Antibiotic Resistance in Aquaculture

know the risks, know the facts

Dr. Jesse Trushenski
Idaho Department of Fish and Game
Introduction: D. Allen Pattillo, USAS Board Member and NCRAC Extension Coordinator
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ANTIBIOTIC RESISTANCE IN AQUACULTURE

Know the Risks, Know the Facts

Jesse Trushenski
EAGLE FISH HEALTH LABORATORY
IDAHO DEPARTMENT OF FISH AND GAME
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WHAT IS ANTIBIOTIC RESISTANCE AND HOW DOES IT DEVELOP?
Incomplete biosecurity, routine introduction of naïve hosts, repeat encounters with the same pathogens, and limited treatment options might increase the odds of developing antibiotic resistance.
Antibiotic resistance has been linked to drug use in animal agriculture.
Resistant bacteria from animals can cause human health impacts directly or by exchanging resistance factors with human pathogens.
HOW DO WE KNOW IF RESISTANCE IS DEVELOPING?
There are several reasons for treatment failure that have nothing to do with antibiotic resistance.
ANTIBIOTIC SUSCEPTIBILITY

Susceptibility

Growth/No Growth
Antibiotic Targets
- Cell Wall
  - β-lactams
  - Vancomycin
- DNA/RNA Synthesis
  - Fluoroquinolones
  - Rifamycins
- Folate Synthesis
  - Trimethoprim
  - Sulfonamides
- Cell Membrane
  - Daptomycin
- Protein Synthesis
  - Linezolid
  - Tetracyclines
  - Macrolides
  - Aminoglycosides

Antibiotic Resistance
- Efflux
  - Fluoroquinolones
  - Aminoglycosides
  - Tetracyclines
  - β-lactams
  - Macrolides
- Immunity & Bypass
  - Tetracyclines
  - Trimethoprim
  - Sulfonamides
  - Vancomycin
- Target Modification
  - Fluoroquinolones
  - Rifamycins
  - Vancomycin
  - Penicillins
  - Macrolides
  - Aminoglycosides

Resistance → Acquiring Mechanisms to Defeat Antibiotic
**A Quick Reference Guide to:**

### Approved Drugs for Use in Aquaculture

#### Florentinicol

<table>
<thead>
<tr>
<th>Product name &amp; supplier</th>
<th>Species</th>
<th>Indication</th>
<th>Dosage</th>
<th>Limitations &amp; Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FLORENTINICOL</strong></td>
<td>Catfish</td>
<td>Control internal disease due to <em>Flavobacterium columnaris</em></td>
<td>2 mg per kg body weight for 5 days</td>
<td>May cause kidney toxicity in susceptible species</td>
</tr>
</tbody>
</table>

#### Oxycetacine Hydrochloride

<table>
<thead>
<tr>
<th>Product name &amp; supplier</th>
<th>Species</th>
<th>Indication</th>
<th>Dosage</th>
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</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxycetacine HCl</strong></td>
<td>All fish</td>
<td>Control external disease due to <em>Saprolegnia</em></td>
<td>2 mg per kg body weight for 3 days</td>
<td>May cause increased gill respiration</td>
</tr>
</tbody>
</table>

#### Oxynitricin Hydrochloride

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<th>Dosage</th>
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<tr>
<td><strong>Oxynitricin HCl</strong></td>
<td>All fish</td>
<td>Control external disease due to <em>Saprolegnia</em></td>
<td>2 mg per kg body weight for 3 days</td>
<td>May cause increased gill respiration</td>
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#### Sulfamethoxazole and Trimethoprim

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<th>Indication</th>
<th>Dosage</th>
<th>Limitations &amp; Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SULFAMETHOXAZOLE &amp; TRIMETHOPRIM</strong></td>
<td>Catfish</td>
<td>Control external disease due to <em>Vibrio</em></td>
<td>2 mg per kg body weight for 3 days</td>
<td>May cause increased gill respiration</td>
</tr>
</tbody>
</table>

### Other Drugs

- **AQUAFLEX**
- **PARASITE-S**
- **FORMALIN-P**
- **FORMACID-S**

### Veterinary Feed Directive (VFD) drugs

- **FLORENTINICOL**: Certain drugs that are intended for use in animal feeds. The use of VFD drugs is permitted only under the professional supervision of a licensed veterinarian. VFD drugs cannot be used under veterinarian-directed prescriptions.
- **Oxycetacine Hydrochloride**: Certain drugs that are intended for use in animal feeds. The use of VFD drugs is permitted only under the professional supervision of a licensed veterinarian. VFD drugs cannot be used under veterinarian-directed prescriptions.

### Additional Information

- [FDA Information](https://www.fda.gov) for additional information and new approvals.
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<thead>
<tr>
<th>Approved Drugs for Use in Aquaculture</th>
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<tr>
<td><strong>AQUAFLOR</strong> (florfenicol)</td>
</tr>
<tr>
<td><strong>35% PEROX-AID</strong> (hydrogen peroxide)</td>
</tr>
<tr>
<td><strong>CHORULON</strong> (chorionic gonadotropin)</td>
</tr>
<tr>
<td><strong>CHLORAMINE-T</strong> (chloramine)</td>
</tr>
<tr>
<td><strong>TRICAINE-S</strong> (tricaine methanesulfonate)</td>
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<tr>
<td><strong>TERRAMYCIN 200</strong> (oxytetracycline dihydrate)</td>
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AQUAFLOWR
(florfenicol)
Systemic bacterial infections

35% PEROX-AID (hydrogen peroxide)
External bacterial infections

CHORULON
(chorionic gonadotropin)
Spawning aid

PARASITE-F
(formalin)
External fungal and protozoan infestations

OXYTETRACYCLINE HCL
TETROXY TERRAMYCIN 343
(oxytetracycline hydrochloride)
Skeletal marking

TERRAMYCIN 200
(oxytetracycline dihydrate)
Systemic bacterial infections

ROMET
(sulfadimethoxine/ormetoprim)
Systemic bacterial infections

TRICAINE-S
(tricaine methanesulfonate)
Sedative

CHLORAMINE-T
(chloramine)
External bacterial infections

CHLORAMINE-T
(chloramine)
External bacterial infections
AQUAFLOR (florfenicol) Systemic bacterial infections

ROMET (sulfadimethoxine/ormetoprim) Systemic bacterial infections

OXYTETRACYCLINE HCL TETROXY TERRAMYCIN 343 (oxytetracycline hydrochloride) Skeletal marking

TERRAMYCIN 200 (oxytetracycline dihydrate) Systemic bacterial infections

ERYMICIN 200 (erythromycin) *Renibacterium salmoninarium* infections
CASE STUDY
IS ANTIBIOTIC RESISTANCE DEVELOPING IN IDFG-OPERATED HATCHERIES?
We used linear regression models to explore 20 years of antibiotic susceptibility data.
RESULTS INDICATE NO MEANINGFUL LOSS OF ANTIBIOTIC SUSCEPTIBILITY

This graph represents ~20 years of sensitivity data for 11 hatcheries.
OBSERVED AND PREDICTED SENSITIVITY OF *F. psychrophilum*

4 ANTIBIOTICS + 11 HATCHERIES

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Zone of Inhibition (mm)</th>
<th>Year</th>
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<tbody>
<tr>
<td>Sulfadimethoxine/Ormetoprim</td>
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<tr>
<td>Tetracycline</td>
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<tr>
<td>Florfenicol</td>
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<tr>
<td>Erythromycin</td>
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Year

0 10 20 30 40 50 60


Zone of Inhibition (mm)
OBSERVED AND PREDICTED SENSITIVITY OF *Aeromonas* spp.
4 ANTIBIOTICS + 8 HATCHERIES

Zone of Inhibition (mm)

Year

Sulfadimethoxine/Ormetoprim

Tetracycline

Florfenicol

Erythromycin
OBSERVED AND PREDICTED SENSITIVITY OF *A. salmonicida*
4 ANTIBIOTICS + 1 HATCHERY

Sulfadimethoxine/Ormetoprim

Tetracycline

Florfenicol

Erythromycin

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<td>2012</td>
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</table>
WHAT PRACTICES COULD LEAD TO RESISTANCE?
THERAPEUTIC VS. NONTHERAPEUTIC USES

Therapeutic Claim(s)
20 g/ton

Production Claim(s)
10-50 g/ton

Therapeutic Claim(s)
100-500 g/ton

Production Claim(s)
10-50 g/ton

Therapeutic Claim(s)
50-200 mg/head/d

Production Claim(s)
25-75 mg/head/d

Therapeutic Claim(s)
2.5-3.75 g/100 lbs./d

No production claims are approved for fish

FDA-APPROVED USES OF OXYTETRACYCLINE DIHYDRATE PRODUCTS
ANTIBIOTICS AS GROWTH PROMOTERS

Nontherapeutic use of antibiotics to enhance terrestrial animal growth performance was a common, but recently controversial practice.

Misconception that antibiotics are used this way in U.S. aquaculture.

Such uses are illegal, generally believed to be ineffective in fish.

Little quantitative data available that unequivocally demonstrate the effect(s) of antibiotic administration on fish growth.
CASE STUDY

DOES OXYTETRACYCLINE PROMOTE GROWTH OR EFFICIENCY IN FISH?
## INCENTIVE TO MISUSE ANTIBIOTICS IN AQUACULTURE?

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>OTC/kg fish/d if fed at 3% BW/d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Commercial feed + 50 mL tap water/kg feed + 5 g fish oil/kg feed</td>
<td>0 mg</td>
</tr>
<tr>
<td>Standard Dose OTC</td>
<td>Commercial feed + 1.2 g OTC/kg feed + 50 mL tap water/kg feed + 5 g fish oil/kg feed</td>
<td>80 mg</td>
</tr>
<tr>
<td>Low Dose OTC</td>
<td>Commercial feed + 0.24 g OTC/kg feed + 50 mL tap water/kg feed + 5 g fish oil/kg feed</td>
<td>16 mg</td>
</tr>
</tbody>
</table>
## INCENTIVE TO MISUSE ANTIBIOTICS IN AQUACULTURE?

<table>
<thead>
<tr>
<th></th>
<th>Commercial feed</th>
<th>50 mL tap water/kg feed</th>
<th>5 g fish oil/kg feed</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Standard Dose OTC</strong></td>
<td>Commercial feed</td>
<td>1.2 g OTC/kg feed</td>
<td></td>
</tr>
<tr>
<td><strong>Low Dose OTC</strong></td>
<td>Commercial feed</td>
<td>0.24 g OTC/kg feed</td>
<td></td>
</tr>
</tbody>
</table>

**If fed at 3% BW/d**

- **Control**: 0 mg OTC/kg fish/d
- **Standard Dose OTC**: 80 mg OTC/kg fish/d
- **Low Dose OTC**: 16 mg OTC/kg fish/d

**THIS STUDY WAS NOT CONDUCTED IN SUPPORT OF PRODUCTION CLAIMS FOR ANTIBIOTICS IN U.S. AQUACULTURE**
INCENTIVE TO MISUSE ANTIBIOTICS IN AQUACULTURE?

Weight gain, specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI), visceroelasticotic index (VSI)

One-way ANOVA with Tukey’s HSD pairwise comparison tests, PROC GLIMMIX, SAS 9.4

Normal vs. abnormal appearance of external and internal features
Chi-square test for differences in the frequency of normal vs. abnormal observations, PROC FREQ, SAS 9.4
INCENTIVE TO MISUSE ANTIBIOTICS IN AQUACULTURE?

Hybrid Striped Bass
- Weight: 27 g
- Temperature: 26.5°C

Channel Catfish
- Weight: 5 g
- Temperature: 26.8°C

Nile Tilapia
- Weight: 54 g
- Temperature: 28.8°C
OTC DOES NOT PROMOTE GROWTH IN HYBRID STRIPED BASS

Weight Gain $P = 0.122$   FCR $P = 0.120$   SGR $P = 0.119$

![Graphs showing Weight Gain, FCR, and SGR comparisons between Control, Standard Dose OTC, and Low Dose OTC groups.](image-url)
OTC DOES NOT PROMOTE GROWTH IN HYBRID STRIPED BASS

**HSI** $P = 0.021$

**VSI** $P = 0.243$

- **Control**
- **Standard Dose OTC**
- **Low Dose OTC**
OTC DOES NOT PROMOTE GROWTH IN NILE TILAPIA

Weight Gain $P = 0.122$  
FCR $P = 0.120$  
SGR $P = 0.119$

Weight Gain

FCR (as fed)

SGR (% BW/d)

- **Control**
- **Standard Dose OTC**
- **Low Dose OTC**

P = 0.112
OTC DOES NOT PROMOTE GROWTH IN NILE TILAPIA

HSI $P = 0.728$

VSI $P = 0.518$

OTC

Control

Standard Dose OTC

Low Dose OTC
OTC DOES NOT PROMOTEGROWTH IN CHANNEL CATFISH

**Weight Gain** $P = 0.201$  
**FCR** $P = 0.281$  
**SGR** $P = 0.197$

---

**Weight Gain (%)**
- Control
- Standard Dose OTC
- Low Dose OTC

**FCR (as fed)**
- Control
- Standard Dose OTC
- Low Dose OTC

**SGR (% BW/d)**
- Control
- Standard Dose OTC
- Low Dose OTC
OTC DOES NOT PROMOTE GROWTH IN CHANNEL CATFISH

\[ \text{HSI} \quad P = 0.972 \quad \text{VSI} \quad P = 0.579 \]
OTC DOES NOT PROMOTE GROWTH IN CHANNEL CATFISH

Skin & Body Surface $P = 0.017$
OTC DOES NOT PROMOTE GROWTH OR EFFICIENCY IN FISH

Continuous feeding of OTC, at a standard therapeutic dose or a low dose, had no significant effect on growth performance of the taxa tested.

- Trends, when apparent, suggested a negative effect of OTC.
- Only significant difference in the frequency of normal vs. abnormal tissues indicated greater incidence of abnormalities among fish fed OTC-mediated feed.

Collectively, our results indicate there is no growth performance benefit associated with continuous feeding of OTC-mediated feed and little-to-no incentive to misuse OTC in fish this way.

Antibiotics are now accessible only with veterinary oversight.

- FDA is encouraging drug sponsors to voluntarily withdraw production claims.
WHAT ARE THE CURRENT RULES FOR ANTIBIOTIC USE?
ANTIBIOTIC ACCESS HAS CHANGED

To address concerns related to use of antibiotics in food-producing animals (including fish) and the development of antimicrobial resistance, the Food and Drug Administration (FDA) has issued a new rule that all medically important antibiotics will be accessible only with veterinary oversight as of January 1, 2017

All **immersion treatments** with antibiotics will require a veterinary **prescription**

All **in-feed treatments** with antibiotics will require a **Veterinary Feed Directive (VFD)**

FDA has released new compliance guidance regarding extra-label use of VFD drugs in minor species, including fish
### WHAT DRUGS ARE AFFECTED?

#### By Prescription Only

*Oxytetracycline hydrochloride*

- Oxymarine™
- Oxytetracycline HCl Soluble Powder-343
- Pennox 343®
- Terramycin 343 (oxytetracycline HCl) Soluble Powder
- Tetroxy® Aquatic

#### By VFD Only

*Florfenicol*

- Aquaflor®

*Oxytetracycline dihydrate*

- Terramycin® 200 for Fish

*Sulfadimethoxine/ormetoprim*

- Romet® 30
- Romet® TC

These products are for specific therapeutic treatments or skeletal marking (Oxytetracycline dihydrate only)

These products are for skeletal marking
WHAT DRUGS ARE NOT AFFECTED?

The January 1, 2017 rule does not apply to...

Other approved drugs, including antimicrobials
- Chloramine-T, formalin, hydrogen peroxide, and others

Investigational New Animal Drugs (INADs)
- Access coordinated through the U.S. Fish and Wildlife Service Aquatic Animal Drug Approval Partnership (AADAP) program is unaffected, including antibiotics

Deferred regulatory status drugs
- Copper sulfate and potassium permanganate

Low regulatory priority drugs
- Ice, salt, carbon dioxide, and others
WHAT DOES THIS MEAN FOR AQUACULTURE?

Hatcheries and fish farms must work with a veterinarian licensed in their state of operations to obtain prescriptions and VFDs.

Extra-label use of VFD drugs is now possible and may be warranted in some cases, under certain circumstances.

For a prescription or VFD to be valid, it must be issued by a licensed veterinarian in the context of a valid vet-client-patient relationship (VCPR).
Federal Definition of Valid VCPR (21 CFR § 530.3(i))

A valid veterinarian-client-patient relationship is one in which:

(1) A veterinarian has assumed the **responsibility** for making medical judgments regarding the health of (an) animal(s) and the need for medical treatment, and the client (the owner of the animal or animals or other caretaker) has agreed to follow the instructions of the veterinarian;

(2) There is sufficient **knowledge** of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s); and

(3) The practicing veterinarian is readily **available** for followup in case of adverse reactions or failure of the regimen of therapy. Such a relationship can exist only when the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of examination of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept.
Idaho Definition of Valid VCPR (IDAPA 46.01.01 150)

An appropriate veterinarian/client/patient relationship will exist when:

01. **Responsibility**. The veterinarian has assumed the responsibility for making medical judgements regarding the health of the animal and the need for medical treatment, and the client (owner or other caretaker) has followed the instructions of the veterinarian.

02. **Medical Knowledge**. There is sufficient knowledge of the animal by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal. This means that the veterinarian has seen the animal within the last twelve (12) months or is personally acquainted with the keeping and care of the animal, either by virtue of an examination of the animal, or by medically appropriate visits to the premises where the animals are maintained within the last twelve (12) months.

03. **Availability**. The practicing veterinarian or designate is readily available for follow-up in case of adverse reactions or failure of the regimen of therapy.
WHERE TO GO FOR MORE INFORMATION

AFS Fish Culture & Fish Health Sections
American Association of Fish Veterinarians
USFWS AADAP Program

Your friendly local veterinarian and fish health professional are also great resources
GUIDE TO USE OF DRUGS, BIOLOGICS, AND OTHER CHEMICALS IN AQUACULTURE

One-stop shopping for up-to-date information on proper use of drugs, biologics, disinfectants, pesticides

Companion treatment calculator helps with application rates

FISHCULTURE.FISHERIES.ORG
USING THE DRUG TREATMENT CALCULATOR ON YOUR DESKTOP

Values auto-calculate treatments based on volumes and flow rates
CONCLUSIONS
KNOW THE RISKS, KNOW THE FACTS

Antibiotic resistance is an important human health risk

Antibiotic use in agriculture, including aquaculture, can contribute to resistance, but recognize that the risks are different from those associated with antibiotic use in human medicine.

It’s all about legal and judicious use

Being able to treat infections quickly, effectively, and compassionately is an important part of fish health management.

Address misinformation when you encounter it

Antibiotics are subject to strict regulation and veterinary oversight, including withdrawal periods to protect consumers.

Little evidence of antibiotic resistance in Idaho, elsewhere?

No incentive to misuse antibiotics as growth promoters.
The Eagle Fish Health Laboratory provides comprehensive, responsive fish health services to the Idaho Department of Fish and Game and its partners.

Questions? Please ask!

Jesse Trushenski
jesse.trushenski@idfg.idaho.gov
208-939-2413
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Questions about this webinar series should be directed to brentoncontact@iastate.edu
Recorded webinars are available at:

• The National Aquaculture Association [www.thenaa.net/industry](http://www.thenaa.net/industry)

• The North Central Regional Aquaculture Center [www.ncrac.org/video](http://www.ncrac.org/video)

• United States Aquaculture Society [http://usaquaculture.org/webinars](http://usaquaculture.org/webinars)