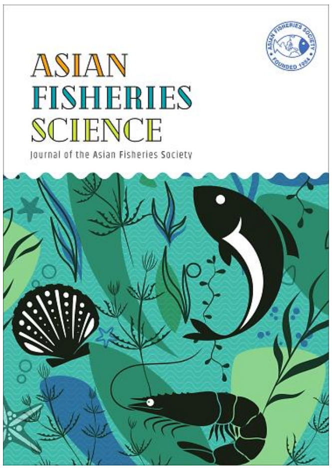

Practical Management of Bacterial Diseases in Finfish Aquaculture to Minimise Antimicrobial Resistance

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Abstract

The development of drug-resistant strains of bacterial pathogens of fish threatens the efficacy of limited aquaculture approved drugs. Development of antimicrobial resistance (AMR) is a natural process in resident bacteria in the environment. Any time antibiotics are used in an aquaculture facility it provides a competitive advantage for pathogens with AMR. This results in a build-up of drug-resistant fish pathogens. Aquaculturists can minimise the build-up of AMR pathogens by reducing the frequency of antibiotic applications and by making sure the antibiotic is properly applied it is when needed. Management practices that reduce antibiotic use are the most important strategies to avoid the build-up of AMR. Disease prevention is a continuous process in all stages of planning and all phases of production. It involves site and strain selection, managing the environment and handling to minimise stress, controlling the feed, using effective vaccines, and applying biosecurity. An effective antibiotic treatment regimen must provide the therapeutic dose and persistence needed to kill the bacteria. When using medicated feed, the fish must still be eating well and the incorporated antibiotic must be of good quality and at the proper dose determined by the weight of the fish. The antibiotic must be provided for the prescribed time even after fish mortality has stopped. Management to reduce the persistence of AMR pathogens also assures that antibiotics will be effective when needed.

Keywords: antibiotics, disease prevention, disease treatment, disease avoidance

Introduction

Aquaculture is the best option for reducing protein deficiencies and relieving the world's demand for seafood. However, the aquaculture environment is not a natural environment for the fish and crustaceans being reared. This environment makes infectious diseases more common than in the wild. Furthermore, the higher density of a single species of fish or crustacean allows for rapid spread of infectious disease once it starts resulting in devastating losses. Some of the most damaging and insidious diseases in aquaculture are caused by bacterial pathogens. Often, the use of antibiotic medicated feed is needed to control the losses, but excessive use of antibiotics can select for antibiotic-resistant strains of bacteria. When this happens, the medicated feeds are no longer effective. Also, the buildup of bacteria that carry antibiotic resistance genes is of concern to human medicine because aquatic pathogens could transfer these genes to human pathogens. There are management protocols to prevent diseases thus reducing the need for using antibiotics. This helps assure that the antibiotics will be effective when they are truly needed.

With a focus on disease prevention, this paper addresses management methods that may result in reducing antibiotic use. Also, because antibiotics are critical tools for reducing losses when needed, this paper discusses the proper and safe use of antibiotics.

Disease Prevention Strategies to Reduce the Need for Antibiotics

It is widely understood that antibiotics should not be used to treat non-infectious diseases and diseases caused by viruses, fungi, or parasites. However, animals are more susceptible to bacterial diseases when another pathogen or predisposing factor weakens their defenses. Often, the predisposing factor is subclinical, not causing disease on its own. For example, it is often seen that moderate levels of external parasites on fish facilitate columnaris disease or focal *Aeromonas* infections. The breaks in the mucus layer caused by the external parasites or by the fish rubbing their skin on submerged structures allow these common opportunistic bacteria to gain a foothold and cause disease. Viral infections also weaken the immune system. Often, virus infected fish will display a variety of bacterial diseases that are secondary pathogens. If a thorough diagnostic evaluation is not done, the producer will focus on treating the bacteria and not recognise the underlying viral infection. Prevention of viral diseases through biosecurity and vaccine use reduces the need for antibiotics. General disease prevention should be part of any comprehensive aquatic animal health plan.

In large operations, developing this plan requires a coordinated team effort. The team must include aquaculturists and aquatic animal health practitioners. Personnel in the aquaculture facility must be trained and continuously encouraged to understand the importance of following the plan and improving the biosecurity of the facility. The components of the plan should include biosecurity, disease recognition, and disease response.

In small operations, a similar plan is needed as well, but this plan may be carried out upon the advice of experts outside of the operation. This may include extension personnel, industry representatives (feed, agriculture suppliers, and/or processing plants), and university experts. With online and email resources, experts outside of the area can be accessed for help. It is important to have this team identified in advance so that their expertise, helpfulness, and availability can be confirmed. Then, when a disease outbreak does occur, the response and management steps can be implemented quickly enough to resolve the problems before it becomes unmanageable.

Disease Prevention by Managing the Environment

Environmental factors that contribute to disease are often recognised as stressors (factors that cause a stress response) in the aquatic animals. Stress is a physiological response of the aquatic animal to a perceived unhealthy environment and this response causes the animal to be predisposed to infectious disease. Also, unhealthy environmental factors may reduce the fish's ability to combat infections without directly causing a stress response. The predisposing factors can work together to greatly suppress the fish's defenses. For example, the combination of marginal water chemistry and marginal water temperature can greatly increase the susceptibility of the fish to infectious diseases. When evaluating potential predisposing environmental factors, the aquaculturist should aim for optimal conditions, not just conditions that do not directly harm the fish. Feeding activity is often a good indication of optimal conditions. When conditions are less than optimal, feeding activity is usually impacted.

Optimising the environment should be done in every phase of an operation and should be a major focus during the planning stages. The temperature range and salinity in the region should be the optimum for the selected species and strains of fish. The site should be free of pollution, distant from agrichemical use and run-off, and located in an area that is not heavily populated by predatory birds and located to avoid natural roosting sites. The site for ponds should provide the optimal alkalinity and hardness for the species of animals cultured. Also, if the ponds are constructed on agricultural lands where organochlorine pesticides, such as dichloro-diphenyltrichloroethane, hexachlorocyclohexane, aldrin, and dieldrin, have been used, the soil must be tested because these compounds can bioaccumulate. These pesticides can cause chronic health effects in animals and humans. Also, transplacental or transmammary transfer can possibly cause disease and negative developmental effects in children that were not directly exposed. Sites for cage culture should avoid areas that have industry discharges, fish processing plants, sewage outfall, and other substantial human activities. The location of shops and traffic areas should be considered to reduce the impact of human activity. Also, site selection in large water bodies should avoid areas that toxic algal blooms are known to occur. The facility design can also be important. Pond shape and size can influence temperature fluctuations, light exposure, and wind exposure.

The water chemistry must be managed to ensure an optimised culture environment. The soil and water source will usually dictate the alkalinity, hardness, and salinity. These parameters are very stable and can be monitored yearly. Alkalinity is the buffering capacity of water. High alkalinity (<50 ppm $CaCO_3$) in freshwater ponds helps maintain a stable pH and provides a more stable microbial community. Hardness indicates the amount of calcium and magnesium present in the pond. These ions are important especially during periods when fish are not actively eating so there is no external nutrient source. Both hardness and alkalinity are usually provided by the limestone content of the soil, but it can be supplemented by the addition of agricultural limestone if natural levels are low. Salinity is an indication of the total ion content of the water. Most freshwater fish can tolerate very low salinity but will thrive in elevated salinities (1 ppt). Likewise, most marine fish can tolerate salinities as low as 10 ppt. Production is influenced by the tolerance of the fish and the tolerance of the pathogens. In some freshwater fish production systems, maintaining elevated chloride levels is done by adding salt (NaCl) because this protects against nitrite toxicity.

The more transient critical components of water chemistry are dissolved oxygen, pH, ammonia, and nitrite. These parameters should be routinely monitored in systems that have high stocking densities. Dissolved oxygen and pH can fluctuate rapidly over the day in ponds. Dissolved oxygen tolerance of aquatic animals can vary considerably based on underlying health parameters that influence gills, blood transport, and tissue oxygen demand through physical activity or digestion. It is important to maintain oxygen levels well above the level that causes stress in the fish. If this is not possible it is important to reduce physical demands on the fish and withhold feed during periods of low oxygen. Although pH in aquaculture systems is rarely a stress factor, it is a critical component in determining the toxicity of ammonia and several other less common stressors such as hydrogen sulphide. The ammonia levels change slowly over days, but pH can vary substantially from early morning to afternoon due to photosynthesis. In a pond with an algal bloom, the pH will be highest in the afternoon and high pH makes ammonia more toxic.

Ammonia and nitrite levels are related to feeding activity and the microbial community in the ponds. These parameters should be monitored once or twice a week in systems with high feeding rates. When ammonia levels are on the rise, feeding levels should be reduced. In nitrite-sensitive species, such as members of the catfish family and salmonids, nitrite toxicity and stress can be prevented by adding salt to maintain at least a 10:1 chloride to nitrite ratio. Proactive water chemistry management and routine monitoring are a critical part of an aquatic animal health management plan (Svobodova et al., 1993).

Disease Prevention by Managing the Host

Managing the host physiology is critical for minimising the impact of diseases. This involves careful choice of the genetics of the aquatic animals and optimising handling, feeding and immune status of the fish.

Like managing the environment, host-based disease management starts in the planning stages. The aquaculture operation should consider disease susceptibility when selecting strains and species to culture. Often, one strain or species, or a hybrid, is much more resistant to an important disease that is endemic in the region. Using this strain may not only reduce losses to the specific disease but may also reduce secondary infections and increase growth because subclinical infections of the causative agent are also reduced. As a caution, many strains of aquatic animals are bred for growth, appearance, or carcass yield (the percent of the body weight that is meat). If disease resistance is not part of the selection process, these varieties may be more susceptible to one or more infectious diseases. Furthermore, some select strains are more resistant to one pathogen, but becomes more susceptible to another. As a rule, it is important to use fish that are not highly inbred. These should be from large breeding populations where parent-offspring or sibling-sibling breeding is avoided, and no known genetic bottlenecks have occurred (Tave, 1998).

Feed management is another important factor in disease prevention. The fish must be provided an adequate amount of good quality feed to allow a strong immune system. Generally, commercial feeds from a reputable source are properly formulated for the target fish species. These feeds, generally, are supplemented with essential minerals, fatty acids, and vitamins to produce healthy fish (Tacon, 1987). However, these feeds must be fresh and stored in cool and dry places. Vitamins C and E and essential fatty acids are quickly lost due to oxidation. Furthermore, rancid fats and toxins produced by mould can also negatively impact the health of the fish. Spoiled or expired feed should be discarded.

Feed pellet size and structure can also be important. The size of the pellets should be small enough for all fish in the population to consume. If dry pellets that are too large are being used, the pellets will cause small abrasions to the mouth and may predispose the fish to bacterial infections. Furthermore, large pellets provide the bigger fish in the population a competitive advantage and this will result in a wide size distribution in the population.

Feeding activity and management have important impacts on fish health and disease management. They not only affect the nutritional status of the fish, but they also impact the physiological needs during digestion, provides excess nitrogen to the water affecting water quality, and affects the shedding and uptake of pathogens. Each fish must receive enough food for optimal immune system functioning. Feed management should be optimised so there is minimal size variation and that there are no malnourished fish are in the population. Malnourished fish, often called runts or pinheads, have weak immunity, and pave the way for a pathogen to get a foothold and start a disease outbreak. Feeding should be done in a dispersed manner to near satiation so that all fish have an opportunity to feed. If there is a need to restrict feeding because of concerns about water quality or to restrict growth, the feeding frequency can be reduced, but continue to feed near satiation whenever they are fed.

The timing of feeding can affect the sensitivity of the fish to marginal oxygen levels. The fish need higher oxygen levels to digest the feed, therefore, aquaculturists often prefer to feed in the morning so the feed is digested during the day when oxygen levels are high. Also, the frequency of feeding can affect the transmission of certain pathogens. If fish are fed before their gut is cleared from the last feeding, they will defecate while feeding this time. This situation promotes the spread of certain pathogens by faecal-oral transmission. If one of these diseases is problematic in an aquaculture facility, the use of alternating day feeding may reduce outbreaks during periods when the disease is most prevalent (Wise and Johnson, 1998).

Another critical factor for optimising host defenses is careful handling of the fish. Whenever possible, handling, moving, and stocking fish should be done during periods when disease problems are less severe. Any handling during an outbreak will likely worsen losses. Any physical handling of the fish causes breaks in the mucus layer which is the main barrier to pathogens. Bringing them in close contact with each other, especially during netting, facilitates pathogen spread and induces the stress response resulting in suppressed immunity.

To minimise skin damage, fish must be transferred in a cushion of water whenever possible and nets must not be overloaded. To minimise pathogen spread, avoid holding the fish in crowded tanks or net pens for a prolonged time as this allows pathogen amplification. The fish must be held off-feed for at least one day before handling to reduce defecation, pathogen shedding and the associated water fouling. The use of an oxidiser such as potassium permanganate before release from holding tanks may help reduce parasites and external bacteria. To reduce the stress response and the negative effect on physiology, the fish, especially valuable ones such as brooders, must be sedated during handling. Proper vigilance in maintaining high levels of dissolved oxygen throughout the handling process must be made as well as making sure the water temperature does not change over 2 °C during the entire process. The osmolarity of the handling water must be adjusted to minimise osmotic stress. For example, the use of 1 to 5 ppt salt in freshwater fish is usually beneficial. Other measures that can reduce stress is to avoid sudden noises and impacting the sides of holding tanks, causing shock waves that startle the fish. In general, it is important to minimise activity around the tanks and cover them with opaque lids so that the fish are not startled by people moving around. Fish are inherently afraid of overhead activity likely because of fear of predatory birds.

Vaccination is also widely used to make the fish resistant to specific pathogens. The use of vaccines must be evaluated for a particular operation. Vaccines are very effective in reducing losses due to certain bacterial or viral diseases. However, in aquaculture, they are rarely so effective that they eliminate the disease risk. The use of vaccines is generally somewhat expensive and the process of vaccinating generally causes some stress to the fish. Vaccines should only be used on healthy fish that are immune-competent. They must be of sufficient age and physical status to develop a good immune response and the water temperature must be in the range where the immune system is functioning well. Also, the fish need to be vaccinated at least 2 to 3 weeks before the risk of being exposed to the target pathogen so that the immunity can be established.

Disease Prevention by Pathogen Avoidance

Effective biosecurity is a critical management tool that reduces the outbreak of infectious diseases. Biosecurity is especially recognised for preventing the introduction of new diseases to the aquaculture system. Moreover, it can also prevent the introduction of more virulent or persistent variants or pathogens or antibiotic-resistant strains of bacteria. Effective biosecurity requires a facility-specific plan that minimises the opportunity for the introduction of pathogens. Components of the biosecurity plan should include preventing pathogen introduction and spread within the facility through cultured animals, water, equipment, personnel, feed, and animals especially predators and scavengers.

The most common sources of pathogens on a facility are either introduced pathogens from the newly stocked fish or resident pathogens being passed down from previous stocks. All efforts should be made to receive healthy stocks and, if possible, stock fish that are certified free of specific pathogens of concern for the species under culture. If there are not certified specific pathogen-free stocks available, it is good to receive fish from a known reliable source that has no disease problems and have a health check performed on the fish before the purchase. The health check can screen infectious diseases and parasites, as well as general health conditions, such as condition factor (length-weight factor), swimming activity, presence of deformities, injuries or signs of previous disease events. A more sophisticated health check may also include a serological evaluation by screening the antibodies present in the fish to determine if the fish have been previously exposed to certain pathogens. The availability, pathogen list, and practical level of inspecting fish stocks should be done with the aid of an aquatic animal health professional that is familiar with the regional industry and fish health resources. It is important to be vigilant when starting an operation because once a pathogen is introduced into a system, it is difficult to be eliminated. An additional disease prevention measure is by employing quarantine by holding the arriving fish stocks in an isolated location and observing them for 10 to 14 days before releasing them into the facility. After the quarantine period, it is good to keep the fish from different sources separated in different ponds, tanks, and circulation systems. Any fish brought on to the facility can be given a prophylactic chemical treatment to remove external parasites. Commonly used treatments include formalin or potassium permanganate (Piper et al. 1982). Although popular in the past, the use of antibiotics for prophylaxis is not recommended and is illegal in many countries because this promotes the build-up of AMR pathogens.

Preventing the transmission of pathogens from one generation to the next is also critical. This involves batch culturing by completely harvesting and disinfecting culture systems between production cycles. If the facility produces fry or larvae, the eggs must be separated from the parents and disinfected before being moved into the hatchery. In salmonids, the use of a buffered solution (pH 6 to 8) containing 100 mg L^{-1} of iodine for 10 minutes after the eggs have water hardened is recommended (OIE Aquatic Animal Health Code, 2019a). A similar treatment procedure can be used in other production systems. Furthermore, brood fish must be from a source that is free from pathogens that can be vertically transmitted from eggs or sperm to the offspring.

The water source and feed are also important sources of introduced pathogens. Water should ideally come from wells or springs that have no exposure to aquatic animals. This includes species of fish other than that the species being cultured. Often, wild fish can be carriers of infectious diseases. The feed is safe if it is a dry extruded pellet due to the heat involved in producing it. However, moist pellets or unprocessed feeds must be pasteurised to kill any potential pathogens. In rare cases where live feed must be used, the aquaculturist should try to produce the feed organisms under specific pathogen-free conditions. The fish should never be fed unpasteurised fish bycatch or live feed obtained from the wild.

Prevention of spread within a facility includes restricting the movement of animals, water, or equipment between rearing tanks and ponds. It is best to have dedicated equipment for each pond, tank, or raceway or a designated portion of a facility. If this is not possible, the equipment should be cleaned and disinfected before being moved from one water body to the next. Disinfection requires the use of both the proper concentration of disinfectant and exposure time to the disinfectant (OIE Aquatic Animal Health Code, 2019b).

Wild animals moving between water bodies on a facility is especially problematic. Reptiles, amphibians, mammals, and birds are common in outdoor facilities. Birds are especially a problem because they often fly from one facility to another. Predators and scavengers that eat infected fish can efficiently transfer many fish pathogens through their faeces. Also, they are often the source of parasites that have predators as the final life stage (trematodes and nematodes). Active programs for minimising the presence of predators and scavengers on the facility is important.

Another obvious step to minimise the spread within and between culture systems is to quickly remove dead and sick fish from the system. Dead and sick fish are often cannibalised by other fish in the system and this results in the rapid spread of the pathogen. Also, scavengers and predators physically drag dead fish between production systems as well as consume infected fish then defecate the pathogens in other production systems. By removing and sanitarily discarding dead and dying fish, pathogens are directly reduced to ease the burden on the facility. Dead fish can be incinerated, securely buried away from the facility, or composted.

Biosecurity and AMR

The availability and use of antibiotics are critical for minimising the losses of fish when bacterial disease outbreaks occur. However, these tools are only effective if there is a proactive component of the fish health management plan to minimise the build-up and persistence of AMR. Any time antibiotics are used on a facility, it provides a selective advantage to microbes that are resistant to that antibiotic. A proactive plan to minimise AMR not only strives to minimise the need to use antibiotics, but also actively uses biosecurity to minimise the introduction, spread, and persistence of AMR pathogens. Such a program should avoid the introduction of AMR pathogens from other sources by either producing the fish on-site or by receiving fish only from facilities that have active an active program to minimise AMR. The health plan should include active surveillance and routine diagnostic procedures to investigate, even minor mortality events. This should include bacterial culture and antibiotic sensitivity testing. The presence of antibiotic-resistant pathogens should be noted in the farm health records and enhanced biosecurity should be implemented on the population of concern to avoid spreading the resistant strain of pathogen.

When and How to Safely Use Antibiotics

The proper use of antibiotics requires a detailed plan on its use as a component in the facility's aquatic animal health plan. The plan must include how managers and aquaculturists will respond to a mortality event, and how the antibiotic will be obtained and used rapidly enough to be of value. The fish health professional must be identified in advance so an accurate disease diagnosis can be made quickly. The diagnosis would include identifying the primary pathogen as well as environmental factors contributing to the disease. As stated earlier, antibiotics should only be used when treating a bacterial disease and when the use of the antibiotic can be provided in an effective dose. Furthermore, it is important to minimise the use of antibiotics. It is best to not use the antibiotic if losses are not expected to be substantial or if another management procedure can effectively reduce the losses. The selected antibiotic should be safe and effective for the treatment of the known disease. If the antibiotic is being used in a fish that will be marketed for food, it must be safe and legal to be used in the species of food fish being produced. Antibiotics are primarily provided to fish using a medicated feed. Therefore, to be effective, the fish must be actively feeding so that the feeding rate can be closely estimated. The fish health professional will consider all these parameters before making a recommendation for the use of an antibiotic. In many countries, the use of the antibiotic must be prescribed by a licensed professional. The prescription will indicate the dosage (antibiotic per kg of fish), the formulation (antibiotic per kg of feed), the feeding rate (kg of feed per day), the duration of the treatment, and the withdrawal time, which is the minimum number of days the fish must be off the medicated feed before the can be harvested for market. If a prescription is not required in the country, the same information should be provided by the fish health professional's recommendation. These parameters are critical for getting effective treatment and minimising AMR because the dose and duration must be sufficient to kill the bacteria. If the dose and duration are below the desired level, there will likely be a selection for bacteria that have intermediate sensitivity and these bacteria can then further evolve into resistant strains. The withdrawal period is critical for the safety of consumers. This prevents antibiotic residue in the fish meat, therefore, reducing the possibility of AMR development in the consumer and prevents other potential health risks.

In the diagnostic evaluation, the diagnostician should culture the bacterial pathogen and run an antibiotic sensitivity test to evaluate AMR, although this process would usually take several days. A treatment may already be initiated before the results are obtained. It will indicate the likelihood that the antibiotic will be effective and will provide possible alternatives for treating the current and future outbreaks on the facility.

In some cases, a pre-made medicated feed can be purchased. However, many times the medicated feed must be made by the producer. If the fish are being fed dry pellets, the antibiotic can be coated on the outside of the pellet using a binder. If the fish are fed a moist feed it can be directly mixed into the ingredients. It is important to use protective clothing, latex gloves, and dust masks when working with the antibiotics. Some antibiotics have toxic effects on humans or their unborn children. Also, exposure to antibiotics may cause a build-up of AMR pathogens in the human body system that may make it hard to treat a disease. To coat the feed, the powdered premix is first mixed into a binder (5 % gelatine solution, vegetable oil or fish oil, or a commercially available binder). Then, this mixture is mixed thoroughly with the feed. The antibiotic must be evenly distributed on all the pellets. Some producers use cement mixers when mixing large amounts of feed. If a water-based binder such as gelatine is used, the coated feed should then be spread out to air dry. The feed can then be used or stored under proper conditions.

The aquaculturist must feed only the medicated feed to the fish for the entire duration of the treatment (do not mix the medicated feed with non-medicated feed). This action must be done for the entire recommended treatment time, even if the fish stopped dying. The aquaculturists must minimise contact with the feed and avoid breathing small feed particles or dust. Feeding must not be rushed to make sure the fish will eat the medicated feed relatively soon after it hits the water because the antibiotic will leach out of the pellet. Also, it must be assured that as many fish will eat the medication as possible and will not leave a lot of uneaten medicated feed in the water.

Conclusion

Preventing disease through management is much more effective than treating diseases after they start. Effective management requires a formal aquatic animal health plan. This plan must identify the availability and roles of the experts, resources and actions to be used during all phases of the aquaculture operation to minimise all diseases and health associated losses, and should include detailed action plans that can quickly be implemented when disease situations arise. The proactive process of managing diseases is important for minimising the need for antibiotics. This reduces costs and minimises the build-up of AMR pathogens and allows the effective use of antibiotics when they are needed. Proactive efforts in minimising introductions of AMR pathogens and preventing the persistence of AMR pathogens on the facility are important components to assure the sustainability of the operation.

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