# OAHN FINAL REPORT

**Project #:** OAHN-06

**Project Title:** Antibiotic Resistance in Ontario Aquaculture

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**Executive Summary**

- The present study surveyed 55 fish specimens from a variety of rearing environments for bacterial pathogens and resistance to antibiotics.
- Thirty four bacterial isolates donated by the University of Guelph's Fish Health Laboratory were also tested for resistance to antibiotics.


**Objectives**

The objective of this study was to survey fish from aquaculture farms in Ontario to determine the prevalence of bacterial pathogens and antibiotic resistant strains of bacteria. Additionally, the project aimed to assist in the establishment of veterinarian-client-patient relationships in the aquaculture industry.

**Materials and methods**

In year one of the study, fish specifies submitted to the University of Guelph's Animal Health Laboratory (AHL) as part of regular screening and fish health testing were examined. Few bacterial isolates were identified using this approach. To make up for the shortfall in sample submissions and encourage the uptake of aquatic veterinary services in the industry, in year two of the study the cost of on-site veterinary visits and diagnostic testing were subsidized to increase the sample size.

Fish specimens were collected from commercial aquaculture farms and from government hatcheries operated by the Ontario Ministry of Natural Resources and Forestry (MNRF). Specimens were primarily salmonids (rainbow trout, brook trout, etc.) in addition to a small number of tilapia and yellow perch. Fish were collected on-farm using dip nets by farm staff, technicians or by veterinarian and were humanely euthanized using an appropriate lethal dose of anesthetic.

Whole fish (sick, moribund and dead) were collected and submitted for diagnostics. The AHL used gills, skin lesions (if present), spleen and kidney tissues for bacterial cultures. If fish were too small to collect

kidney and spleen, pooled viscera were used. Samples were kept cold on ice packs and were submitted to the lab within 24 hours of sample collection. In some cases, archived bacterial isolates from prior MNRF testing were submitted. The fish species was not available for isolate submission.

Bacterial pathogens were cultured on agar by the AHL. When bacteria was cultured from the tested fish it was identified to genus or species using matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry. Those considered primary fish pathogens were further tested for minimum inhibitory concentrations (MICs) using the Sensititre™ Avian Plate (Thermo Scientific) to monitor for resistance to the following antibiotics: amoxicillin, ceftiofur, clindamycin, enrofloxacin, erythromycin, florfenicol, gentamicin, neomycin, novobiocin, oxytetracyline, penicillin, spectinomycin, streptomycin, sulphadimethoxine, sulphathiazole, tetracycline, trimethoprim/sulfamethoxazole, tylosin. The MIC is a quantitative method of susceptibility testing and provides the ability to precisely determine the concentration of antibiotic required to inhibit growth of a pathogen.

The Clinical and Laboratory Standards Institute (CLSI) has set guidelines for the interpretation of MIC results for *A. salmonicida* but guidelines do not exist for any other fish pathogens. Other pathogens tested as part of this project were defined as sensitive, intermediate or resistant based on the range of MIC results compared to the concentrations on the test plate.

**Results and Discussion**

A total of 55 fish specimens and 34 bacterial isolates were tested. Bacterial isolates were cultured from 54 of the 55 sample submissions. *Flavobacterium* spp. was isolated from 54.5% of the fish specimens and *Aeromonas* spp. was isolated from 29.1% of the samples. Other bacterial pathogens, including *Edwardsiella* spp., *Streptococcus* spp., *Vibrio* spp. and *Yersinia* spp. were detected infrequently and ranged from 1.8% to 5.5% of fish samples.

In Ontario, four antibiotics are currently approved for use in food fish (florfenicol, oxytetracycline, sulphadimethoxine and sulphathiazole). Sulphathiazole is no longer available as suppliers are not carrying it and sulphadimethoxine is being phased out in 2018. This leave limited options for fish farmers to use for the treatment of bacterial diseases in fish. While the MIC results are presented for all antibiotics tested, the focus is on the three drugs currently used in Ontario - florfenicol, oxytetracycline, and sulphadimethoxine.

Of the *Aeromonas* spp. tested, *A. salmonicida* and *A. hydrophila* were the most common species isolated. *A. salmonicida* is an etiological agent for furunculosis, a disease that causes hemorrhages, muscle lesions, inflammation of the lower intestine, spleen enlargement, and death in freshwater fish populations. *A. hydrophila* can cause hemorrhagic septicemia, where reddening, hemorrhaging, and ulcers can be observed on the fish. Stress and overcrowding are common causes of both furunculosis and hemorrhagic septicemia. One hundred percent of *A. salmonicida* (n = 13) and *A. hydrophila* (n = 10) isolates were susceptible to florfenicol. The results for oxytetracycline were different, where 54% of *A. salmonicida* isolates (n = 7) were sensitive and 46% (n = 5) were resistant to the antibiotic. Eighty percent of *A. hydrophila* isolates (n = 8) were sensitive to oxytetracycline, one isolate was intermediate and one was resistant (Table 1).

Of the *Flavobacterium* spp. tested, *F. columnare* and *F. psychrophilum* were the most common species isolated. *F. columnare,* the causal agent of columnaris disease, may result in skin lesions, fin erosion and gill necrosis, with a high degree of mortality. *F. psychrophilum* is the etiological agent of cold water disease and fry syndrome in rainbow trout and may cause mortalities greater than 50% among infected fish. *Flavobacterium* spp. isolates were sensitive or intermediately sensitive to treatment by florfenicol and oxytetracycline.

Anecdotal reports have indicated an increase in *Yersinia ruckeri* detections in Ontario and other areas bordering on the Great Lakes (personal communication, Great Lake Fish Health Committee). *Y. ruckeri* the causative agent of enteric redmouth disease in fish. Most of the *Y. ruckeri* isolates were sensitive or intermediate for both florfenicol and oxytetracycline with only four isolates that were resistant.

**Table 1:** Results of MIC testing for 18 antimicrobics for the five most commonly cultured fish pathogens. S = sensitive, I = intermediate, and R = resistant. Florfenicol and oxytetracycline (highlighted) are the most commonly used antimicrobials approved for use in food fish production. The number in brackets represents the sample size.

| Antimicrobic | *Aeromonas salmonicida* (n = 13) | *Aeromonas hydrophila* (n = 10) | *Flavobacterium columnare* (n = 11) | *Flavobacterium psychrophilum* (n = 7) | *Yersinia ruckeri* (n = 20) |
|---|---|---|---|---|---|
| Amoxicillin | S | R | S | S | S/I |
| Ceftiofur | S | I/R | I | S | S/I |
| Clindamycin | R | R | S | S | R |
| Enrofloxacin | S | S | S | S | S |
| Erythromycin | I | I/R | S | I | I/R |
| Florfenicol | S | S | S | S | S/I |
| Gentamicin | S | S/I | S | S | S/I |
| Neomycin | S | S | I | S/I | I |
| Novobiocin | I/R | R | S/I | I | S/R |
| Oxytetracyline | S/R (7/5) | S/I/R (8/1/1) | S | S/I (5/2) | S/I/R (6/10/4) |
| Penicillin | S/I | R | S | S | S/I/R |
| Spectinomycin | R | I/R | S/I | S/I | S/I/R |
| Streptomycin | S/I | S | /S | S | S/I |
| Sulphadimethoxine | S/I | S/R | R | R | S |
| Sulphathiazole | S | S/R | S/I | I | S |
| Tetracycline | S/R | S/I/R | S | S/I | S/I/R |
| Trimethoprim/ Sulfamethoxazole | S | S | S | S | S |
| Tylosin | I/R | R | S | S | I/R |

**Applications**

The information gathered during the two year study has a number of applications. In the short term, funding through this project assisted in the establishment of veterinarian-client-patient relationships where none existed previously. Additionally, as a quantitative method of susceptibility testing, MIC

results help determine which class of antibiotic is most effective. This information can lead to an appropriate choice of an antibiotic that will increase chances of treatment success and help in the fight to slow antibiotic resistance. In the present study, the MIC results were able to direct treatment in real-time. In some cases, farm operators initiated treatments with oxytetracycline and found the treatments to be ineffective. Following test results indicating the bacteria were resistant to oxytetracycline, they were able to switch to florfenicol.

Currently, prevention and control are essential to avoid large scale losses of farmed fish as a result of bacterial infections. This is because there are no commercially available vaccines currently available and there are a limited number of antimicrobials that have been approved for treating food fish. Therefore, another application of this project is generating relevant data to support a request to the federal government to bring in fish vaccines currently used in other jurisdictions. Currently, there are only four therapeutant products registered for food fish use in Canada and all of these products are primarily targeting cold water diseases. Registered products for newly cultured warm water species, such as tilapia, are currently lacking. This project has assisted in the identification of new and emerging warm water pathogens not previously found in Canada.

**Suggestions for next steps**

Fish species cultured in Ontario are susceptible to a wide range of bacterial pathogens. Samples for the present study were collected from a variety of environments including cage-based aquaculture farms, and tank-based hatcheries and grow-out facilities which are representative of the industry in Ontario. Through this project, we identified and tracked a number of emerging pathogens, including branchial copepods and epitheliocystis (a β-proteobacteria) only recently described in Ontario. Additionally, we identified some resistance to the only antibiotics available for use in food fish production in Canada.

These findings demonstrate the importance of routine antimicrobial resistance testing in facilities where there is regular use of a small number of antibiotics for a narrow spectrum of diseases. The importance of acquiring and tracking this type of data over time includes developing disease prevention programs in order to decrease the use of therapeutants in food fish, tracking resistance to therapeutants and adapting treatment protocols.

**Communications**

- Preliminary results have been presented at the Canadian Animal Health Laboratorian's Network (CAHLN) conference (June 4-7, 2017) and at the Great Lakes Fish Health Committee (GLFHC) meeting (August 3, 2017).
- The project lead will submit an abstract to present the final results at the Aquaculture Association of Canada (AAC) conference (May 2018).
- The network has communicated the preliminary results of the project to the aquaculture sector through the OAHN producer report.
- The project lead is drafting a scientific manuscript with the intention to submit for publication in a peer-reviewed journal (North American Journal of Aquaculture).

- A poster summarizing the project has been created to be used as part of a display to showcase the OAHN funded projects.

**Acknowledgements**

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