Cir 84

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Use of Antibiotics in Ornamental Fish Aquaculture[1]

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Introduction

Antibiotics are very useful additions to any fish-health manager's toolbox, but they are only tools and not 'magic bullets.' The ability of antibiotics to help eliminate a fish disease depends on a number of factors: 1) Does the problem actually have a bacterial component? 2) Are the bacteria involved sensitive to the antibiotic chosen? 3) Are the proper dosage and treatment intervals being used? 4) Have other contributing stresses been removed or reduced?

Antibiotics, in and of themselves, do not cure a fish. Antibiotics merely control the population growth of bacteria in a fish long enough for its immune system to eliminate them.

Before antibiotics are even considered, sources of stress such as poor water quality (including drastic temperature change), nutrition, genetics, and handling or transport must be removed or reduced. Affected fish should also be examined for parasites. Any of these factors may be the primary cause of disease, as bacterial infections are often secondary responses to such management problems. Contacting a fish health specialist early during the disease outbreak will help identify contributing stressors and the rate of bacterial infection so total loss of fish will be reduced.

Gram-positive Bacteria versus Gram-negative Bacteria

Most bacteria that infect fish fall into one of two groups - gram-positive or gram-negative. These groups are named based on their response to a protocol called gram staining. Gram-positive bacteria stain blue, and gram-negative bacteria stain pink. They stain differently because each group has a different type of outer structure known as the cell wall. This difference is important for the producer and aquaculturist because some antibiotics work better against gram-positive bacteria and others work better against gram-negative bacteria. Most bacteria that infect fish are gram-negative, including *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Flavobacterium columnare* (which causes columnaris), *Vibrio*, and *Pseudomonas* species. (See UF/IFAS Fact Sheets FA-14 *Aeromonas* Infections, FA-31 *Vibrio* Infections of Fish and FA-11 Columnaris disease). The major group of gram-positive bacteria that cause disease in fish are *Streptococcus*. (See UF/IFAS Circular 57 Streptococcal Infections in Fish.)

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A third group, the acid-fast bacteria, which includes *Mycobacterium* species, will not be discussed in this publication because they are considerably different from most other bacteria (see UF/IFAS Fact Sheet VM-96 Mycobacteriosis in Fish).

Optimal Approach to Fighting Bacterial Infections

Most bacteria that cause diseases in fish are normal inhabitants of aquatic systems and, ordinarily, they do not cause a problem. However, fish that are stressed by one or more factors (such as temperature changes, poor water quality, recent transport or handling), may have compromised (less effective) immune systems, making them more susceptible to bacterial infections. In addition, stress factors that compromise the immune system of fish may actually favor bacterial growth, further increasing the risk of a disease outbreak.

The ideal solution to bacterial diseases involves working with a fish health specialist to culture the organism and to run sensitivity tests. 'Culture' refers to growing the infective bacteria on a special type of media (or 'food'; usually agar or gelatin based), and 'sensitivity' refers to treating the bacteria with a series of antibiotics to determine which one will work best. Although culture and sensitivity tests generally take two or three days, they are, by far, the best methods for selecting an antibiotic that will successfully and economically treat an infection.

A fish health specialist should be contacted and given a complete history of the problem during the initial stages of the disease. The specialist will provide instructions on submitting samples to a diagnostic laboratory (see UF/IFAS Fact Sheet FA-55 Submission of Fish for Diagnostic Evaluation).

Affected fish should not be treated with any antibiotic until after a sample has been analyzed. The sample should include at least 3 to 5 fish showing typical signs of the disease. Fish that are submitted after they have been given antibiotics often provide poor culture results. While waiting for the culture results, the fish health specialist may suggest a broad-spectrum antibiotic that can be used until culture and sensitivity tests have been completed.

Legalities must also be considered when selecting antibiotics. Producers of food fish have fewer options than producers working with ornamental fish because the FDA has elected to use regulatory discretion to keep products intended for the treatment of aquarium fish available. Your fish health specialist will be able to provide information on legal constraints for specific antibiotics, information for correct dosages, proper methods of administration, and concerns about effluent from ponds with treated fish.

Proper Dosages and Treatment Regimens: Pharmacokinetics

Although selecting the correct antibiotic is an important first step in controlling bacterial disease, proper administration of any antibiotic for the recommended number of days is equally important. Your fish health specialist should provide you with instructions on the dose (amount of antibiotic to use), the frequency (how often) and duration (how long) it should be administered (given), withdrawal time (if applicable - this is the length of time required, after the last dose of antibiotic has been given, that a producer must wait before selling the fish, and is usually more of a concern in food fish species) and any other relevant information. The technical term for the branch of medical science that determines all of these variables is 'pharmacokinetics'; defined as the study of how drugs are absorbed by, distributed within, chemically altered within, and eventually excreted by the body (in this case, the body of a fish).

How are the pharmacokinetics of a specific antibiotic scientifically determined? Firstly, different concentrations of the selected antibiotic are tested against various bacteria in order to determine what concentration will work best against these bacteria. Next, the antibiotic is administered (given) in one of three ways: 1) by injection, 2) mixed in feed, or 3) in a bath treatment. Then, antibiotic levels in the body (usually in the blood) are measured at different time intervals after treatment. The purpose of these measurements is to determine how long the antibiotic stays active in the body of the fish and whether or not the concentration in the body is high enough to kill or inhibit bacteria.

Although the pharmacokinetics of many antibiotics have not been scientifically determined for most species of fish (and especially not for most ornamental species), good estimations of the activity of many antibiotics have been determined from clinical experience and from work with food fish. Following such guidelines for dosages and their corresponding treatment intervals (e.g., Table 1) should yield good results.

Percent Active Ingredient

Many antibiotics commonly used for ornamental fish are sold by different companies; therefore, the percent of active ingredient will vary from product to product. This means that you may not have 100% of pure antibiotic required for disease control. You need to ask your supplier to provide you with the actual percentage of antibiotic that is active in the product you have purchased and calculate your dosage accordingly. For example, if your product contains less than 100% of active ingredient, your dosage needs to be increased to bring the level up to the equivalent 100% active ingredient. (See Equation 1) If you are unsure how to do this calculation, call your local fish health specialist.

Major Routes of Administration

Injection: Injection is the most direct and effective method for getting antibiotics into the blood stream. Unfortunately, this process is very labor intensive and impractical for fish grown on a commercial scale. However, for small numbers of fish, or for important or expensive fish, injection may be the best method.

Mixed in food: In aquaculture production, the most cost effective and commonly used method to deliver antibiotics is orally by mixing them into food. The proper dose of antibiotic is mixed into the feed during production, or else it is added after production, using fish oil or canola oil as a binding agent. The mixture is then fed to the fish for the prescribed number of days. Oral administration of antibiotics requires that most of the fish are still eating, so every attempt should be made to catch bacterial diseases early, before the majority of fish stop eating. Only fish that are eating will be treated. Very sick fish that are no longer eating will contribute to ongoing mortalities. It is a good idea to train fish to eat a prepared diet (i.e., one that can be used to incorporate antibiotics), so that, should it becomes necessary to use antibiotics, the fish will be more likely to eat the medicated food as it is familiar to them.

Bath treatments: Although bath treatments are a popular method of administering antibiotics, much more drug is required to achieve the desired result as compared to oral treatments or injections. In many cases, even a large amount of antibiotic in the water does not guarantee that enough of it will get into the fish to be an effective treatment. At the same time, excessive amounts of antibiotic in the water can increase the likelihood of water-borne bacteria developing resistance to that drug. Furthermore, to avoid poor water quality and any potential toxicity, between 70% and 100% of the water should be changed at the end of each daily treatment and also prior to redosing. Finally, bath treatments are not recommended in recirculating systems or in any aquarium system where the treated water will contact the biological filter, because the antibiotics may kill or inhibit the nitrifying bacteria in the biological filters (see UF/IFAS Fact Sheet FA-16 Ammonia). If treating fish in a bath, ideally a separate container should be used, or tanks and vats should be taken off-line during treatment. In summary, bath treatments should be considered only when the majority of the fish are not eating or when treating primarily external bacterial infections and fish should be switched to oral medications as soon as they resume eating.

Consequences of Improper Dosage and Treatment Time

If the dose is too high or treatment times are too long, there is a danger of toxicity to the fish, frequently causing liver, kidney, or other organ damage that may or may not be reversible.

On the other hand, if the dose of antibiotic is too low or treatment time is too short, the bacteria will not be killed or weakened enough for the immune system of the fish to remove them, and this greatly increases the risk of the bacteria developing resistance to the antibiotic. When bacteria become resistant to a specific antibiotic, even high concentrations of that drug will not be effective.

Antibiotic resistance can also occur when antibiotics are used improperly, such as the 'shot-gunning' method. 'Shot-gunning' involves administering one antibiotic after another to a population of fish, frequently at improper dosages, for shortened treatment times, and without the aid of proper diagnosis (i.e., without culture and sensitivity tests). If shot-gunning is used frequently at a facility, resistance is almost certain to occur. In some cases, the problem may not be due to bacteria, but rather poor water quality or other management issues that have not been properly investigated.

While shot-gunning may work occasionally, over time, it introduces too great a risk of producing populations of bacteria that are resistant to multiple antibiotics, which can result in the very real possibility of a 'superinfection,' where the bacteria cannot be controlled with antibiotics. Once a system has a superinfection, it is usually necessary to sacrifice the entire affected population, completely break down and disinfect the affected system, and start over. This is obviously not a desirable outcome. The importance of using antibiotics wisely (running culture and sensitivity tests, using proper dosages and adhering to proper treatment times) cannot be overstressed.

Combining Antibiotics

Combining different antibiotics is generally not recommended. Antibiotics work at many different sites on and in the targeted bacterial cell. Using more than one antibiotic can result in interference between them and, as a worst case scenario, the antibiotics can essentially 'cancel each other out.' Most bacterial infections can be treated effectively with a single antibiotic.

Proper Handling of Antibiotics

When preparing or administering any type of medication, it is always a good idea to wear gloves in order to avoid unnecessary exposure to the user.

It is important to use antibiotics that are as fresh as possible and that have been stored properly. Antibiotics used after their expiration date or after being stored in hot and humid conditions will have greatly reduced efficacy at best and, at worst, they may be toxic.

Notes on Specific Antibiotics

The following are some notes on specific antibiotics used in the ornamental fish trade. Many of them are strictly forbidden for use by food fish producers or are otherwise of concern to the Food and Drug Administration (FDA). The FDA has elected to use regulatory discretion to keep products intended for the treatment of aquarium fish available. Officially there are no FDA-approved antibiotics for treating ornamental fish. Therefore, should you have any questions regarding the legalities of using any antibiotics, consult a fish health specialist. For information on antibiotic use in channel catfish, including specifics on use of oxytetracycline (Terramycin®) and Romet B®, see UF/IFAS Fact Sheet VM-70 Use of Medicated Feed in Channel Catfish.

Erythromycin is most effective against gram-positive bacteria, such as *Streptococcus* species. The vast majority of bacteria that cause disease in fish are gram-negative, so erythromycin should only be used after culture and sensitivity test results confirm it will be effective. Also, erythromycin is not very effective in a bath treatment, and it should only be administered by injection or in feed. Erythromycin is not FDA-approved for use with food fish.

The penicillins, including penicillin, amoxicillin, and ampicillin, are most effective against gram-positive bacteria such as *Streptococcus* species; therefore, for the same reasons as those given above, these antibiotics are not a good first choice for most bacterial infections in fish. None of the penicillins are FDA-approved for use in food fish.

Oxytetracycline and related antibiotics are considered broad-spectrum antibiotics (effective against a wide variety of bacteria), and they work well when mixed with food. (See UF/IFAS Fact Sheet VM-70 Use of Medicated Feed in Channel Catfish.) However, bath treatments may not be as effective for all species. One study (Nusbaum and Shotts, 1981) demonstrated that channel catfish absorbed approximately 15-17% of the oxytetracycline added to water with hardness of 20 mg/L and pH of 6.7. However, at least two freshwater fish species (yellow perch and hybrid tilapia) did not have the expected levels of this antibiotic in their blood when exposed experimentally to oxytetracycline as a bath treatment for up to 8 hours (K. Hughes, unpublished data; S.A. Smith, Virginia Tech, pers. comm. 2002; and R.P.E. Yanong, University of Florida, unpublished data). In addition, calcium and magnesium bind to tetracycline and oxytetracycline rendering them inactive. This means that with increasing water hardness (i.e., increases in calcium and magnesium levels), it is necessary to increase the dosages of these drugs in bath treatments. Tetracyclines are ineffective when used as a bath treatment for saltwater fish.

Tetracyclines are light sensitive, and they turn brown when decomposing. This contributes to poor water quality and may be harmful to the fish. Water should be changed immediately after the bath treatment period is concluded. Due to years of misuse, several bacteria in many different facilities are now resistant to tetracyclines. Oxytetracycline still works adequately against most cases of *Flavobacterium columnare* (columnaris disease).

Terramycin ® is a brand of oxytetracycline manufactured by Pfizer that is FDA approved for use in the production of salmonids, channel catfish and lobsters (See UF/IFAS Fact Sheet VM-70 Use of Medicated Feed in Channel Catfish).

The aminoglycosides, including gentamicin, neomycin, kanamycin, and amikacin, are very effective against gram-negative bacteria infections when administered by injection. Unfortunately, this group has also been shown to cause kidney damage in fish when administered by this technique.

As a group, these antibiotics are not considered effective when used in oral or bath treatments. A couple of exceptions may be kanamycin and neomycin, both of which may be effective against external infections if used in bath treatments. In addition, kanamycin is also believed to be effective when mixed with feed to treat gastrointestinal bacterial infections (Gilmartin, Camp and Lewis, 1976).

None of the aminoglycosides are approved by the FDA for use with food fish.

The quinolones, including nalidixic acid and oxolinic acid are considered broad-spectrum antibiotics, like the tetracyclines, and they work against a wide variety of bacteria. These antibiotics work best at acidic pHs of 6.9 or less, and they are inhibited by hard water. Although they appear to work well in both bath and oral treatments, some fish may sink to the bottom and appear lethargic after bath treatments.

These antibiotics have been shown to damage the nervous system of other animals and none are approved by the FDA for use with fish. Quinolones are closely related to a category of antibacterials known as 'fluoroquinolones,' which are categorized as 'of high regulatory concern' by the FDA. Use of fluoroquinolones or quinolones to treat any food animal is illegal and completely irresponsible.

The nitrofurans, including nitrofurantoin, nitrofurazone, furanace, and furazolidone, are commonly used in the ornamental fish trade, but the FDA strictly forbids their use by producers of food fish. Nitrofurazone is specifically categorized as 'of high regulatory concern' by the FDA and should not be present at any facility where food species are raised.

Although nitrofurans are commonly used in bath treatments, they are probably most effective against superficial infections and, in at least one study, nitrofurazone was not readily absorbed into the body of either gilthead sea bream (*Sparus aurata*) or tilapia (*Oreochromis mossambicus*) in experimental trials (Colorni and Paperna, 1983). Within the group, furanace appears to be the most effective for use in bath treatments.

To achieve the most effective result when using a nitrofuran in a bath treatment, either some sort of cover or a darkened treatment location should be considered, as the nitrofurans can be inactivated by light. Nitrofurans as oral treatments may not be as effective as previously believed, although the proper pharmacokinetic research needs to be performed to verify this conclusion.

The sulfa drugs, including Romet®, are also considered to be broad-spectrum antibiotics. There are many different types of sulfa drugs. The more common sulfas are not as effective as they once were, due to their misuse or overuse resulting in the creation of many bacteria that are now resistant to them. On the other hand, the potentiated sulfas, including Romet®, are still very effective.

Romet® works well when mixed with feed, but it does not work well as a bath treatment. It is FDA-approved for use with channel catfish and salmonids.

Summary

Many bacterial infections in ornamental fish are avoidable with proper management. However, if a population does become infected, it is important to eliminate or reduce all contributing stresses. A fish health specialist should be consulted to assist with proper diagnosis, to run culture and sensitivity tests, and to provide the best information regarding dosages and treatment intervals.

Misuse of any antibiotic can lead to the creation of resistant bacteria in a facility. In an attempt to avoid this, some farms will rotate the antibiotics they use every few months or every year. However, the best solution is to positively identify the bacteria by running culture and sensitivity tests, and thereby avoid unnecessary, costly and potentially harmful treatments.

The most extreme cases of misuse and 'shot-gunning' can result in 'superinfections' in fish. These infections are caused by bacteria that are resistant to most of the commonly used antibiotics. Obviously, this situation is not desirable, and it is completely avoidable in most cases. Once it occurs, however, little can be done to reverse the situation, and the farmer is left with no treatment options other than depopulation of the fish followed by sterilization of the culture system and equipment or scrupulously good management.

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Table 1.

ANTIBIOTIC	ORAL (food) DOSE	BATH DOSE (water change recommended prior to each dosing)
Amoxicillin	1.2 - 3.6 grams/lb food per day for 10 days	Not recommended
Ampicillin	150 mg/lb food per day for 10 days	Not recommended
Erythromycin	1.5 grams/lb food per day for 10 days	Not recommended
Gentamicin	45 mg/lb food per day for 10 days	Not recommended
Kanamycin	300 mg/lb food per day for 10 days	189-378 mg per gallon every 3 days for 3 treatments
Nalidixic Acid	300 mg/lb food per day for 7-10 days	500mg per 10 gallons, repeat as needed
Neomycin	1.5 grams/lb food per day for 10 days	2.5 grams per 10 gallons every 3 days for up to 3 treatments
Nitrofurazone	1.12 grams/lb food per day for 10 days	189-756 mg per 10 gallons for 1 hour, repeat daily for 10 days OR 378 mg per 10 gallons for 6-12 hours, repeat daily for 10 days
Oxolinic Acid	150 mg/lb food per day for 10 days	38 mg per 10 gallons for 24 hours, repeat as needed OR 95 mg per gallon for 15 minutes, repeat twice daily for 3 days
Oxytetracycline (Terramycin®)	1.12 grams/lb food per day for 10 days	750-3,780 mg per 10 gallons for 6-12 hours, repeat daily for 10 days (dose will depend on hardness of water)
Romet B® (sulfadimethoxine/ormetoprim)	0.148 lb Romet B® Premix/20 lb food per day for 5 days	Not recommended

Note: As a rule, oral/food treatments are more effective, more efficient, less detrimental to water quality, and preferable to bath treatments; remember, bath treatments may hurt biological filtration. Also, some of these drugs are available locally in premixed medicated foods.

1 gram = 1000 milligrams (mg)

Dosage references: Carpenter et al. 1996; Darwish and Hobbs 2005; Darwish and Ismaiel 2003; Noga 1996; Post 1987; Stoskopf 1988; and University of Florida.

Equation 1.

To determine the correction factor for an antibiotic that is not pure (i.e., is not 100% active ingredient) use the following:

Correction factor = Dosage rate X 100/P

Where P is the percentage of active ingredient.

Example:

To calculate the correct oral dose of tetracycline with 50% active ingredient using Equation 1
Dosage rate (from Table 1 above) is 1.12 grams/lb of food and P is 50
Therefore, Correction factor = 1.12 x 100/50
First divide 100 by 50, which gives you 2
Then multiply 1.12 by 2 to get 2.24
This means with 50% active ingredient of tetracycline, you need to dose with 2.24 grams/lb of food
This makes sense- you have to use twice as much since your antibiotic is only half strength (50%)