the greatest roadblocks we ran into was the difference between time-varying covariates and time-varying coefficients. Many of the time-

varying covariate models that we used were unable to handle our daily data and were actually built for time-varying coefficients. The distinction between these two variables, and the irregularity with which they are properly referenced in literature, highlighted the need for more statistical education and support on modeling these two complex and disparate variable types. Difficulties encountered during modelling are well experienced in the world of Statistics. Sir David Cox, creator of the Cox proportional hazards model, wrote the following when musing over his journey in academics over the years:

> "In another sense I feel very dissatisfied: there are all sorts of problems that I nearly solved and gave up, or errors of judgement in doing a little something and not taking it far enough. That I nearly did something you see, this is the irritating thing. You know, if you'd no idea at all, well it doesn't matter, it's irrelevant, but if you feel you were within an inch of doing something and didn't quite do it …" (Reid, 1994, p. 454).

We recommend a mixture of statistical methods when analyzing AMR and disease in the marine environment. The need to comprehensively assess all variable involved in the marine environment requires screening methodologies such as decision tree analysis. Analytical methods for model building must be capable of utilizing a variety of extensions that can handle complex interactions with time, as well as allowing analysis of factors over time. When utilizing Cox proportional hazards survival models, we strongly recommend exploring and implementing R© (version 4.3.1, Vienna, Austria) instead of STATA® BE (version 17.0, College Station, TX) due to its ability to accommodate time-varying covariates and due to the amount of informational literature available for syntax. However, we also acknowledge that this software comes with a learning curve that must be satisfied, which required time.

5.3 The relationship between AMR and AMU

We expected to find AMU to be associated in models where the outcome was resistance to that antimicrobial. This expectation was driven not only from personal and broad rhetoric, where use of a drug would be assumed to select for resistance to that drug, but also out of the plethora of studies which claim this association to be true in the aquaculture environment (Miranda et al., 2018; Miranda & Zemelman, 2002; Tuševljak et al., 2013). This largely unchallenged association is seen in many high impact articles (Cabello, 2006; Cabello et al., 2016; Santos & Ramos, 2018). Our study attempted to address the call for empirical research on this gap in farmed salmonids (Brunton et al., 2019; Caputo et al., 2023; Fraser et al., 2004; Larsson et al., 2018; Mardones et al., 2018; Miranda et al., 2013; Miranda et al., 2018; Preena et al., 2020; Smith, 2008; Watts et al., 2017) and supports studies which have already done so (Buschmann et al., 2012; Gordon et al., 2007; Miranda & Rojas, 2007). The findings of our study highlight the need for both further research to address this gap, as well as to evaluate the hypothesis that AMR may be more strongly reliant on factors other than AMU. The indirect relationship discovered in this thesis is supported by other studies (Gordon et al., 2007; Henriksson et al., 2018). It is important to note that the evaluation of AMR and AMU linkages conducted in Chapter 3 utilized collated provincial level data, unlike the area, farm, and even pen-level data provided in Chapter 4. The ability to analyze resistance and use of antimicrobials at the pen or area level may provide stronger evidence for or against a link, as this would allow for controlling of area-specific variables that we found to be important throughout the thesis, as well as evaluating resistance at the site and time of use. The results of our literature search in Chapter 1 informed us of a high degree of background resistance independent of use (Buschmann et al., 2012; González-Gaya et al., 2022; Gordon et al., 2007; Kerry et al., 1996; Miranda & Rojas, 2007; Shah et al., 2014). Pen-level data would also provide the ability to

adjust for outliers that may not be visible in the provincial level data. Similarly, we found that the ability to analyze AMR at the species-level is crucial due to a wide range of resistance among isolates identified in Chapter 2.

We encourage future researchers to refrain from a tunnel-visioned approach to AMR research in the marine environment by solely studying local AMU. Instead, we recommend that researchers incorporate AMU with an evaluation of antimicrobial genes (ARGs), augmentations of horizontal gene transfer (HGT), and other marine factors that may influence the resistome (see Chapter 3). This approach may not only provide benefits to scientific understanding of marine AMR but may help negate public health and consumer concerns as well. Additionally, we encourage future researchers to study AMR and AMU at the pen or area level to allow for a more direct evaluation of resistance to instances of use and to control for or investigate important area-specific variables. AMR data should be evaluated at the species-level to account for a wide range of isolate-specific characteristics, such as innate resistance or capability and propensity for HGT.

5.4 **The complications of marine infectious disease**

Although our survival analysis did not provide completed models to allow statistical evaluation of factors associated with YM, it did support the findings of Chapter 3 in that statistical analysis of the marine environment is complex. As with the incidence of AMR, the causal pathway for YM was shown to be multifactorial and highly interrelated. Our use of random forest decision tree models in a collaborative project for variable screening in this analysis allowed us to evaluate more variables than what would have been possible with manual screening and model building. The utilization of Cox proportional hazards models allowed us to consider the assessment of time-varying covariates and variables with time-dependent

coefficients. The significance of time as a variable in all of the models, as well as the syntax complications introduced by multiple interactions with time, drives home the importance of chronological factors in YM modeling. The complexity of causal associations with disease in the marine environment is well supported in the literature (Henriksson et al., 2018; Kerry et al., 1996; Sajid et al., 2024; Smith, 2008). We were repeatedly confronted in this study with an inability to simplify models due to a high prevalence of significance and multi-term interactions between variables. The ability to comprehensively model all significant variables discovered through traditional screening methodology would not be compatible with the limited power of our sample sizes and subsequent violations of model assumptions (i.e., non-proportional hazards). We recommend that future modeling in this space utilizes large sample sizes and software better suited to the complex survival data in order to capture an adequate amount of environmental, managerial, and biological factors for the study of disease in the marine environment. Such a holistic approach, as we attempted in this thesis, may be the only way to elucidate possible interventions from the aquaculture industry and reduce AMU.

5.5 **Industry application and importance to public health**

For the aquaculture industry, the pressure to reduce AMU has been immense. Antimicrobial abuse and misuse in production areas such as Chile has been the focus of multiple studies (Billi et al., 2022; Cabello & Godfrey, 2023; Higuera-Llanten et al., 2018; Lozano-Muñoz et al., 2021; Millanao et al., 2018; Miranda et al., 2018; Quiñones et al., 2019). Considering present scrutiny, it is vital that the BC aquaculture industry evaluate and respond to these concerns. Antimicrobial use remains an important tool for disease management, without which the production of farmed salmon would be substantially reduced or economically unfeasible (Jonah et al., 2024; Wade & Weber, 2020). This thesis identified that AMR levels in

farmed aquaculture in BC are relatively low and did not have a strong linkage to AMU at the provincial level. However, the presence of multidrug resistance and linkage between resistance to antimicrobials of different classes is still concerning and requires future research. These results suggest that strategies that focus on reduction of AMU alone are likely not enough to solve AMR. The BC aquaculture industry may be able to better address social concerns about AMU by instead targeting factors associated with infection and disease from YM. This redirection opportunity may provide an effective step in alleviating social concerns and criticisms of aquaculture in BC and allow production to continue to meet ever-increasing demand.

Although this thesis is focused on the AMR from bacteria isolates from farmed salmon, it is important to note the concern of the risk of transmission of AMR and ARGs to human pathogens in seafood products and the broader environment (Brunton et al., 2019; Lozano-Muñoz et al., 2021; Reverter et al., 2020). The so-called AMR hotspots of aquaculture farms may directly propagate human pathogen ARGs or exacerbate challenges of food safety by making seafood-borne pathogens more difficult to eradicate (Brunton et al., 2019; Cabello et al., 2013; Ochs et al., 2021; Reverter et al., 2020; Watts et al., 2017).

The findings of this study and the application of our results to reduce AMU are presently relevant due to the recent decision of the Canadian Government not to renew open water aquaculture licenses in BC in 2024 and to ban all currently existing open-water facilities by 2029 (Fisheries and Oceans Canada, 2024).The global dependence on finfish aquaculture has raised the stakes on such decisions, where a reversion back to wild stock fisheries may cause unprecedented environmental depletions and exacerbation of global food security (Garlock et al., 2022; High Level Panel of Experts (HLPE), 2014; Reverter et al., 2020). As of 2019, the demand

for aquatic foods has grown at twice the rate of population growth (Food and Agriculture Organization of the United Nations, 2022). Only as recently as 2022 did we produce more fish on farms than we took out of wild fish stock (Food and Agriculture Organization of the United Nations, 2024). Even with this impressive growth of the aquaculture industry, wild fish populations continue to decline, with over a third of all fish stocks being fished at unsustainable levels in 2021(Food and Agriculture Organization of the United Nations, 2024). These three metrics, the accelerating demand for fish, the inadequate yet strained growth of aquaculture, and the continuing decline of wild fish populations highlight the need for aquaculture operations to expand operations in order to attempt to meet demand. The decision of the Canadian Government to dismantle finfish aquaculture as it presently exists in BC threatens to push this sustainability goal to unreachable levels (Fisheries and Oceans Canada, 2024). As much of this decision relies on social concerns, it is incumbent on the scientific community to evaluate public concerns, such as those of AMU, objectively and empirically. British Columbia is uniquely situated to provide a leading example of industry and government collaboration to tackle this problem due to the interconnection of aquaculture surveillance in this region. This thesis would not have been possible without data provided from both entities, and we are hopeful that further collaboration will be utilized to quantify the risk of AMU in aquaculture, alleviate public concerns, and retackle the issue of wild fish population sustainability.

5.6 **Strengths and limitations**

This thesis was subject to many of the common limitations involved in statistical analyses. We were reliant on government provided provincial-level historical AMR and AMU data for Chapters 2 and 3, which negated our ability to include organisms of current interest such as *T. maritimum*, conduct genome analysis for ARGs alongside phenotypic susceptibility testing,

or evaluate AMR-AMU relationships at an area or pen-level. The cross-sectional nature of our data in the AMR-AMU analysis in Chapters 2 and 3 limits our ability to infer causal relationships between any of our chosen variables. In addition, the sparse and highly varied, and skewed distribution of isolate data at the species level caused some modeling convergence issues and limited the statistical power of our models in Chapter 3.

Despite these limitations, this thesis provides the first database of 11 years of historical AMR and AMU data on the Canadian West Coast. These government data have never been made analyzed and made publicly available and will contribute to nation-wide AMU and AMU surveillance and analysis programs in the future. Furthermore, the industry data in Chapter 4 presents the first evaluation of AMU at the farm level in BC. Lastly, this data is presented in in cohort design, allowing for the evaluation of temporal trends.

5.7 **Future directions**

We have provided in this study a long-term historical evaluation of AMR in BC, an evaluation of factors associated with AMR, and a clear database and pathway for survival analysis to evaluate YM, one of the most important drivers of AMU. The findings of this thesis can be used to provide direction for future research on AMR in the marine environment, support stewardship strategies to reduce AMU in the aquaculture industry, and to directly support further comprehensive analysis of YM breakouts in BC. Specifically, we encourage future research to: 1) continue surveillance of AMR in BC and advocate for the adoption of bacteria of interest, such as *T. maritimum* in routine sampling; 2) to utilize whole genome sequencing, metagenomic sequencing, and other molecular techniques for comprehensive profiling of both phenotypic and genotypic AMR in order to understand the flow, transfer, and emergence of AMR in marine settings; 3) to adopt holistic approaches in their evaluation of AMR surrounding aquaculture

facilities in order to capture extraneous variables that may promote AMR proliferation, as targeting reduced AMU may be both economically unfeasible and epidemiologically ineffective; 4) to consider the utilization of the database we have provided to evaluate the time-dependent effects of environmental, biological, and managerial factors resulting in YM breakouts in BC finfish operations, and; 5) to promote the fulfillment of gaps in statistical analysis of time-varying covariate models and the incorporation and standardization of cross-database syntax translation materials.

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APPENDIX 1

Table A1.1. Bacterial genera isolated (n=1,237) from all farmed salmonid species in British Columbia by the Animal Health Centre from 2007-2018 and tested for antimicrobial susceptibility.

Bacterial genera	Total isolates	Atlantic salmon n (%)	Pacific salmon n (%)	Rainbow trout n (%)	Other species* n (%)
Aeromonas	246	206 (83.7)	15 (6.1)	20 (8.1)	5 (2.0)
Vibrio	227	172 (75.8)	45 (19.8)	0 (0.0)	10 (4.4)
Aliivibrio	195	186 (95.4)	8 (4.1)	0 (0.0)	1 (0.5)
Photobacterium	117	108 (92.3)	5 (4.3)	0 (0.0)	4 (3.4)
Yersinia	82	79 (96.3)	1 (1.2)	2 (2.4)	0 (0.0)
Pseudomonas	78	53 (67.9)	8 (10.3)	12 (15.4)	5 (6.4)
Pseudoalteromonas	46	44 (95.7)	2 (4.3)	0 (0.0)	0 (0.0)
Psychrobacter	46	41 (89.1)	4 (8.7)	0 (0.0)	1 (2.2)
Serratia	34	32 (94.1)	1 (2.9)	1 (2.9)	0 (0.0)
Carnobacterium	25	20 (80)	0 (0)	5 (20.0)	0 (0)
Shewanella	24	23 (95.8)	0 (0)	0 (0)	1 (4.2)
Arthrobacter	11	8 (72.7)	0 (0)	3 (27.3)	0 (0)
Brochothrix	10	8 (80.0)	0 (0)	2 (20)	0 (0)
Moritella	9	9 (100)	0 (0)	0 (0)	0 (0)
Acinetobacter	5	4 (80.0)	1 (20.0)	0 (0)	0 (0)
Chryseobacterium	4	1 (25.0)	0 (0)	2 (50.0)	1 (25.0)
Flavobacterium	4	2 (50.0)	0 (0)	2 (50.0)	0 (0)
Iodobacter	4	0 (0)	0 (0)	4 (100)	0 (0)
Lactococcus	4	1 (25.0)	0 (0)	3 (75.0)	0 (0)
Staphylococcus	4	2 (50.0)	0 (0)	2 (50.0)	0 (0)
Edwardsiella	3	0 (0)	0 (0)	3 (100)	0 (0)

Hafnia	3	2 (66.7)	0 (0)	1 (33.3)	0 (0)
Providencia	3	2 (66.7)	1 (33.3)	0 (0)	0 (0)
Bacillus	2	2 (100)	0 (0)	0 (0)	0 (0)
Enterococcus	2	2 (100)	0 (0)	0 (0)	0 (0)
Idobacter	2	2 (100)	0 (0)	0 (0)	0 (0)
Microbacterium	2	2 (100)	0 (0)	0 (0)	0 (0)
Rhodococcus	2	2 (100)	0 (0)	0 (0)	0 (0)
Aerococcus	1	0 (0)	1 (100)	0 (0)	0 (0)
Citrobacter	1	1 (100)	0 (0)	0 (0)	0 (0)
Enterobacter	1	0 (0)	0 (0)	1 (100)	0 (0)
Erwinia	1	0 (0)	1 (100)	0 (0)	0 (0)
Exiguobacterium	1	0 (0)	1 (100)	0 (0)	0 (0)
Kluyvera	1	1 (100)	0 (0)	0 (0)	0 (0)
Macrococcus	1	0 (0)	0 (0)	0 (0)	1 (100)
Micrococcus	1	0 (0)	1 (100)	0 (0)	0 (0)
Morganella	1	1 (100)	0 (0)	0 (0)	0 (0)
Pantoea	1	1 (100)	0 (0)	0 (0)	0 (0)
Plesiomonas	1	1 (100)	0 (0)	0 (0)	0 (0)
Proteus	1	1 (100)	0 (0)	0 (0)	0 (0)
Raoultella	1	0 (0)	0 (0)	1 (100)	0 (0)
Sphingobacterium	1	0 (0)	0 (0)	1 (100)	0 (0)
Stenotrophomonas	1	1 (100)	0 (0)	0 (0)	0 (0)
Unidentified genera	28	22 (78.6)	0 (0)	6 (21.4)	0 (0)
Total**	1,237	1,042 (84.2)	95 (7.7)	71 (5.7)	29 (2.3)

n = number. * Other species: Sablefish, Tilapia, and White Sturgeon. ** Total includes two isolates for which susceptibility data were not reported

APPENDIX 2

Table A2.1 Listing of characteristics and of all variables considered for screening for Cox proportional hazards model with failure defined as treatment with antimicrobials as a proxy for and outbreak of Yellow Mouth in salmon pens in British Columbia, Canada.

Variable	Description	Variable type	TVC	Mean	Standard Deviation	Min, Max
Gcs	ID variable for each subject (pen)	ID	No	--	--	--
Area	Our broadest geographic variable; n=5	Categorical	No	--	--	--
site	Second level geographic variable; informally (i.e., not encoded) to be nested within area; n=17	Categorical	No	--	--	--
Msmt	Date of environmental measurement; used as our time variable	Time		--	--	--
Place	Date that the pen was placed in the ocean; used as our subject entry date (day 0)"	Origin	No	--	--	--
Yearp	Year in which the pen was placed in the ocean	Categorical	No	--	--	--

Monthp	Month in which the pen was placed in the ocean	Categorical	No	--	--	--
Weekp	Week in which the pen was placed in the ocean (out of 52)	Discrete-continuous	No	--	--	--
Brood1	Source broodstock for smolts; 1 = MCXMOWI, 2 = Mowi, 3 = Mixed Mowi & MCXMOWI, 4 = Atlantic	Categorical	No	--	--	--
Brood2	Source broodstock for smolts; 0 = Mowi or Mowi cross, 1 = Atlantic	Binary	No	--	--	--
Ingrams	Average weight in grams of smolts when placed in the ocean	Continuous	No	116.11	43.30	68.54, 431.05
Temp5	Daily temperature measurement for the pen at a depth of 5m Q1: [3.10, 8.95] Q2: (8.95, 10.05] Q3: (10.05, 11.70] Q4: (11.70, 20.4]	Continuous	Yes	10.60	2.34	3.1, 20.4
Temp10	Daily temperature measurement for the pen at a depth of 10m Q1: [4.50, 9.10) Q2: (9.10, 10.00] Q3: (10.00, 11.45]	Continuous	Yes	10.40	1.88	4.5, 20

	Q4: (11.45, 20.00]					
Sal5	Daily salinity measurement for the pen at a depth of 5m Q1: [5.50, 25.50] Q2: (25.50, 28.00] Q3: (28.00, 30.5] Q4: (30.50, 35.75]	Continuous	Yes	27.64	3.97	5.5, 35.75
Sal10	Daily salinity measurement for the pen at a depth of 10m Q1: [11.50, 27.50] Q2: (27.50, 30.00] Q3: (30.00, 32.00] Q4: (32.00, 35.50]	Continuous	Yes	29.55	3.14	11.5, 35.5
Oxy5	Daily oxygen measurement for the pen at a depth of 5m Q1: [3.25, 7.95] Q2: (7.95, 8.70] Q3: (8.70, 9.55] Q4: (9.55, 77.10]	Continuous	Yes	8.83	2.05	3.25, 77.1
Oxy10	Daily oxygen measurement for the pen at a depth of 10m Q1: [2.30, 7.50]	Continuous	Yes	8.40	2.05	2.3, 64.55

	Q2: (7.50, 8.35] Q3: (8.35, 9.15] Q4: (9.15, 64.55]					
Mortcount	Count of mortality in a pen	Continuous	Yes	45.84	190.10	0, 9960
Mortcountpr	Mortality count of a pen as a percentage of the pen population	Continuous	Yes	0.00	0.00	0, 0.11
Mr30	Mortality ratio in the first 30 days after placement in the ocean	Continuous	No	0.03	0.03	0, 0.17
Mr120	Mortality ratio in the first 120 days after placement in the ocean	Continuous	No	0.06	0.04	0.01, 0.37
Mr	Mortality ratio: mortality as a percent of input count	Continuous	No	0.22	0.11	0.01, 0.54
Bfcr	Biological feed conversion ratio after placement in the ocean	Continuous	No	1.22	0.09	1.06, 1.52
Dbwg	Days that the average weight of fish in the pen stayed between 100g and 500g	Continuous	No	121.06	26.24	19, 198
denskg	Density measured as biomass in kg per cubic meter in the first 120 days at sea, recorded as an average percent for the first 120 days	Continuous	No	1.29	0.72	0.5, 5.97

denscount	Density measured as count of fish per cubic meter in the first 120 days at sea, recorded as an average percent for the first 120 days	Continuous	No	5.04	2.35	1.37, 15.75
Treat	Failure/outcome variable for whether a pen was treated or not on this date; 1= treated, 0 = not treated	Binary	--	--	--	--

TVC = Time-varying covariate.