**ANTIMICROBIAL DRUGS IN AQUACULTURE**

Administration of Drugs: Methods for Aquaculture

The two most common routes for the administration of antimicrobials in aquaculture are water medication and medicated feed.

Water Medication

Medicating fish through the water they inhabit is the most common and traditional administration method. Immersion therapy is commonly used for problems involving ectoparasites and is used occasionally to treat bacterial diseases when the fish are in their juvenile stages. However, land-based hatcheries and tank systems, especially hatcheries for marine finfish and crustaceans, also use antimicrobial baths. This method is used where the biomass is small, such as with fry, and when adequate oral therapy is impractical, such as with larvae. Water medication is a simple method, in which only the volume of water needs to be known in order to calculate the final concentration of the medicine. It is normally recommended for medicines of low molecular weight that can be distributed thoroughly throughout the water. The distributed medicines are absorbed by the fish through the epithelia of the gills, skin and mucosa, and will also kill free-living pathogens. In addition to its simplicity, the other advantage of water medication is that a large number of fish can be treated at the same time.  
  
The absorption rate, however, varies from drug to drug. Generally, lipophilic compounds will diffuse across the membranes of fish but ionic compounds with large molecular weights will not. The absorption rate also varies between fish species. These disadvantages mean that the method is wasteful and relatively expensive. Moreover, water medication results in an undesirable exposure of the environment to the drugs. In an attempt to overcome these disadvantages, alternative methods of water medication can be used, including immersion or dipping, flushing and bath treatment.

In-feed Medication

Medicated feeds are prepared by the addition of a small amount of the antimicrobial drug to a homogenised and extruded diet, or the drug may be sprayed or top-coated onto the feed. The merit of in-feed medication is the reduced wastefulness when compared with water medication. It also reduces undesirable exposure of the environment and other fish to the drug. In-feed medication is standard practice for a large number of diseases and species of fish. The important limitation of this method is that the fish to be treated must be feeding actively. The successful administration of medicated feed is largely dependent on the level of feeding of the infected population. However, almost all fish afflicted with disease cease to eat, and bigger and healthier individuals eat more. Therefore the medication is usually prophylactic, not therapeutic.  
  
A second problem is lack of homogeneity. The ideal way to produce medicated feed is to add the medicine to the feed prior to mixing. However, if the drug is heat labile or only small batches of feed are needed for medication, the surface-coating process is a suitable method of feed medication. Among the many problems associated with the surface-coating method is the difficulty of achieving homogeneity. Even if all the fish in a pen eat the same quantity of feed, they will not receive the same dosage of the drug. This causes overuse or under-threshold use of drugs, which may result in conditions that lead to drug resistance. Moreover, there is an environmental impact, regardless of whether the fish have been medicated successfully or not. Uneaten food accumulates on the floor, along with medicated faeces containing unmetabolised drugs such as oxytetracycline. Various techniques have been developed for the recovery of uneaten food or medicated faeces.

Gavage

Gavage is a form of oral administration used extensively in experimental work because the precise dose is known. The technique uses a stomach tube, which is attached to a syringe containing a drug, and the drug is pumped into the stomach of the fish. Before administration of the drug, the fish must be anaesthetised or sedated. This method is rarely used in the aquaculture industry because it is labour intensive and stressful to the fish.

Injection

The usual routes of injection for fish are intramuscular, intraperitoneal, and dorso-median sinus injections. Venepuncture and cardiac puncture are also possible but are not recommended for routine injections since they are always stressful to the fish. Injection methods are indicated for vaccination, treating a limited number of fish or valuable fish, and for some diagnostic test procedures, but they are labour intensive.

Topical Application

Topical application is rare in the aquaculture industry. It is used for the treatment of skin ulcers on valuable fish. Anaesthesia is essential, and the drugs are usually oilbased.

Antibacterial Drugs

To successfully treat diseased fish, accurate diagnosis and identification of the aetiological agent is essential. In addition, various host factors must be considered, such as the species of fish, whether it is a systemic or localised disease, the appetite of the diseased individuals, as well as the culture system and environment. The third aspect to consider in antibacterial drug use is the nature of the drug itself, which is the focus of this section.

Choice of Antibacterial Drugs

Spectrum of Activity

All antibacterial drugs have a distinct spectrum of activity. For example, benzylpenicillin and macrolides are useful for the treatment of Gram-negative bacteria, which constitute the majority of the pathogenic bacteria involved in fish disease. The only important Gram-positive pathogens are Renibacterium and Streptococcus spp.

Susceptibility Testing

From among the available drugs the most effective should be chosen on the basis of susceptibility testing against identified pathogens in a standard manner. Determination of the minimum inhibitory concentration (MIC) for aquatic pathogens is not the same as for terrestrial species. Susceptibility testing is important not only in choosing the most effective drugs for therapy, but also to prevent the induction of antibacterial resistance caused by the application of sub-therapeutic concentrations of drugs in the aquaculture environment. Recently, the Clinical and Laboratory Standards Institute (CLSI) provided guidelines for methods of disc diffusion and broth dilution susceptibility testing (2, 3). Usually, the growth temperature and the nutrient requirements are different for aquatic pathogens and mammalian pathogens. For example, 22°C, 28°C and 35°C are adopted as culture temperatures for testing aquatic pathogens; these are lower than the temperatures used to grow the terrestrial pathogens of mammals. In seawater, moreover, where divalent and trivalent metallic cations are abundant, the activity of some drugs such as quinolones and tetracyclines is inhibited. For this reason, the effective concentration of antimicrobial drugs administered to marine fish should be tested using media containing the appropriate ion concentrations.

Mechanisms of Antibacterial Drug Action

Generally, the sites of action for drugs used in fish are the gut mucosa and the gills, where there is a high rate of blood circulation. If oral administration is used, changes in the gut flora should be considered.

*Tetracyclines*

The tetracyclines are a group of broad-spectrum bacteriostatic drugs. Tetracycline, chlortetracycline and oxytetracycline (OTC) are naturally occurring products, and there are several semi-synthetic derivatives. Tetracycline antimicrobials are protein synthesis inhibitors, which inhibit the binding of aminoacyl-tRNA to the mRNA –ribosome complex. Two of the natural tetracyclines, OTC and chlortetracycline, have been used in aquaculture: OTC, which has been used as a first choice drug for nearly all bacterial diseases of fish, is not only widely available but also cheaper than other broad-spectrum antibacterial drugs. This widespread use has led to bacterial resistance in the aquaculture industry.  
  
The disadvantage of OTC is its low bioavailability in all fish. The extent to which OTC has been used is remarkable since its formation of a complex with Ca2+ and Mg2+ ions makes it generally an unsuitable antimicrobial for fish. In marine fish, cations are a requirement for osmoregulation. The fish therefore drink continually, and hence the content of their alimentary tracts is modified seawater. In this medium, the tendency of OTC to form complexes creates a requirement for high doses and exacerbates the low bioavailability. Since the drug is hardly metabolised at all by fish, virtually the entire large dose is excreted or defecated into the environment. The effect is weaker in freshwater than in seawater not only because of the lower concentrations of cations present but also because freshwater fish do not drink.

*Penicillins*

Penicillins are a group of antimicrobials derived from Penicillium fungi and are members of the beta-lactam family. Beta-lactam antimicrobials work by inhibiting the cross-linking of peptidoglycan in the bacterial cell wall. Cephalosporins, which also belong to the beta-lactam family, are not used in aquaculture. Penicillin has a synergistic effect with aminoglycosides because the inhibition of peptidoglycan synthesis allows aminoglycosides to penetrate the bacterial cell wall more easily and to cause the disruption of bacterial protein synthesis within the cell. This results in a lower minimum bactericidal concentration for susceptible organisms. Penicillins are sensitive to hydrolysis by bacterial betalactamase enzymes, but some can be potentiated by betalactamase inhibitors, notably clavulanic acid.  
  
Benzylpenicillin (penicillin G) is a natural antimicrobial produced by Penicillium notatum. It has a narrow spectrum of action, mainly against Gram-positive bacteria, and hence is of little use in aquaculture. Other semi-synthetic penicillins, ampicillin and amoxicillin, have similar spectra of activity that are broader than that of benzylpenicillin, and they are widely used in aquaculture. They do not form complexes with divalent cations, and therefore their cost–benefit ratio, when compared with OTC, is better in seawater than in freshwater. These semi-synthetic penicillins are used in most species of farmed fish.

*Macrolides*

Macrolides are intermediate-spectrum antimicrobials active mainly against Gram-positive bacteria. The antibacterial spectrum of macrolides is slightly wider than that of penicillin; thus, they are common substitutes for penicillin. Unlike penicillin, macrolides have been shown to be effective against mycoplasma, mycobacteria, Chlamydia and rickettsias. The mechanism of action of macrolides is the inhibition of bacterial protein biosynthesis: they are thought to prevent peptidyltransferase from adding the peptidyl attached to tRNA to the next amino acid (in a similar way to chloramphenicol), as well as inhibiting ribosomal translocation. Another potential mechanism is premature dissociation of the peptidyl-tRNA from the ribosome. Since the majority of bacterial pathogens of fish are Gramnegative, the use of macrolides is limited and specific. Only three are used to any extent in aquaculture: erythromycin, spiramycin and josamycin.

*Quinolones*

Quinolones are a group of chemically related synthetic antibacterial agents and inhibit the bacterial enzyme DNAgyrase. The supercoiling of bacterial DNA requires a nick in the DNA double helix that enables it to fold over; DNAgyrase repairs the nick afterwards. Inhibition of the enzyme results in breaks in the DNA. Nalidixic acid was the first quinolone to be developed. It has been used for fish only in Japan. Its spectrum of activity is mainly against Gram-negative bacteria, but in practice this covers the majority of the bacterial pathogens of fish. Only a limited number of quinolone drugs have been investigated for use in aquatic medicine. Among them, oxolinic acid is of particular interest and importance because it was originally developed in Japan specifically for use in fish.

*Sulfonamides*

Sulfonamides consist of a large range of synthetic compounds, which are chemically related derivatives of sulfanilamide. The sulfonamides have a broad spectrum of activity, but the only application of these compounds in fish is topical application to skin wounds, abrasions and ulcers. The doses required often leave little margin below the level of toxicity, and many bacteria develop resistance fairly easily. One advantage of sulfonamides over other antibacterial drugs is that they are absorbed through the gills. Administration by immersion is, therefore, feasible and is of particular value for juvenile fish.  
  
The potentiated sulfonamides are combinations of two antibacterial drugs, a sulfonamide and a pyrimidine potentiator. The combination is synergistic in that the antibacterial potency is greater than the potencies of the two drugs separately. Potentiated sulfonamides are active against a wide range of bacterial infections in fish.

*Nitrofurans*

The nitrofurans are synthetic antimicrobial agents with a broad spectrum covering not only Gram-positive and Gram-negative bacteria but also several types of protozoan parasite. They are normally bacteriostatic but can be bactericidal at high doses. Although rare, adverse effects such as pulmonary toxicity can be caused by high doses. Furthermore, prolonged exposure to nitrofurans, in common with malachite green and gentian violet, has been associated with a carcinogenic effect in laboratory animals (10). The two nitrofurans most commonly used in aquatic medicine are furazolidone and nifurpirinol. Furazolidone is well absorbed by fish, and it is normally administered in feed, unlike most nitrofurans, which are poorly absorbed from the gastrointestinal tract. In addition, nitrofurantoin, which is absorbed but immediately excreted, is useful for the treatment of urinary tract infections and has been investigated for that use.

*Chloramphenicol*

Chloramphenicol is a broad-spectrum antimicrobial agent that is used systemically in veterinary medicine. Owing to resistance and safety concerns, this drug is no longer commonly used. Chloramphenicol is bacteriostatic. It is a protein synthesis inhibitor that inhibits the peptidyl transferase activity of bacterial ribosomes. Chloramphenicol has been used as a prophylactic agent against carp dropsy (caused by Aerobacterium liquefaciens). It has also been used in the treatment of trout ulcer disease (caused by Haemophilus piscium) and furunculosis (caused by A. salmonicida).

*Florfenicol*

Florfenicol is a fluorinated synthetic analogue of thiamphenicol. Thiamphenicol and florfenicol differ from chloramphenicol in having a methyl-sulfonate group in place of a nitro group in their molecular structure. This is believed to prevent the development of aplastic anaemia, which makes the compounds acceptable for use in foodproducing species. Florfenicol is indicated for the treatment of fish diseases such as furunculosis in Atlantic salmon and rainbow trout fry syndrome.

Risks and Regulations

Safety

Exposure of untargeted animals and the environment to drugs used in aquaculture is inevitable due to the characteristics of the culture systems and the manner of introduction of the drugs. Moreover, residual drugs in treated fish can affect the people who consume aquatic food products.

Safety of the Consumer

The drugs administered can accumulate as the parent form or as metabolites in the tissues of fish, which may be used for human consumption. The withdrawal period is the time between the last treatment of the fish with the drug and the point at which the fish is processed into a food product. This is determined by the drugs used, the species of fish and the environmental temperature. The duration of the withdrawal period is closely related to the maximum residue limit (MRL) of the drug or its metabolite. Different countries apply different MRL values and withdrawal periods to achieve safe levels and reduce the risk of residues.  
  
The MRL is calculated from several parameters, such as the acceptable daily intake (ADI) and the maximum no-effect level (NOEL). The procedure for determining MRLs from the ADI is much better established for mammals than for fish. Usually, the withdrawal period for fish considers two tissues, the muscles and the skin. In some cases, separate residue data are needed to establish the withdrawal periods for specific drugs; for example, trimethoprim is known to be selectively concentrated in fish skin. In addition, the pharmacokinetic parameters of each drug vary for different species. Temperature is another important variable; a higher temperature is related to higher absorption, distribution, metabolism and excretion (ADME) rates.

Safety Concerns for the Environment

The aquaculture industry may cause environmental problems due to water pollution from the various chemicals in the antimicrobial preparations and their metabolites. There are few drugs that are completely absorbed into fish, and it is impossible to recover all the administered medicines from the environment. Lipophilic compounds are likely to form slicks on the water surface, and soluble compounds may be distributed widely throughout the water.

Bacterial Resistance

In addition to direct toxicity of the drugs to non-target organisms, including humans that consume seafood, the propagation of antimicrobial resistance is of global concern. It is thought that the release of antimicrobials into the environment can lead to selection for antimicrobial-resistant bacteria.  
  
There are two potential risks associated with antimicrobial resistance in aquaculture:  
  
– direct dissemination of resistant bacteria from fish or aquatic products to people  
  
– the transfer of gene(s) responsible for resistance from the bacterial flora in fish to human pathogens.

Induced Resistance

Since significant proportions of the bacteria associated with humans and other animals, including fish, are zoonotic bacteria, selection for resistance in bacteria associated with human disease is of major concern in aquaculture. For example, Salmonella and Vibrio species are common zoonotic pathogens that are isolated from aquatic wastes and food products, and antimicrobial resistance has been reported recently in these bacteria. It has been assumed that there is an association between the aquacultural use of florfenicol and the emergence of floR-encoded florfenicolresistant Salmonella enterica serovar Typhimurium DT104 and Vibrio damsela. In aquaculture, however, pathogens that can infect humans are not as prevalent as in terrestrial animals and agriculture. There has been some controversy over these issues and whether the risk is indeed relatively small or not. Further investigation and risk analysis should be performed, based on scientific evidence, to determine the risks to public and animal health.

Dissemination of Resistance

Although many aquatic pathogens have a narrow host range, significant risk to public health can develop through evolutionary processes in which genes, including antimicrobial resistance genes and virulence genes, can be transferred between different species and genera of bacteria. Antimicrobial use in aquaculture is considered by some to risk the development of a reservoir of transferable resistance genes in bacteria in aquatic environments, from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens. Several papers have reported that the use of antimicrobials in aquaculture has led to an increase in the frequency of occurrence of resistance genes, and that these genes can be transferred from aquatic bacteria to terrestrial bacteria with relatively high efficiency. The direction of flow of the resistance genes, however, is not always one way, and transmission in the reverse direction is also possible. Thus, the prudent use of antimicrobials should be emphasised in humans and in both terrestrial and aquatic animals.

Regulations and Control

Recently, there has been global interest in antimicrobial resistance in aquaculture, and in controlling antimicrobial resistance through risk analysis and the establishment of regulations and guidelines. Several international organisations have produced recommendations on the responsible and prudent use of antimicrobial agents in veterinary medicine to reduce the overuse and misuse of antimicrobials in animals in order to protect public health. They include:  
  
– the Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food published by the World Health Organization  
  
– the International Standards on Antimicrobial Resistance produced by the World Organisation for Animal Health  
  
– the Code of Practice to Minimize and Contain Antimicrobial Resistance (CAC/RCP 61-2005) and the Code of Practice for Fish and Fishery Products (Section 6  
  
– Aquaculture Production) (CAC/RCP 52-2003), published by the Codex Alimentarius Commission.  
  
In the United States, all drugs legally used in aquaculture must be approved by the United States Food and Drug Administration (FDA) Center for Veterinary Medicine. There are five drugs currently available for legal use in aquaculture in the United States. These include just three antimicrobials: oxytetracycline HCl, sulfamerazine, and a combined preparation that contains sulfadimethozine and ormetoprim. The FDA also provides information, including the ingredients of each preparation, manufacturer, species, route of delivery, dosage form, withdrawal times, tolerances, and uses in each species, including dose rates and limitations.  
  
Although antimicrobial therapy should be guided by the principles of disease diagnosis and the rational selection and administration of chemotherapeutics, empirical therapy and prophylactic use have caused concern regarding the development of antimicrobial resistance. Data on the quantities of antimicrobial agents used in aquaculture are not available in most countries, but the available evidence suggests that the amount of antimicrobials used in aquaculture in most developed countries is limited, and in some countries the quantity has been decreasing. Nevertheless, large quantities of antimicrobials are used in aquaculture in some countries, often without professional consultation or supervision. An important proportion of the aquatic animals produced within the global aquaculture industry are raised in countries with insufficient regulation and limited enforcement of the authorised use of antimicrobial agents in animals. In some countries, the availability of registered antimicrobials is insufficient, which contributes to the illegal use of antimicrobials.  
  
Prevention and control of bacterial diseases in aquatic animals is essential to minimise the use of antimicrobials and to avoid the negative impact of antimicrobial resistance. Effective vaccines and improved systems for mass vaccination of finfish should be developed, and optimisation of vaccine licensing procedures should be promoted. Programmes to monitor antimicrobial usage and antimicrobial resistance in bacteria from farm-raised aquatic animals and their environment should be implemented, and national databases should be developed to encourage efficient communication.