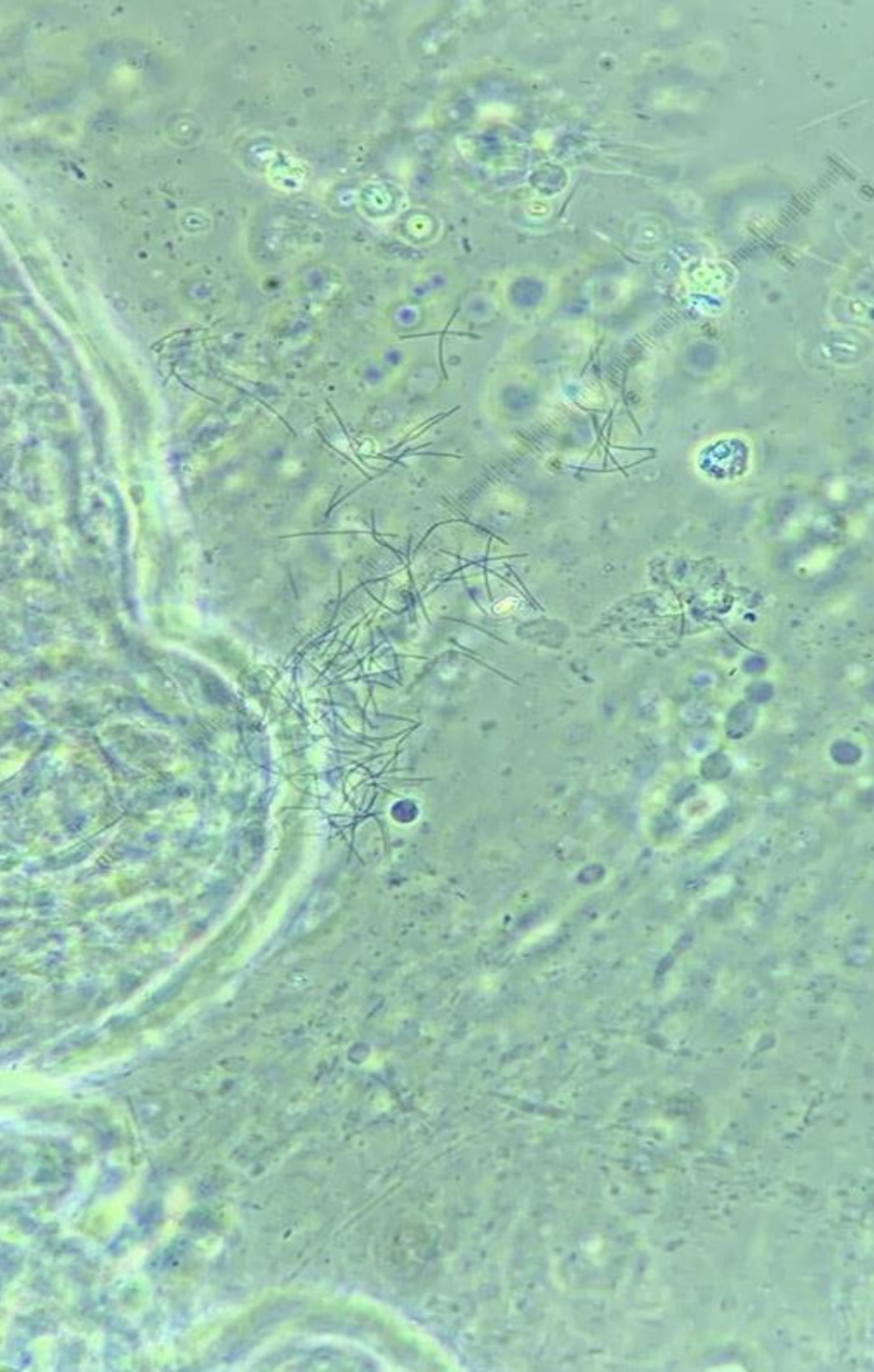


# Establishment of QC Parameters for Antimicrobial Susceptibility Testing of *F. psychrophilum* and *F. columnare* by Disk Diffusion

---

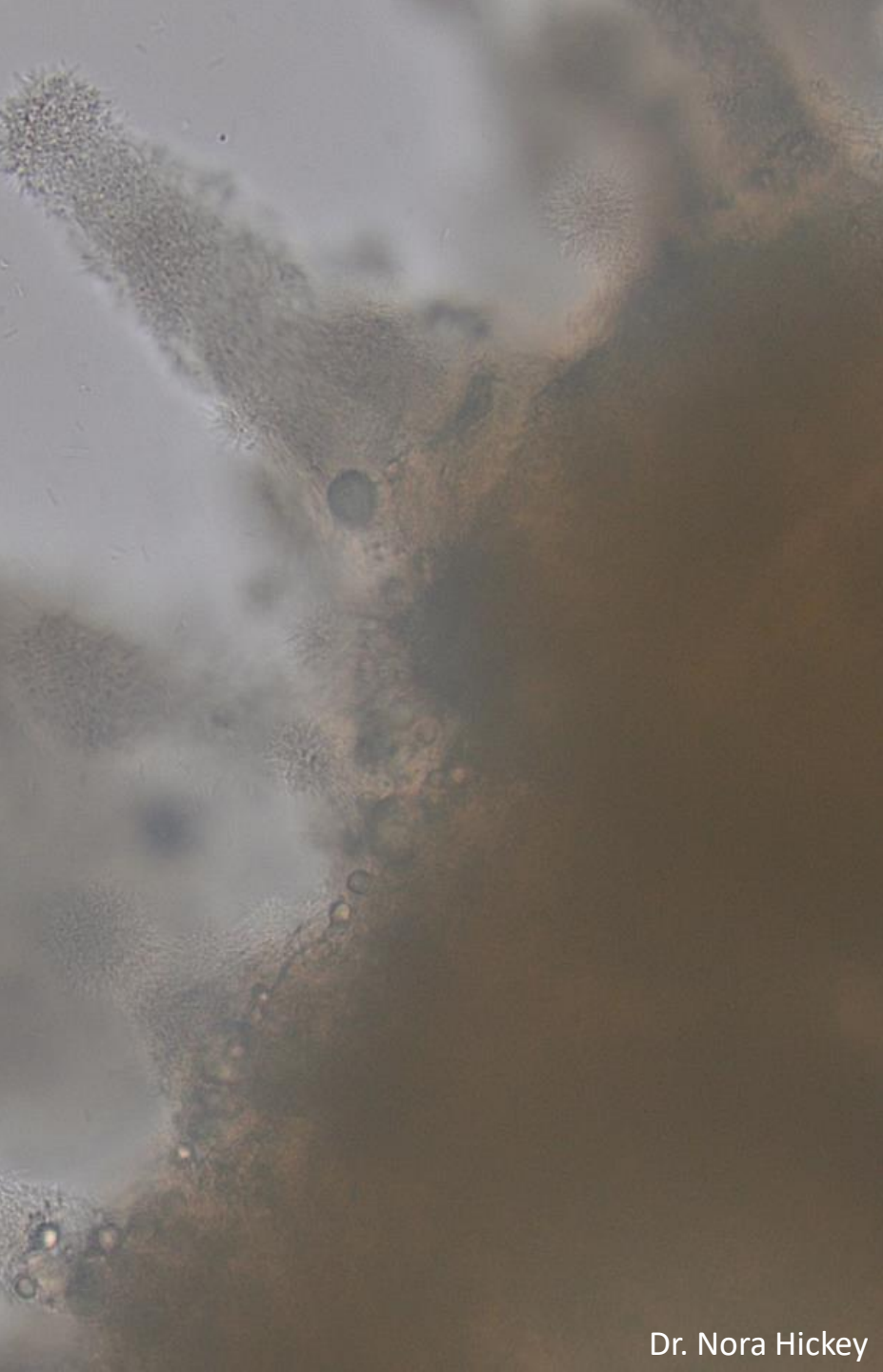
Kayleigh Carranza, BS  
Washington Animal Disease Diagnostic Laboratory  
Washington State University



# *Flavobacterium psychrophilum*

---

- Ubiquitous in the aquatic environment
- Causes Bacterial Coldwater disease (BCWD)
  - Affects various fish species, primarily salmonids, that inhabit cold freshwater temperatures (10-20°C)
- Disease can be associated with environmental stress, such as fish handling or crowding
- Causes skin ulcers, fin degradation, systemic infection
- Causes large economic losses in farmed salmonids



# *Flavobacterium columnare*

---

- Also ubiquitous in the aquatic environment
- Causes Columnaris disease
  - Affecting various freshwater fish
- Disease is associated with environmental stress such as:
  - Increased water temperature (20-25°C)
  - Decreased water quality – pH or salinity
  - Fish density
- Causes acute to chronic infections involving gills, skin, and fins, even systemic infections
- Causes large economic losses in farmed fish worldwide

# Cultivation of *Flavobacterium*

---

- Cultivation and isolation of *Flavobacterium* has historically been problematic due to its fastidious nature and specific growth requirements.
  - Requires specialized media:
    - Cytophaga agar
    - Tryptone Yeast Extract Salts (TYES) agar
  - Requires incubation at specific temperatures
  - Overgrowth of environmental bacteria





3rd Edition

## VET04

### Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated From Aquatic Animals

This document includes updated tables for the Clinical and Laboratory Standards Institute veterinary antimicrobial susceptibility testing guideline VET03.

A CLSI supplement for global application.

# Antimicrobial Susceptibility Testing: *F. psychrophilum* & *F. columnare*

## Pros:

- CLSI standardized MIC methods & QC ranges available
- Epidemiological cutoff values (ECVs) available

## Cons:

- No commercial broth microdilution plates available with antibiotics commonly used in aquaculture
- Custom plates are extremely expensive
- No disk diffusion methods or QC available
- No disk diffusion ECV's available

# AST - Broth Microdilution for *Flavobacterium*

Current CLSI Methods:

## *F. psychrophilum*

- Growth medium: Diluted CAMHB (4 g/L)
- Incubation: 18°C; ambient air; 92-96 hours

## *F. columnare*

- Growth medium: Diluted CAMHB (4 g/L)
- Incubation: 28°C; ambient air; 44-48 hours

MIC QC Ranges available for:

- Ampicillin
- Enrofloxacin
- Erythromycin
- Florfenicol
- Flumequine
- Gentamicin
- Ormetoprim-sulfa
- Oxolinic acid
- Oxytetracycline
- Trimethoprim-sulfa





# Antimicrobial Susceptibility Testing for *Flavobacterium*

---

Currently, many labs perform disk diffusion on TYES media

- Varying incubation times, using historical zone sizes for interpretation
- Little or no QC

...How sad



CLINICAL AND  
LABORATORY  
STANDARDS  
INSTITUTE

4th Edition

## VET02

### Development of Quality Control Ranges, Breakpoints, and Interpretive Categories for Antimicrobial Agents Used in Veterinary Medicine

This guideline discusses the necessary and recommended data for selecting appropriate quality control ranges, breakpoints, and interpretive categories for antimicrobial agents for veterinary use.

A guideline for global application developed through the Clinical and Laboratory Standards Institute consensus process.

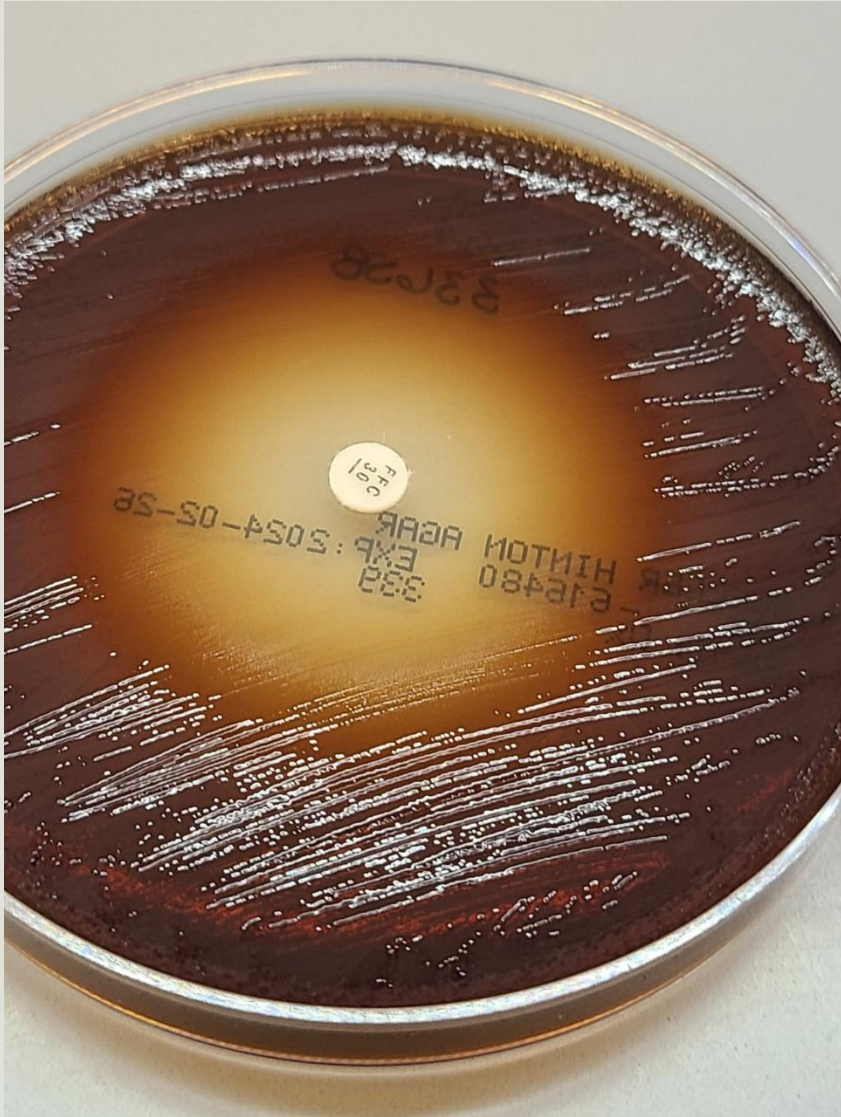
# Disk Diffusion QC: Development

- Used CLSI VET02 Tier 2 Quality Control Study
- Designed to provide adequate data to establish expected QC ranges
  - Method reproducibility within a laboratory
  - Method reproducibility between separate laboratories
  - Between reagent lots & manufacturers
  - Perform 10 replicates of each QC strain

# Disk Diffusion: Test Goals

- Media that supports growth of both *F. psychrophilum* and *F. columnare*
- Media can be made with readily available materials
- Materials must be low cost
- Methods must be simple
- Must have QC ranges to monitor performance of method and reagents





# Disk Diffusion QC: Plan

Using *E. coli* (ATCC 25922) and *Aeromonas salmonicida* (ATCC 33658), develop testing protocols that includes:

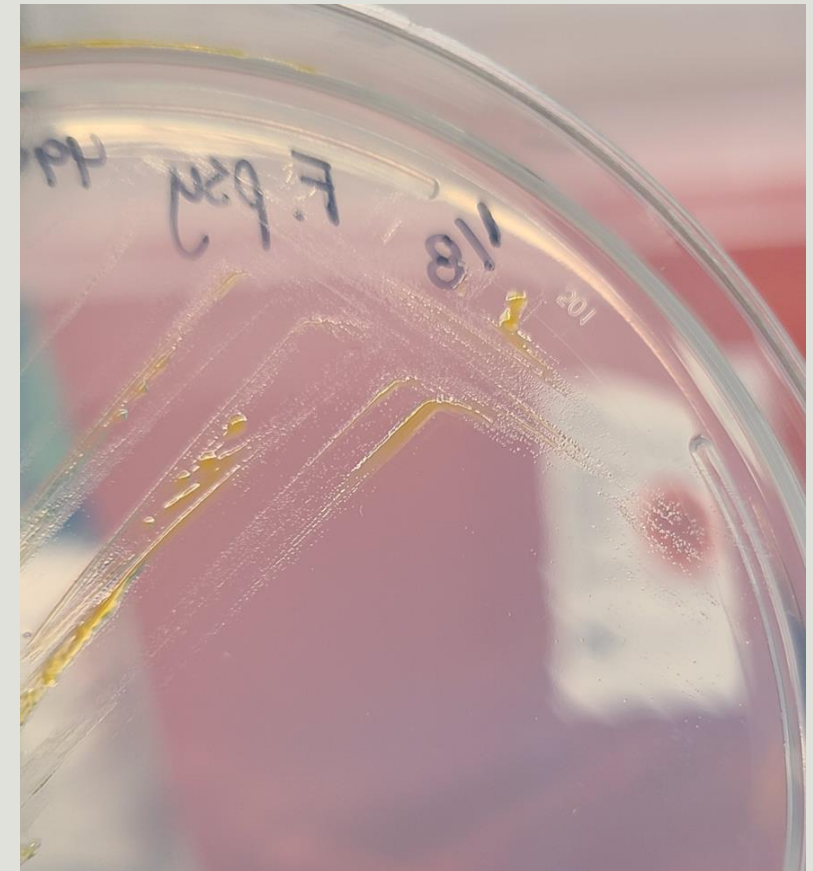
1. Specific temperatures and incubation times
  1. Any complications?
2. Antibiotics appropriate for aquaculture
  1. Enrofloxacin
  2. Florfenicol
  3. Ormetoprim-sulphadimethoxine
  4. Oxytetracycline
  5. Trimethoprim-sulfamethoxazole
3. Multiple lots and brands of disks and media reagents

# Media

---

**Goal: Media can be made with readily available materials.**

- Multiple publications show successful growth of *F. psychrophilum* and *F. columnare* on dilute cation-adjusted Mueller-Hinton agar (DCAMHA)
  - Similar to the broth currently used in broth microdilution susceptibility testing (published in CLSI VET04)
- Multiple dilutions of DCAMHA were evaluated
  - Promotes bacterial growth of all four organisms
    - *E. coli* ATCC 25922
    - *A. salmonicida* ATCC 33658
    - *F. psychrophilum* ATCC 49418
    - *F. columnare* ATCC 23463
  - Allows distinct zone margins
- Dilution chosen - 4 g/L DCAMHA, consistent with CLSI



For each ATCC organism...

Diluted Cation-Adjusted Mueller-Hinton Agar (DCAMHA) - Plate Size

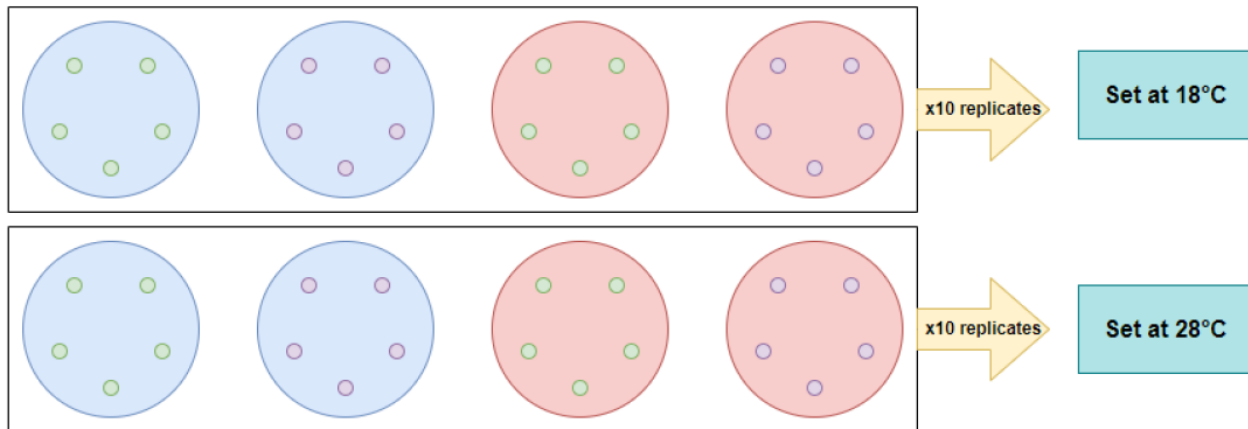
150mm

Plate A - DCAMHA (BD BBL)

Plate B - DCAMHA (Thermo Oxoid)

Disk A - BD BBL (or Lot #1, depending on manufacturer)

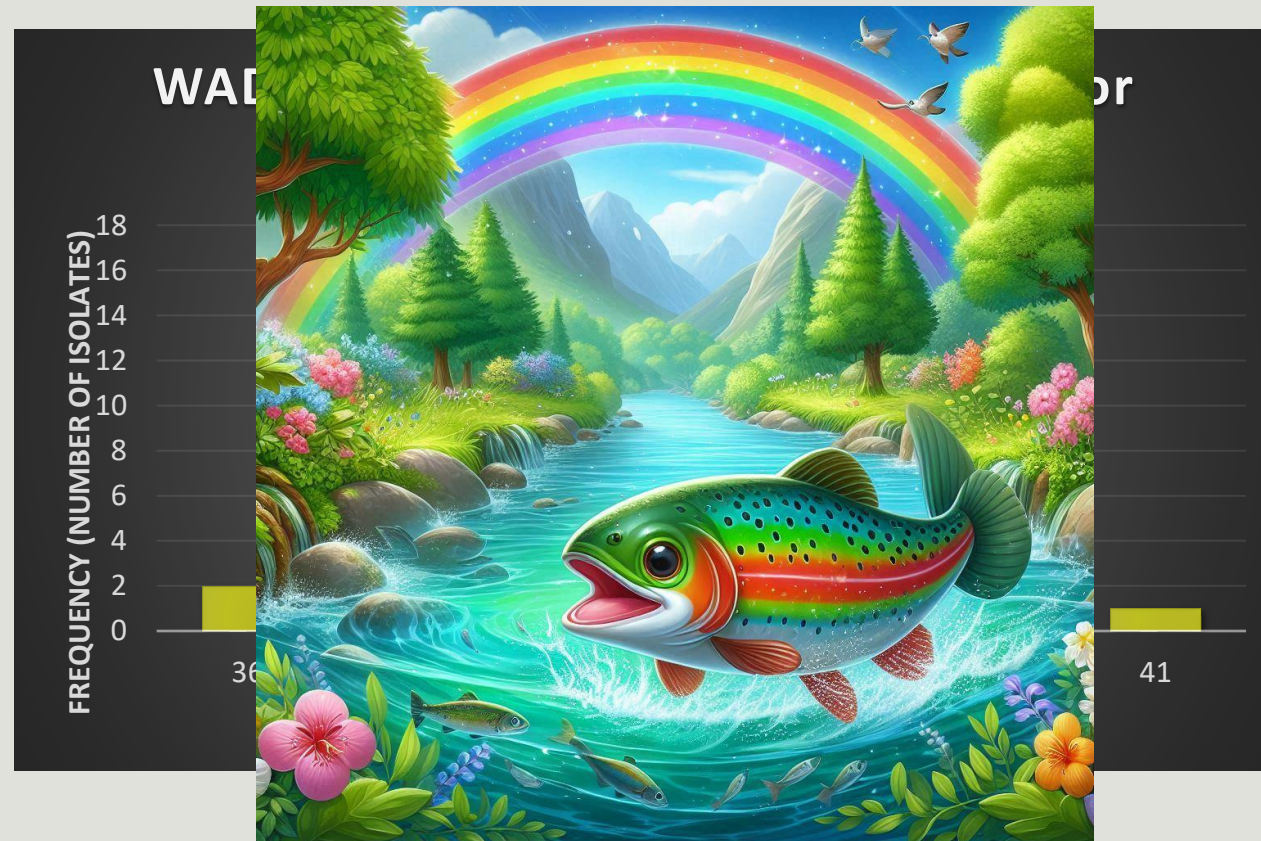
Disk B - Thermo Oxoid (or Lot #2, depending on manufacturer)



# Testing and Data Collection

# Results

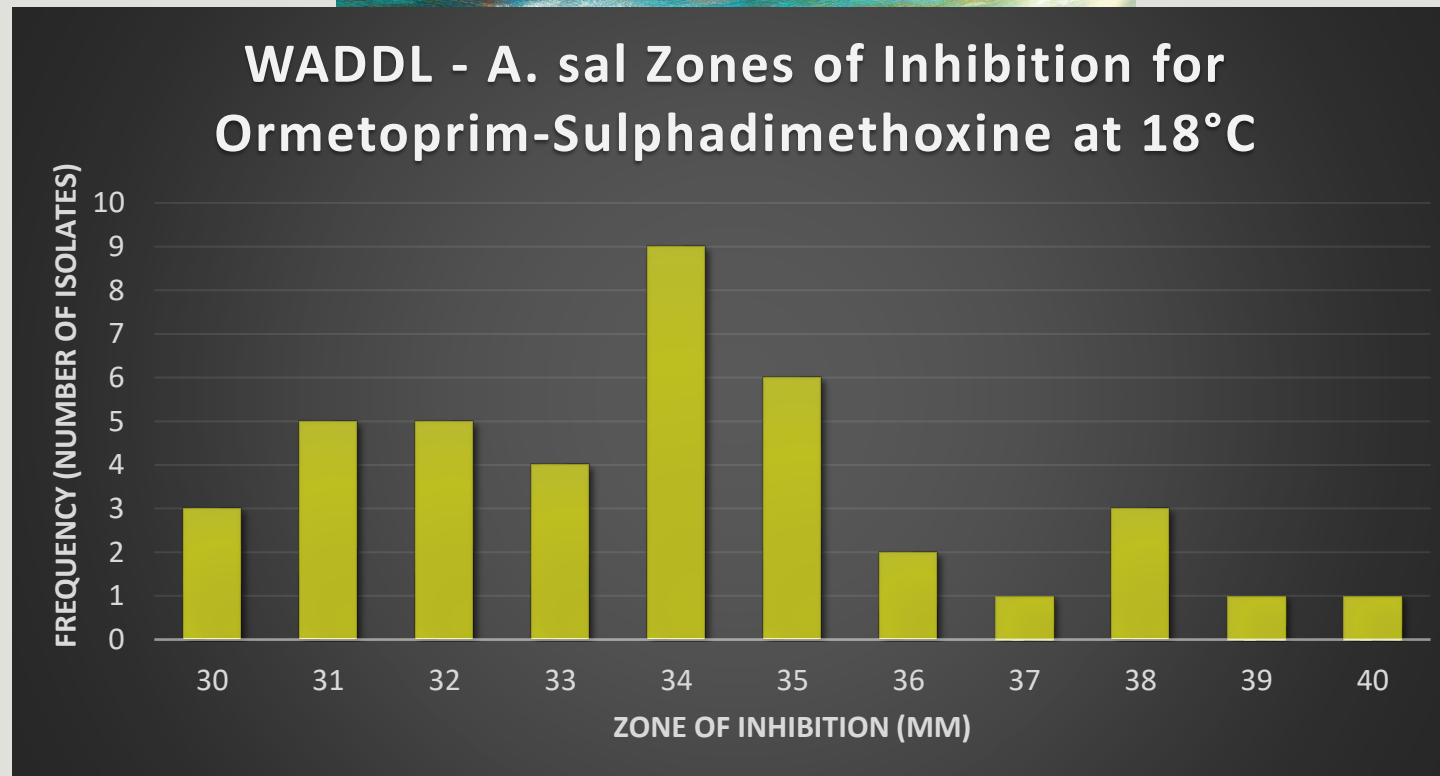
## In a Perfect World



# Results

---

## Reality



# Challenges

---

## QC Strain Challenges

- Long incubation times at lower temperatures can lead to varying zone sizes
- Large zone sizes can result in overlap of other antibiotic zones complicating measurement
- Zone margins are not as distinct - Sulfonamides

## *Flavobacteria* Challenges

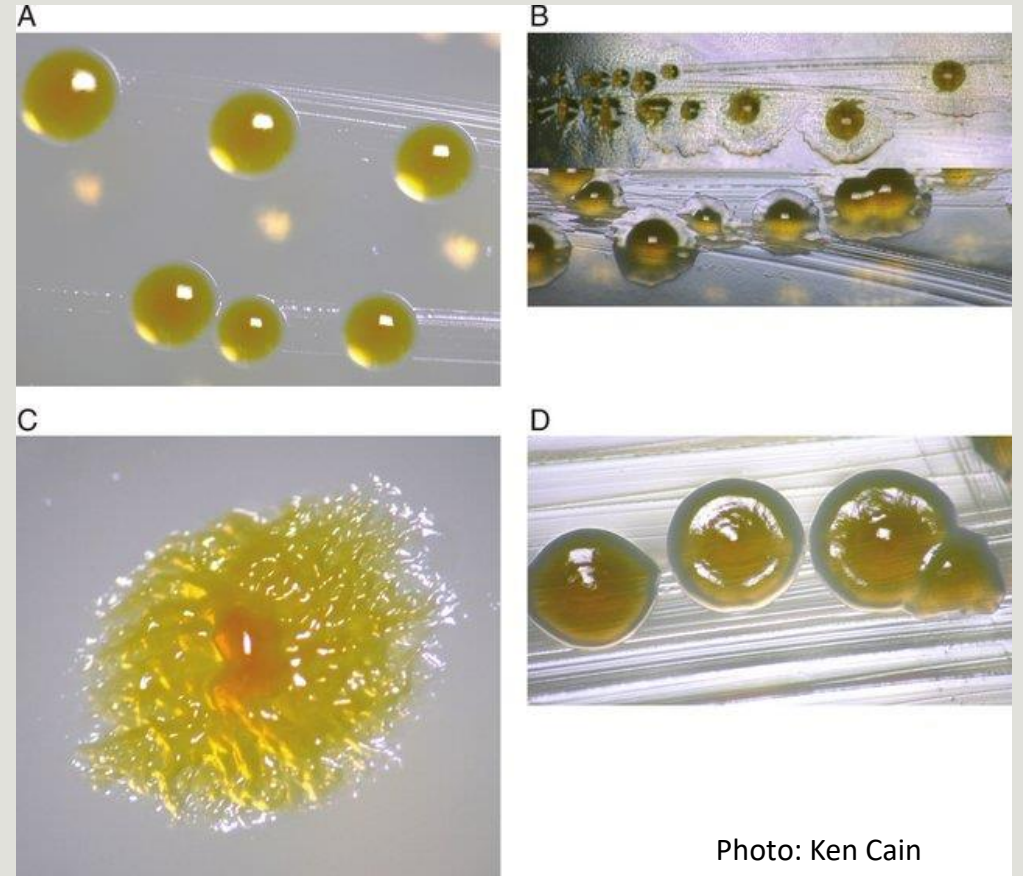
- Highly sensitive/fastidious organisms - varying and inconsistent growth patterns of clinical isolates result in zone irregularities
- Obtaining the appropriate CFU when creating 0.5 McFarland

## Logistical Challenges

- Labs with both the ability to incubate at 18°C & 28°C.
- Labs with the ability to read zone sizes accurately and consistently

# Ongoing testing

- Testing Clinical Isolates
  - 30 isolates of both organisms – *F. psychrophilum* & *F. columnare*
  - Test other species of *Flavobacterium columnare*: *F. covae*, *F. davisii* and *F. oreochromis*
- Perform additional disk diffusion with QC organisms to account for variation between microbiologists
  - Goal: to learn what may contribute to variability in interpreting zone sizes





# Future

---

Ultimately, we are hoping our work paves the way for a complete CLSI Tier 2 study to be performed so QC ranges can be developed for disk diffusion.

# Acknowledgments

---

## **WADDL Staff:**

- Brendon Meldrum
- Trevor Alexander
- Dr. Claire Burbick
- Dr. Nora Hickey



College of Veterinary Medicine

**Washington Animal Disease Diagnostic Laboratory**

## **USDA APHIS:**

- Dr. Diana Short



Animal and Plant Health Inspection Service  
U.S. DEPARTMENT OF AGRICULTURE

# Acknowledgments

---

- **Oregon VDL**
  - Mathew Miller
- **ODFW**
  - Emma Miller
  - Aimee Reed
- **Bozeman FHC**
  - Renee Yamamoto
  - Tammy Weiss
- **Cornell AHDC**
  - Christina Ann Dean

