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Antibacterial Drugs in Fish Farms: Application and Its Effects

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1. Introduction

Antibacterial chemotherapy has been applied in aquaculture for over 60 years. The discovery of antibacterials changed the treatment of infectious diseases, leading to a dramatic reduction in morbidity and mortality, and contributing to significant advances in the health of the general population. Antibacterials are used both prophylactically, at times of heightened risk of disease and therapeutically, when an outbreak of disease occurs in the system. The removal of antibacterials from fish medicine would cause great welfare problems. There are many antibacterial drugs for animal health. However, pharmacological research on aquaculture drugs has focused mainly on a few antibacterials widely used in aquaculture. It is well recognised that the issues relating to antibacterial use in animal food are of global concern. Currently, there is a general perception that veterinary medicines, and in particular antibacterials, have not always been used in a responsible manner. In some cases, rather than providing a solution, chemotherapy may complicate health management by triggering toxicity, resistance, residues and occasionally public health and environmental consequences. As a result, authorities have introduced national regulations on the use of antibacterials.

2. Antibacterial use in aquaculture

Aquaculture continues to be the fastest growing animal food producing sector, and aquaculture accounted for 46% of total food fish supply (FAO, 2011). The intensification of aquaculture has led to the promotion of conditions that favour the development of a number of diseases and problems related to biofouling. It is worth remembering the age-old adage that "prevention is better than cure?" and certainly it is possible to devote more attention to preventing the occurrence of disease in fish. Fish may be reared under ideal conditions, in which case the stock are inevitably in excellent condition and without signs of disease (Austin & Austin, 2007). However, disease is a component of the overall welfare of fish (Bergh, 2007). Consequently, a wide range of chemicals are used in aquaculture, including antibacterials, pesticides, hormones, anaesthetics, various pigments, minerals and vitamins, although not all of them are antibacterial agents. As is the case in terrestrial animal production, antibacterials are also used in aquaculture in attempts to control bacterial disease (Burka et al., 1997; Horsberg, 1994; Defoirdt et al., 2011). Usage patterns also vary between countries and between individual aquaculture operations within the same country.

Antibacterials are among the most-used drugs in veterinary medicine (Sanders, 2005). The principal reasons behind the control of infectious diseases in hatcheries are to prevent losses in production; to prevent the introduction of pathogens to new facilities when eggs, fry, or broodstock are moved; to prevent the spread of disease to wild fish via the hatchery effluent or when hatchery fish are released or stocked out; and to prevent the amplification of pathogens already endemic in a watershed (Phillips et al., 2004; Winton, 2001; Lupin, 2009). Antibacterial usage requires veterinary prescription in aquaculture as with usage in terrestrial animals (Sanders, 2005; Prescott, 2008; Rodgers & Furones, 2009). We have limited data about antibacterial use in world aquaculture. For most of the species farmed, we also lack adequate knowledge of the pharmacokinetics (PK) and pharmacodynamics (PD) of administration (Smith et al., 2008). Along with widespread use comes a growing concern about irresponsible use, such as the covert use of banned products, misuse because of incorrect diagnose and abuse owing to a lack of professional advice. That said, there are still not enough approved products for a range of species and diseases in aquaculture (FAO, 2011).

Antibacterials are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms. Antibacterials that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of infectious diseases amongst humans, animals and plants (Table 1). Drug choices for the treatment of common infectious diseases are becoming increasingly limited and expensive and, in some cases, unavailable due to the emergence of drug resistance in bacteria that is threatening to reverse much of the medical progress of the past 60 years (FAO, 2005). In aquaculture, antibacterials have been used mainly for therapeutic purposes and as prophylactic agents (Shao, 2001; Sapkota et al., 2008; FAO, 2005). The voluntary use of antibacterials as growth promoters in any aspect of aquaculture is generally rare. Prophylactic treatments, when they are employed, are mostly confined to the hatchery, the juvenile or larval stages of aquatic animal production. Prophylactic treatments are also thought to be more common in small-scale production units that cannot afford, or cannot gain access to, the advice of health care professionals. There are no antibacterial agents that have been specifically developed for aquacultural use and simple economic considerations suggest that this will always be the case (Smith et al., 2008; Rodgers & Furones, 2009). Despite the widespread use of antibacterials in aquaculture facilities, limited data is available on the specific types and amounts of antibacterials used (Sapkota et al., 2008; Heuer et al., 2009). General considerations in the selection and use of antibacterial drugs are given by Figure 1. (Walker & Giguére, 2008). Treatment options will be different for animals that are held in net pens at sea as opposed to those held in an indoor facility or an aquarium. A treatment must also be feasible: an appropriate treatment route for aquarium fish or selected broodstock individuals may be cost- or labour-prohibitive in commercial aquaculture ventures. The stress associated with treatments must be balanced with the need for and the expected benefits of treatment (Smith et al., 2008). Also, before making a decision to treat a group of fish, the following questions should be asked (Winton, 2001):

1. Does the loss-rate, severity or nature of the disease warrant treatment?
2. Is the disease treatable, and what is the prognosis for successful treatment?
3. Is it feasible to treat the fish where they are, given the cost, handling, and prognosis?
4. Is it worthwhile to treat the fish or will the cost of treatment exceed their value?
5. Are the fish in a good enough condition to withstand the treatment?
6. Will the treated fish be released or moved soon, and is adequate withdrawal or recovery time available?

Antibacterial class	Mode / mechanism of action	Mechanisms of resistance	Multiple resistance[a]	PK-PD relationship[b]
ß-Lactams (penicillins, cephalosporins, and carbapenems)	Bactericidal. Inhibition of the penicillin binding proteins (PBPs) located on the cytoplasmic membrane	ß-lactamase production, PBPs modifications, reduced permeability, and efflux	Yes	Time-dependent
Aminoglycosides (streptomycin and neomycin)	Bactericidal. Protein synthesis inhibition through binding to the 30s subunit of the ribosome	Decreased permeability, efflux, modification of enzymes, and target (ribosome) modification	Yes	Concentration-dependent
Macrolides (erythromycin, tylosin and spiramycin)	Bacteriostatic. Protein synthesis inhibition through binding to the 50s subunit of the ribosome	Target (ribosome) modification of enzymes, decreased permeability and efflux	Yes	Time-dependent
Fluoroquinolones (enrofloxacin and ciprofloxacin)	Bactericidal. Inhibition of DNA gyrase and topoisomerase	Target point mutations decreased permeability, efflux and plasmid mediated mechanism	Yes	Concentration-/time-dependent
Tetracyclines (oxytetracycline and chlortetracycline)	Bacteriostatic. Protein synthesis inhibition at the ribosomal level (interference with peptide elongation)	Efflux, drug detoxification, and ribosome modification	Yes	Time-dependent
Folate synthesis inhibitors (sulphonamides and ormetoprim)	Single bacteriostatic, combination bactericidal. Inhibition of dihydro-pteroate synthase and dihydrofolate reductase	Decreased permeability, formation of enzymes with reduced sensitivity to the drugs	Yes	Concentration-dependent
Phenicols (florfenicol and chloramphenicol)	Bacteriostatic. Inhibit the peptidyltransferase reaction at the 50s subunit of the ribosome	Decreased target binding, reduced permeability, efflux and modifying enzymes	Yes	Time-dependent

[a]Resistance to antibacterials belonging to different classes in at least one of the isolates. [b]Represents a generalisation only, the actual relationship can be variable when an individual drug is involved.

Table 1. Properties of the major classes of antibacterial agents (Modified from (Yan & Gilbert, 2004) and (Defoirdt et al., 2011)).

2.1 The pathogen and the host

Organisms responsible for disease in aquatic species include fungi, bacteria, nematodes, cestodes and trematodes as well as parasitic protozoans, copepods and isopods. Some can cause death, while others may stress the affected animal to the point that it becomes more susceptible to additional diseases (Stickney, 2005). Disease forms a part of the lives of wild fish and farmed fish. Often, it is not cultured fish that are most susceptible, due to efficient prophylactic strategies and good culture practices. Unprotected wild fish, as exemplified in the case of salmon lice, will be more susceptible to infections and mortality (Bergh, 2007). The difference between health and disease typically depends on the balance between the pathogen and the host, and that balance is greatly influenced by environmental factors, such as temperature and water chemistry (Winton, 2001). The diagnostic techniques for pathogens that are used range from gross observation to highly technical bimolecular-based tools. Pathogen screening is another health management technique, which focuses on the detection of pathogens in subclinical or apparently healthy hosts (Subasinghe, 2009).

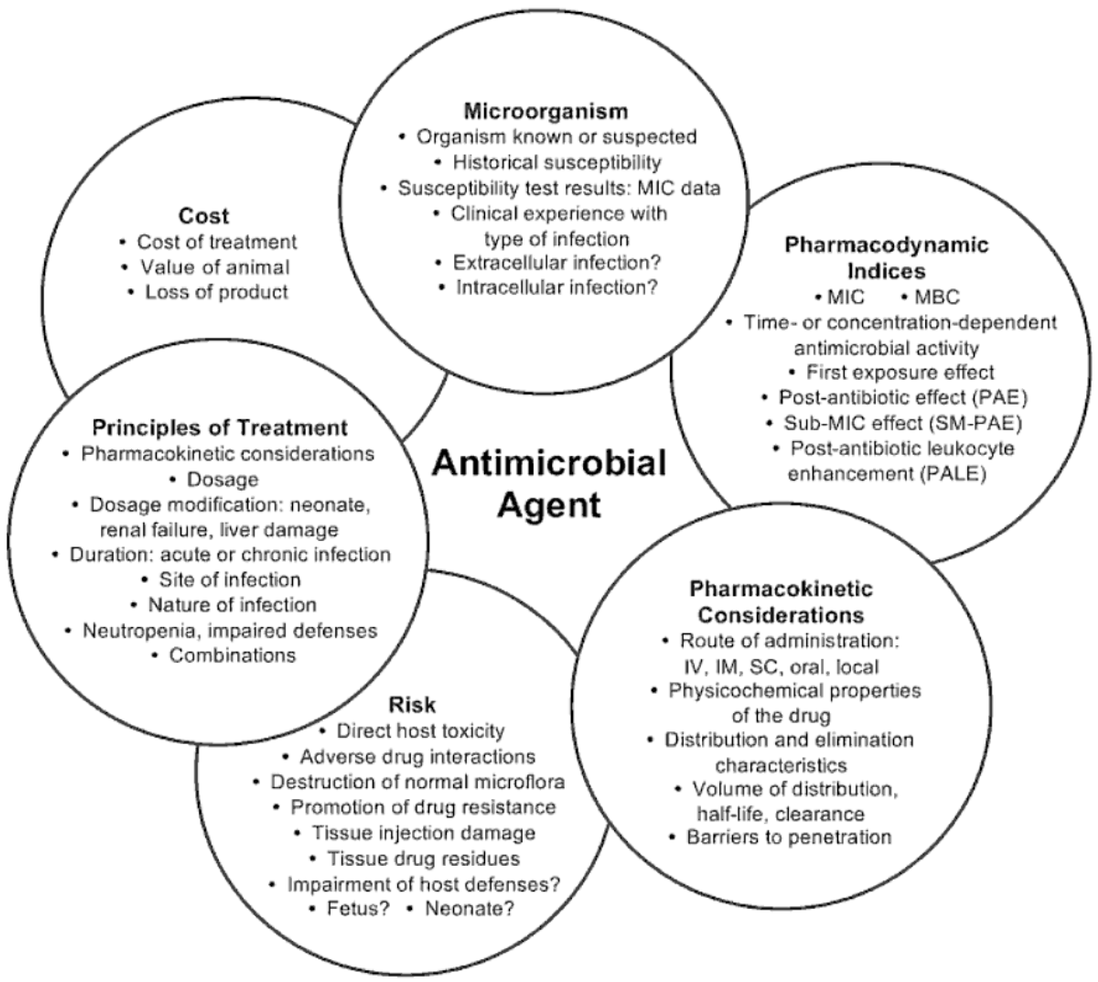


Fig. 1. Some considerations in selecting and using antibacterial drugs (Walker & Giguére, 2008).

The primary pathogens in aquaculture are bacteria and viruses (Shao, 2001). More than 100 bacterial pathogens of fish and shellfish have been reported (Alderman & Hastings, 1998; Winton, 2001). The artificially high host-densities associated with aquaculture are evolutionarily beneficial for pathogens (Bergh, 2007). Bacterial pathogens probably cause more disease problems overall than all other causes combined. In virtually every type of aquaculture, bacterial diseases rank number one amongst aetiological agents. In each type of culture, and for virtually every species, specific bacterial pathogens are responsible for serious disease problems. Gram-negative bacilli are the most frequent cause of bacterial diseases in finfish. Although only a few Gram-positive forms affect finfish, such bacteria cause serious diseases among crustaceans (Meyer, 1991; Rodger, 2010; Roberts, 2004). Whereas similar types of pathogens affect freshwater and marine fish, relatively few pathogens are transmissible from freshwater fish to marine fish, and vice versa (i.e. most pathogens affect either marine or freshwater fish, but not both). This is the rationale for why many freshwater pathogens can be treated with salt, and many marine pathogens can be treated with freshwater (Noga, 2010). Choosing the right drug depends in part on such factors as age, size and the housing of the animal. Common bacterial fish diseases, their definition, aetiology and treatment, as well as control issues, are resumed in Table 2.

Disease / Aetiology	**Treatment and control**
Mycobacteriosis. Mycobacteriosis in fish is a chronic progressive disease caused by certain bacterial species within the genus Mycobacterium. *Mycobacterium marinum, M. fortuitum, M. salmoniphilum and M. chelonae* are all considered pathogenic for fish. All are aerobic, acid-fast, Gram-positive and non-spore forming.	There is no fully effective treatment. Therefore, the best course is to cull and disinfect the premises. Rifampicin in combination with tetracycline (Boos et al., 1995) and clarithromycin may reduce infection (Collina et al., 2002).
Coldwater Diseases & Rainbow Trout Fry Syndrome (RTFS). Bacterial coldwater disease is a serious septicaemic infection of hatchery-reared salmonids, especially young coho salmon, which has also been referred to as peduncle disease. *Flexibacter psychrophilus, Cytophaga psychrophila* and *Flavobacterium psychrophilum* are all terms that have been used for the causal agents of these diseases. Most of the recent classificatory work indicates that *Flavobacterium psychrophilum* is the correct name for these bacteria. These bacteria are Gram-negative.	Broad-spectrum antibacterials have been partially ineffective in controlling an outbreak, but the improvement of the environment and using 3-4 times the recommended doses of antibacterials have shown benefits (Bebak et al., 2007). Florfenicol also appears to be effective for recommended dose regimes.
Bacterial Kidney Disease (BKD). A serious disease of freshwater and seawater fish, farmed and wild salmonids, that results in an acute to chronic systemic granulomatous disease. *Renibacterium salmoninarum* is a Gram-positive diplococcus that grows best at 15-18°C, is causative agent of BKD and a significant threat to the healthy and sustainable production of salmonid fish worldwide (Wiens et al., 2008).	Chemotherapy (erythromycin) provides limited and only temporary relief. The bacteria can survive and multiply within phagocytic cells.

Enteric Redmouth Disease (ERM). A bacterial septicaemic condition of farmed salmonids, and in particular rainbow trout. There are recent reports amongst channel catfish. *Yersinia ruckeri* is the causal agent and the Gram-negative, motile rod-shaped bacterium is catalase positive and oxidase negative. Several serotypes have been identified.	Broad-spectrum antibacterials are effective in controlling an outbreak, although increasing antibacterial resistance is observed. The bacteria can survive and multiply within phagocytic cells (Tobback et al., 2009; Rykaert et al., 2010).
Furunculosis. Furunculosis is a fatal epizootic disease, primarily of salmonids. It also causes clinical diseases in other fish species, where it is named ulcer disease or carp erythrodermatitis. *Aeromonas salmonicida* is a Gram-negative bacteria. Atypical furunculosis is caused by a slower growing non-pigmenting isolate, *A. salmonicida achromogenes*.	Broad-spectrum antibacterials are effective in controlling an outbreak, but increasing antibacterial resistance is observed.
Piscirickettsiosis. A disease of salmonids caused by *Piscirickettsia salmonis* and a significant disease problem in farmed marine salmonids. *Piscirickettsia salmonis* is a Gram-negative, acid-fast, non-motile, spherical to coccoid, non-capsulated (although often pleomorphic) organism.	Broad-spectrum antibacterial therapy is used, although some resistance is developing. Outbreaks are usually associated with stressful events, such as algal blooms, sudden changes in the environment or grading.
Bacterial Gill Disease (BGD). BGD is an important disease in farmed freshwater salmonids. The bacterium *Flavobacterium branchiophila* causes a chronic, proliferative response in gill tissue. *Flavobacterium branchiophila* is a Gram-negative, long, thin, filamentous rod.	BGD usually responds well to antiseptic and surfactant baths, such as chloramine T and benzalkonium chloride. Providing adequate oxygen is a useful supportive therapy.
Vibriosis. Vibriosis is the term most commonly used to describe infections associated with *Vibrio spp.* In recent years, vibriosis has become one of the most important bacterial diseases in marine-cultured organisms (Stabili et al., 2010). *Vibrio anguillarum*, *V. ordalii* and other *Vibrio sp.* may cause similar clinical signs in wild and farmed fish. It is Gram-negative, and has straight or slightly curved rods which are motile.	Broad-spectrum antibacterials are effective in controlling an outbreak, but increasing antibacterial resistance is observed. Vaccines are widely used. Caprylic acid may be helpful as an alternative or as an adjunct to antibacterial treatment (Immanuel et al., 2011).
Epitheliocystis. Epitheliocystis is a chronic and unique infection caused by the *Chlamydia spp.* organism and which results in hypertrophied epithelial cells - typically of the gills but sometimes also of the skin - or certain freshwater and marine fishes.	Broad-spectrum antibacterials have been used with some degree of success, though the avoidance of infected fish should be adhered to at all costs.

Tenacibaculosis. An infection of marine fish by *Tenacibaculum maritimum* is common in farmed fish and many species. The bacteria appear to be opportunistic, commonly infecting fish after minor epidermal or epithelial trauma or irritation, and they can rapidly colonise such tissue. It is Gram negative, with slender bacilli which multiply in mats on the damaged tissue.	Oral treatment with broad-spectrum antibacterials is generally successful if the fish are maintained in a low-stress environment.
Francisellosis. Francisellosis is the term used to describe infection associated with *Francisella philomiragia subspecies noatunensis*, which has emerged as a major pathogen of farmed cod. *Francisella spp.* is also a major pathogen of farmed tilapia. It is Gram-negative, with intracellular coccobacilli.	There is no effective treatment due to the intracellular nature of the infection. Removal of the affected fish and the disinfection of the premises and equipment, and fallowing.
Rainbow Trout Gastro-Enteritis (RTGE). RTGE is an enteric syndrome of freshwater farmed rainbow trout, reported in several European countries, and which results in significant economic loss and daily mortalities. It is not fully established and the role of the segmented filamentous bacteria remains unclear, as they are also found in apparently healthy fish. However, *Candidatus arthromitis* may have some role to play in the disease. This bacterium have not yet been cultured in vitro.	Changing the diet-type or the addition of salt to the diet as well as broad-spectrum antibacterials all appear to be effective, once the disease is present. However, none appear to be preventative. Biosecurity is important in preventing the disease from entering a farm.
Red Mark Syndrome (RMS) or Cold Water Strawberry Disease. RMS is an infectious dermatitis of rainbow trout which does not cause mortality but which presents as dramatic haemorrhagic marks on the skin. It is not fully established, although *Flavobacterium psychrophilum* and rickettsia-like organisms have been associated with it.	The lesions will resolve eventually without treatment; however, broad-spectrum antibacterials do induce the rapid healing of the condition. The avoidance of any livestock from infected farms reduces the chance of the introduction of RMS onto a site.

Table 2. Common bacterial fish diseases, their aetiology and treatment – control issues (Modified from (Rodger, 2010)).

2.2 Antibacterial susceptibility testing of aquatic bacteria

Resistance is a description of the relative insusceptibility of a microorganism to a particular treatment under a particular set of conditions. Therefore, it should be noted that resistance or at least the resistance level depends strongly upon the test type and test conditions, as well as the type of compound and its mode of action (Kümmerer, 2008). The empirical use of antibacterials should be avoided. The use of antibacterials should always be based upon an examination of the clinical case, the diagnosis of a bacterial infection and the selection of a clinically efficacious antibacterial agent. However, in certain

situations (such as when the animal is seriously ill or where there is an outbreak with a high mortality or a rapid spread) therapy may be initiated on the basis of clinical signs (Guardabassi & Kruse, 2008; Smith et al., 2008). The target organisms must be known or shown to be susceptible, and adequate concentrations must be shown to reach the target (Phillips et al., 2004). A definitive diagnosis requires the isolation and identification of the causative organism, preferably from three to five infected fish (Smith et al., 2008). Samples for a bacteriologic culture should be collected from the actual site of infection before administering an antibacterial drug (Walker & Giguére, 2008). Currently, a wide range of standardised methods are available (Smith et al., 2008; Guardabassi & Kruse, 2008; CLSI, 2006a, 2006b). It should be expected that there will be differences between the bacteria isolated and their antibacterial sensitivities, between freshwater and saltwater fish, between different taxa of fish, and possibly even between different species of fish (Mulcahy, 2011). Furthermore, due to the varying activity spectrum of the different compounds in some tests, microbial population dynamics may overrule their effects in some populations. They may thereby mask effects (Kümmerer, 2008). The discrepancies between testing methods may also require further studies (Kum et al., 2008). Differences in the measurement of zone sizes by individual scientists also represent a possible source of inter-laboratory variation (Nic Gabhainn et al., 2004).

2.3 The treatment route

In intensive fish farming, the antibacterials used to treat bacterial infections are administrated generally by either water-borne or oral means, or else through injection (Shao, 2001; Treves-Brown, 2001; Zounkova et al., 2011). Agents that are intended to treat diseases must reach therapeutic levels in target tissues. It is always advisable to perform a bioassay of a small number of individuals before treating any fish species without a known history of response to the treatment. A bioassay can be performed by placing five or six fish in an aquarium that has the treated pond water. The fish should be observed for 1-2 days before treatment so as to be sure that none have died from the stress of collection. Fish should never be left unattended during treatment and, if an adverse response occurs, the drug should be immediately removed by transferring the fish to clean water or diluting the treatment water. It is necessary to take the presence of these additives into account when calculating the active drug quantity required for any treatment (Noga, 2010; Rodgers & Furones, 2009). Adequate plans for detoxification and the removal and disposal of used drugs must be in place before treatment is begun (e.g., ammonia and nitrite levels must be monitored closely during therapy). When hospitalisation is completed, the aquarium, the filter, and all other materials in contact with the hospitalisation aquarium should be disinfected before re-use. Used drugs must be disposed of responsibly. Deteriorated or otherwise uncontrolled water quality poses particular challenges to farmed fish and their surroundings. Outputs from these systems can further harm local wildlife and the ecosystem (Cottee, 2009). There is no one specific drug application method which is better than other; rather, the method of treatment should be based on the specific situation encountered. Here, experience is exceptionally valuable. A fish health professional or other knowledgeable source should be consulted if one is unfamiliar with the disease or treatment proposed (Winton, 2001). Methods for the application of antibacterials to fish are resumed in Table 3.

Method of application	Comments
Oral route (on food)	Needs palatable components; minimal risk of environmental pollution
Bioencapsulation	Needs palatable compounds; minimal risk of environmental pollution
Bath	Need for a fairly lengthy exposure to the compound, which must be soluble or capable of being adequately dispersed; problem of the disposal of spent drug
Dip	Brief immersion in a compound, which must be soluble or capable of being adequately dispersed; problem of disposal of the dilute compound
Flush	Compound added to a fish holding facility for brief exposure to fish; must be soluble or capable of being adequately dispersed; poses a problem of environmental pollution
Injection	Feasible for only large and/or valuable fish; usually requires prior anaesthesia; slow; negligible risk of environmental pollution
Topical application	Feasible for the treatment of ulcers on valuable/pet fish

Table 3. Methods for application of antibacterials to fish (Haya et al., 2005).

2.3.1 Water medication

The water-borne route is the most common method for administering treatments to fish and it has distinct advantages, such as being relatively non-stressful and easy to administer. Drugs are added to water for two distinct purposes. The first and most obvious one is so that the drug will be absorbed by, and so medicate, the fish; the second is to kill the free-living and, hence, transmissible stages of parasites (Treves-Brown, 2001). Seawater fish drink significant amounts of water and may absorb large amounts of a drug via the gastrointestinal tract (Noga, 2010). Application by the water-borne route becomes necessary if the fish refuse to eat, and, therefore, would be unlikely to consume any medicated food. With these methods, the fish are exposed to solutions/suspensions of the drug for a predetermined period. This may be only briefly, i.e., a few seconds duration (a "dip") or for many minutes to several hours (a "bath") (Haya et al., 2005). Waterborne antibacterial treatments will vary depending upon the animal and its holding conditions. Treating fish by applying the drug to the water avoids stressing the fish by handling (Reimschuessel & Miller, 2006). However, there are disadvantages. Relative to other treatment routes, dosing is less precise (too little or too much). Baths and dips are not as effective as some of the other treatment methods – particularly for systemic infections – because of generally poor internal absorption of the antibacterial being used. Water-borne treatments are mainly used for surface-dwelling (skin and gill) pathogens, including parasites, bacteria and water moulds. Certain species, such as scaleless fish are often especially sensitive to water-borne treatments (Rodgers & Furones, 2009). Antibacterials which are absorbed from the water include chloramines, dihydrostreptomycin, enrofloxacin, erythromycin, flumequine, furpyrinol, kanamycin, oxolinic acid, oxytetracycline, nifurpirinol, sulphadimethoxine, sulphadimidine, sulpha-monomethoxine, sulphanilamide, sulphapyridine, sulphisomidine and trimethoprim. Antibacterials that are absorbed poorly or not at all include chloramphenicol and gentamicin (Reimschuessel & Miller, 2006). With bath-type treatments, more antibacterials

are required when compared with oral (feed) treatments or injections. Bath treatments are also not recommended for recirculation systems or aquarium systems using biological filters. The accurate calculation of the volume of water in the tank, pond or cage is also required (Rodgers & Furones, 2009). If both short- and long-term exposures are probably equally feasible and effective, it is preferable to use a short-duration drug exposure. The advantages of this type of treatment lie in reduced waste (and thus reduced expense) and less environmental contamination (Reimschuessel & Miller, 2006). Even where absorption is known to occur, the technique does have some important disadvantages. In particular, in most cases less than 5% of the administered dose will be absorbed by the fish. In this case, the technique is wasteful, expensive (at least twenty times the dose required by the fish must be provided) and environmentally undesirable (Treves-Brown, 2001).

2.3.2 Oral medications

In food fish or ornamental aquaculture, many of the bacterial diseases of fish can be successfully treated with medicated feeds, and it is usually the preferred method of treatment. However, care must be taken because some of the causes of disease – such as stress – can lead to treatment failures or the recrudescence of disease after the completion of treatment (Rodgers & Furones, 2009). Fish in ponds are best treated using oral medications. However, sick fish may not eat, and withholding food for 12-24 hours may increase the acceptance of a medicated feed (Reimschuessel & Miller, 2006). The incorporation of an antibacterial in the feed is usually via a powdered premix in conjunction with a binder, such as gelatine (up to 5%), fish or vegetable oil (Shao, 2001). The dosage required for treatment with a medicated feed depends upon the original level of active ingredient/kg fish body weight. The dosage rates used in medicated feeds will vary according to the specific antibacterial used, but usually the rate is based on a number of grams per 100 kg of fish per day. The exact dosage will also require the number and average weight of the fish to be treated, as well as a daily feeding rate and consideration of whether the fish are marine or fresh water species. It is also important that treated fish must not be harvested for food use until a specified withdrawal period has elapsed (Rodgers & Furones, 2009). One problem for the treatment of marine species is that antibacterials have been shown to be less effective in seawater, which is related to their reduced bioavailability, e.g., tetracycline has a low bioavailability in fish (< 10%) due to binding with sea-water-borne divalent cations such as Mg^{2+} and Ca^{2+}. It is noteworthy that non-bioavailable tetracyclines contaminate the environment (Toutain et al., 2010). The bioavailability of some aquaculture drugs in salmon held in seawater is shown in Table 4. (Rodgers & Furones, 2009). The dosage can vary within certain limits and depending upon the feeding rate. It is usually best to use a feed that has enough medication so that feeding at a rate of 1% of body weight per day will give the needed dosage (Noga, 2010). Absorption from the intestinal tract may vary between species. Saltwater fish will drink and, therefore, drugs may bind cations in the water in their intestinal tracts, affecting bioavailability (Reimschuessel & Miller, 2006). For particular applications, like the treatment of young larvae and fry, some success has been obtained with the bio-encapsulation of drugs in live feeds, especially with artemias. Other innovative methods of oral delivery – like microspheres or coated beads – offer the possibility of protecting fragile molecules from deterioration in the gastric juices, carrying them up to their target sites in the intestine. Though still quite recent, large developments are expected from these innovative technologies in the near future (Daniel, 2009).

Antibacterial	Bioavailability (%)
Oxytetracycline	1
Amoxicillin	2
Sarafloxacin	2
Oxolinic acid	30
Flumequine	45
Sulfadiazine	50
Trimethoprim	96
Florfenicol	97

Table 4. Examples of reduced bioavailability for some aquaculture drugs in seawater (Rodgers & Furones, 2009).

2.3.3 Injection

The injection of antibacterials can be a more effective treatment for bacterial infections than the use of a medicated feed, particularly for advanced infections and as the best way of being sure of the given dose (Douet et al., 2009). However, it is usually only practical for valuable individual fish, such as brood stock or ornamental fish, rather than for fish in large-scale production facilities. Injection quickly leads to high blood and tissue levels of antibacterials (Yan & Gilbert, 2004; Haya et al., 2005). Normally, an individual fish will also need to be anaesthetised before treatment. Typical injection sites include the intraperitoneal (IP) cavity and the intramuscular route (IM) (Rodgers & Furones, 2009; Treves-Brown, 2001). Disadvantages include the stress imposed by capturing the fish and, for aquarium fish, the need to bring the fish to the clinic for every injection, since the owner is usually unable to perform the treatment (Noga, 2010). The IP route is the widely used route for injection. Fish should be fasted for 24 hours prior to injection. The landmarks for an IP injection are the pelvic fins and the anus. All fish should be at least 35g. Improper injection can lead to peritoneal adhesion, ovulation problems, mortality from injection, reduced efficacy, side effects (local reactions), reduced carcass quality and therapy failure (Treves-Brown, 2001; Noga, 2010). IM injection is best used only on fish more than 13 cm long or else more than 15g. The best site is the dorsal musculature just lateral to the dorsal fin. Only relatively small amounts can be injected (0.05 ml/50 grams of fish). Injections should be done slowly. The IM route has the disadvantage of causing damage to carcass quality and has the potential of forming sterile abscesses (Noga, 2010). The volume required for the injection of antibacterials is based on the weight of fish to be treated, the recommended dosage for the antibacterial being used and its supplied concentration (Rodgers & Furones, 2009). This is usually expressed as:

Volume of antibacterial required = recommended dosage (mg/kg) x weight of fish (kg) /supplied solution concentration (mg/ml)

2.3.4 Topical application

The topical application of drugs to fish is rare. Anaesthesia is an essential preliminary procedure. Topical treatments are usually only necessary for more valuable individual fish, such as ornamental varieties or brood stock. Ointments containing antibacterials have sometimes been used in fish surgeries, applied to the sutures and incision site. Commercial antibacterial ointments are most commonly used (Mulcahy, 2011). Open sores or ulcers that

are secondarily infected by bacteria or water moulds can be treated. A cotton swab should be dipped in a drug solution and then used to gently touch the lesion, allowing the solution to soak the lesion via capillary action. Nevertheless, it is possible that ulcers may heal themselves with improved water quality and the elimination of parasites (Treves-Brown, 2001; Haya et al., 2005; Noga, 2010).

2.3.5 Water treatment

Disinfection can reduce the risk of disease transmission within aquaculture facilities, and from facilities to the environment, by deactivating or destroying pathogens with disinfecting agents. Disinfection can be done routinely, but also in response to the outbreak of specific diseases (Winton, 2001). In this procedure the drug is applied to all the water in the aquarium. It is, therefore, not applicable to antibacterial drugs, as these would inactivate the filter (Treves-Brown, 2001).

2.4 Dosage

PK and PD data has allowed the design of therapeutic regimens, with the PK/PD variable providing the most appropriate surrogate for drug effectiveness being dependent upon several factors (Rigos & Troisi, 2005; Martinez & Silley, 2010; Toutain et al., 2010). Within some species there may be considerable differences both within and between breeds in PK and PD profiles; veterinary pharmacogenetics aims to identify genetic variations (polymorphisms) as the origin of differences in the drug response of individuals within a given species. These between- and within-species differences in drug response are largely explained by variations in drug PK and PD, the magnitude of which varies from drug to drug (Toutain et al., 2010). We will not be able to apply the full power of the PK/PD approach to either the design of treatment regimens that minimise the development of resistance or the setting of clinical breakpoints that provide an empirical definition of resistance (Smith et al., 2008). There is a considerable amount of information available on the PK of various antibacterials delivered by different routes to different species of fish, but little information about the plasma levels of antibacterials that are required to be of benefit for the implanted fish or the calculation of the dosage of antibacterial required to obtain a positive benefit (Mulcahy, 2011). It is important to remember that it is the host immune system that is ultimately responsible for success in combating bacterial disease (Martinez & Silley, 2010). If one is unsure about the dose to use, it is usually best to start with the lower recommended dose. If the disease does not respond adequately, repeat the treatment with a higher dose. For oral medications, dosage varies with feed intake. Fish that are eating less need a higher percentage of the drug in their diet, but there are limits on the legally allowable amount as well as practical considerations, since some drugs are unpalatable at high doses (e.g., many antibacterials) (Noga, 2010; Winton, 2001). Drug dosage regimens also are host-dependent. Fish species reared in warm water may absorb, metabolise and excrete drugs at a different rate (often faster) than those in cold water. The salinity of the holding water also affects drug kinetics. Fish kept in saltwater drink the water while freshwater fish do not. Thus, antibacterials in the gastrointestinal tract of fish species held in saltwater may bind cations, which can reduce their uptake (Smith et al., 2008; Toutain et al., 2010). This is especially true for antibacterials – such as the tetracyclines – that have low bioavailability even in freshwater. The half-lives of drugs in fish are highly dependent upon the dosage regimen, the route and the temperature. Therefore, these parameters are included in the Phish-Pharm Database and should be considered when administering antibacterials to fish. Table 5 shows

some drug dosages that have been reported for fish (Reimschuessel et al., 2005). It is important to realise that the dosages listed in Table 5 may not have been shown to be safe or effective in all fish species. No generalisations are possible. Successful therapy often depends on maintaining adequate blood levels over a course of seven to ten days. Temperature is a very important factor in deciding on the dose and treatment intervals (Toutain et al., 2010).

Drug	**Species**	**$t_{1/2}$ (hr)**	**Dosage**	**Route[b]**	**°C**
Amoxicillin	Atlantic salmon	120	12.5 mg/kg sd[a]	IM	13
	Atlantic salmon, sea bream	14-72	40-80 mg/kg sd	IV/PO	16-22
Chloramphenicol	Carp	48-72	40 mg/kg sd	IP	9
Ciprofloxacin	Carp, rainbow trout, African catfish	11-15	15 mg/kg sd	IM/IV	12-25
Difloxacin	Atlantic salmon	16	10 mg/kg sd	PO	11
Enrofloxacin	Atlantic salmon, red pacu, rainbow trout, sea bass, sea bream	24-105	5-10 mg/kg sd	IM/IV/PO	10-26
Erythromycin	Chinook salmon	120	0.1 g/kg 21 d	PO	10
Florfenicol	Atlantic salmon	12-30	10 mg/kg sd	IV/PO	10-11
	Cod	39-43	10 mg/kg sd	IV/PO	8
Flumequine	Eel	255	9 mg/kg sd	IM	23
	Atlantic halibut, brown trout, corkwing wrasse, Atlantic halibut,	21-96	5-25 mg/kg sd	IP/IV/PO	5-25
	Atlantic salmon, cod, goldsinny wrasse, sea bass, sea bream, turbot				
	Eel	208-314	10 mg/kg sd	IV/PO	23
	Rainbow trout	285-736	5 mg/kg sd	IV/PO	13 vs 3
Furazolidone	Channel catfish	1-24	1 mg/kg sd	IV/PO	24
Gentamicin	Channel catfish, brown shark, goldfish	12-54	1-3.5 mg/kg sd	IC/IM	20-25
	Toadfish	602	3.5 mg/kg sd	IM	19
Miloxacin	Eel	35	30-60 mg/kg sd	IV/PO	27
Nalidixic acid	Rainbow trout, amago salmon	21-46	5-40 mg/kg sd	IV/PO	14-15
Nifurstyrenate	Yellowtail	2	100 mg/kg sd	PO	23
Ormetoprim	Atlantic salmon, channel catfish, rainbow trout, hybrid striped bass	4-25	4-50 mg/kg sd	IV/PO	10-28
Oxolinic acid	Atlantic salmon, corkwing wrasse, channel catfish, cod, rainbow trout, red sea bream, sea bass	15-87	4-20 mg/kg sd	IP/IV	8-24
	Atlantic salmon, cod, rainbow trout	82-146	25-75 mg/kg sd	PO	5-8

	Atlantic salmon, gilthead sea bream, rainbow trout, sharpsnout, sea bream, turbot	13-48	10-40 mg/kg up to 10d	PO	9-19
Oxytetracycline	African catfish, carp, rainbow trout, red pacu, sockeye salmon	63-95	5-60 mg/kg sd	IM	12-25
	African catfish, Atlantic salmon, ayu, carp, Chinook salmon, eel, rainbow trout, red pacu, sea bass, sea bream, sharpsnout, sea bream	6-167	5-60 mg/kg sd	IV	8-25
	Arctic charr	266-327	10-20 mg/kg sd	IV	6
	Atlantic salmon, ayu, black sea bream, carp, channel catfish, eel, perch, rainbow trout, sea bass, sea bream, hybrid striped bass, summer, flounder, walleye	43-268	10-100 mg/kg up to 10d	PO	7-27
	Arctic charr, sockeye salmon, Chinook salmon	428-578	10-100 mg/kg sd	PO	6-11
Piromidic acid	Eel, goldfish	24	5 mg/kg sd	PO	26
Sarafloxacin	Atlantic salmon, cod	12-45	10-15 mg/kg sd	IV/PO	8-24
Streptozotocin	Toadfish	24	50 uCi	IV	
Sulphachlor-pyridazine	Channel catfish	4-5	60 mg/kg sd	IC/PO	22
Sulfadiazine	Atlantic salmon, carp, rainbow trout	26-96	25-200 mg/kg sd	IV/PO	8-24
Sulfadimethoxine	Atlantic salmon, channel catfish, rainbow trout, hybrid striped bass	1-48	25-200 mg/kg sd	IV/PO	10-20
Sulphadimidine	Carp, rainbow trout	18-57	100-200 mg/kg sd	IV/PO	10-20
Sulphametho-xypyridazine	Rainbow trout	72	200 mg/kg sd	PO	13
Sulphamono-methoxine	Rainbow trout, yellowtail	5-33	100-400 mg/kg sd	IV/PO	15-22
Sulphanilamide	Rainbow trout	36	200 mg/kg sd	PO	13
Sulfathiazole	Rainbow trout	60	200 mg/kg sd	PO	13
Thiamphenicol	Sea bass	21	30 mg/kg 5d	PO	19
Tobramycin	Brown shark	48	1-2.5 sd	IM	25
Trimethoprim	Atlantic salmon, carp, rainbow trout	21-50	1-100 mg/kg sd	IV/PO	8-24
Vetoquinol	Cod	79	25 mg/kg sd	PO	8
	Atlantic salmon	16	40 mg/kg sd	PO	10

[a]sd: single dose. [b]Abbreviations, IM: intra muscular, IV: intra venous, PO: per os (oral), IP: intra peritoneal, IC: intra coelom, uCi: a unit of radioactivity (Curie= $3{,}7 \times 10^{10}$ disintegration per second).

Table 5. Half-lives and dosages of antibacterials in fish (Reimschuessel et al., 2005).

2.5 Drug metabolism in fish

Liver is the primary organ for the detoxification of drugs in fish. Similarities exist in the metabolism of drugs by fish and mammals. The metabolism of aquaculture antibacterials by the cytochrome P450 system could affect their activation, tissue distribution and elimination rates, and determine the persistence of residues as well as the length of the withdrawal period before the fish can be used for human consumption (Moutou, 1998). The elimination rate of antibacterials from fish tissues varies greatly with the temperature. The temperature dependency of drug PK is an important consideration for drug residues. The elimination half-life of antibacterial drugs increases significantly as the temperature decreases. Ideally, the drug dose should be adjusted according to the water temperature, but in clinical practise the dose is normally fixed (Toutain et al., 2010). However, unmetabolised oxytetracycline can be passed unabsorbed through the body of treated sparids and then excreted via the faeces into the local marine environment (Rigos & Troisi, 2005; Rigos et al., 2004).

2.6 Duration of antibacterial treatment

It is universally recognised that a drug must be present in a sufficient concentration for an adequate length of time at the site of the infection, although the variables affecting the length of treatment have not yet been fully defined (Walker & Giguére, 2008). The responses of different types of infections to antibacterial drugs vary, and clinical experience with many infections is important in assessing the response to the treatment. For serious acute infections, treatment should last at least 7 to 10 days. If no response is seen by that time, both the diagnosis and treatment should be reconsidered (Walker & Giguére, 2008). It is important to remember that it is the host immune system that is ultimately responsible for any success in combating bacterial diseases (Martinez & Silley, 2010).

2.7 Failure of antibacterial therapy

Treatment failure has many causes. The selected antibacterial may be inappropriate because of misdiagnosis, poor drug diffusion at the site of the infection, inactivity of a given drug at the site of infection, failure to identify the aetiological agent including inaccurate results of laboratory tests, resistance of pathogens, intra-cellular location of bacteria, metabolic state of the pathogen, or errors in sampling. Other factors that may contribute are inadequate dosage or the use of drugs with low bioavailability. When failure occurs, diagnose must be reassessed and proper samples collected for laboratory analysis. Patient factors such as the persistence of foreign bodies, neoplasia, and impairment of host defences are important to consider. It is important also to ensure that persons medicating their own animals comply with dosing instructions (Walker & Giguére, 2008; Winton, 2001; Treves - Brown, 2001; Noga, 2010).

3. Treatment options in various aquaculture systems

Another important factor influencing treatment is the type of culture system. The four major types of culture system are aquaria, ponds, cages and flow-through systems (Noga, 2010). The main factors that may influence a treatment's success are given in Table 6.

Aquaria	Ponds	Cages	Raceways
The most highly controllable culture systems for maintaining temperature, biological filtration and oxygen. Amenable to various waterborne treatments. Ease of manipulability.	Influenced by natural factors such as light, temperature and rainfall. Natural biological cycles are less controllable. Interventional strategies are more limited compared with aquaria.	Susceptible to the vagaries of natural environmental changes. Water-borne treatments are possible in such systems, but are much more difficult. The fish that need to be treated in such systems must have their cage enclosed. Alternatively, the fish must be treated in a closed system (e.g., a bath treatment) or the medications must be delivered orally.	Raceways and other flow-through systems are the least manipulable systems by virtue of the constant and rapid water turnover. Similar adverse environmental consequences can follow such treatments. Flow-through systems are even more limited than cages in the ability to use water-borne treatments.

Table 6. Major types of culture systems influencing the diseases' treatment (Table established from (Noga, 2010)).

4. Legal use of antibacterials

A number of international and regional codes of practice, agreements and technical guidelines exist for aquatic animals (Subasinghe, 2009). The drugs available for use and their treatment protocols are tightly regulated. The consumers of fish – and particularly in the world's richer economies – are increasingly demanding that retailers guarantee that the fish which they offer are not only of a high quality and safe to eat, but also that they derives from fisheries that are sustainable (FAO, 2011). As health threats have appeared, management practices have evolved and fish husbandry has greatly improved over the past 20 years, resulting in a reduction in the use of some chemicals, and particularly the use of antibacterials in most jurisdictions (Burridge et al., 2010). The banning of any antibacterial usage in animals based upon the "precautionary principle" in the absence of a full quantitative risk assessment is likely to be wasted at best, and even harmful at worst, both to animal and human health (Phillips et al., 2004). The antibacterials used in veterinary medicine are only prescribed by veterinarians in the European Union (EU). The prescription scheme could be discussed and improved, and non-approved and even banned antibacterials are purchased "over-the-counter" (without the need for a prescription) or their use is undeclared in fish feed formulations. The use of specifically banned antibacterials in aquaculture is a violation of regulations (Lupin, 2009). The user safety data included on labelling and packaging inserts should provide sufficient information for such occupational safety assessments to be made (Alderman & Hastings, 2009). Before approval, drugs are assessed for the definition of their maximum residue limits (MRLs) (Table 7), and their

environmental impact and efficacy (Sanders, 2005). MRLs are generated by a number of bodies, such as the EU, and more globally within the framework of the FAO/WHO Codex Alimentarius Commission, which is advised scientifically by the JECFA (Joint FAO/WHO Expert Committee on Food Additives). The use of antibacterial agents in food animal species, including fish, is controlled by regulations, particularly in Europe and the USA.

Antibacterial	**Species***	**Tissue****	**MRL**	**Comments**
Amoxicillin	All FPS	Muscle	50 pg/kg	
Ampicillin	All FPS	Muscle	50 pg/kg	
Benzylpenicillin	All FPS	Muscle	50 pg/kg	
Chlortetracycline	All FPS	Muscle	100 pg/kg	
Cloxacillin	All FPS	Muscle	300 pg/kg	
Colistine	All FPS	Muscle	150 pg/kg	
Danofloxacin	All FPS	Muscle	100 pg/kg	
Dicloxacillin	All FPS	Muscle	300 pg/kg	
Difloxacin	All FPS	Muscle	300 pg/kg	
Enrofloxacin	All FPS	Muscle	100 pg/kg	Enro.+ciprofloxacin
Erythromycin	All FPS	Muscle	200 pg/kg	Erythromycin A
Florfenicol (Fish)	Fish	Muscle+skin	1000 pg/kg	
Flumequine	Fish	Muscle+skin	600 pg/kg	
Lincomycin	All FPS	Muscle	100 pg/kg	
Neomycin (Incl. Framycetin)	All FPS	Muscle	500 pg/kg	Neomycin B
Oxacillin	All FPS	Muscle	300 pg/kg	
Oxolinic Acid	Fish	Muscle+skin	100 pg/kg	
Oxytetracycline	All FPS	Muscle	100 pg/kg	
Paromomycin	All FPS	Muscle	500 pg/kg	
Sarafloxacin (Fish & Poultry)	Salmonids	Muscle+skin	30 pg/kg	
Spectinomycin	All FPS	Muscle	300 pg/kg	
Sulphonamides (All)	All FPS	Muscle	100 pg/kg	
Tetracycline	All FPS	Muscle	100 pg/kg	
Thiamphenicol	All FPS	Muscle	50 pg/kg	
Tilmicosine	All FPS	Muscle	50 pg/kg	
Trimethoprim	All FPS	Muscle	50 pg/kg	
Tylosin	All FPS	Muscle	100 pg/kg	

*All FPS: all food producing species (with some exclusions and depending on each compound).
**For all fish MRLs, the target tissues "muscle" or "muscle and skin" shall be understood as "muscle and skin in natural proportions."

Table 7. Main antibacterial compounds having fixed MRLs (Modified from (Daniel, 2009)).

The approval process is very costly and time consuming, and the sales potential for the aquaculture market in global terms is limited, which in some cases has meant a certain lack of interest on the part of pharmaceutical companies for developing new antibacterials

and registering them (Alderman & Hastings, 2009; Rodgers & Furones, 2009). In the USA, the regulatory authority for the approval of Veterinary Medicinal Products (VMPs) is the FDA (FDA, 2009, 2011). The body that is responsible for the authorization procedure in the EU is the European Medicines Agency (EMEA) and the European Commission, or else the national competent authorities in the EU Member States (depending on the procedure chosen for the marketing authorisation application). The EMEA's Committee for Medicinal Products for Veterinary Use (CVMP) carries out the scientific evaluation (Sanders, 2005; Prescott, 2008; Valois et al., 2008; Alderman, 2009). Compared to agricultural use and medicinal use, the market for aquaculture antibacterials is fairly small and the approval process can be expensive. The availability of antibacterial agents for aquacultural use is affected by the setting of MRLs. However, these withdrawal times are based on studies that are mainly performed on fish held in temperate freshwater. The excretion of a drug by a fish can vary greatly with its environmental conditions, and especially the temperature (Daniel, 2009; Noga, 2010). Because of the variability of drug excretion, especially with temperature, a rule of thumb called "degree days" has been advocated for estimating the required withdrawal time. If the data does not indicate a temperature effect on depletion, then a day-based withdrawal can be accepted (Alderman & Hastings, 2009).

5. Problems associated with antibacterial use in aquaculture

Consumers demand guarantees that their food has been produced, handled and sold in a way that is not dangerous to their health, and which respects the environment and addresses various other ethical and social concerns (FAO, 2011). Even if the occurrence, effects and fate of antibacterials have been considered from the perspective of scientific interest, little is still known about the actual risk to both humans and the environment (Kemper, 2008). However, medicines legislation requires that user-safety be assessed in the safety package and that the product label must include advice and warnings to the user, giving guidance for safe use. Any hazards associated with feed medication – whether in feed mills or on farms – must be considered, as must any hazards to the final user (the fish farm staff) (Alderman & Hastings, 2009). The majority of fishers and aquaculturists are in developing countries, and mainly in Asia which has experienced the largest increases over recent decades, reflecting the rapid expansion of aquacultural activities (Sapkota et al., 2008; FAO, 2011; Smith et al., 2008). Fish diseases are generally coupled with cultured fish and viewed as a result of aquaculture (Bergh, 2007). As a consequence, it is probable that the majority of antibacterial use in world aquaculture is not associated with any classification of the target bacterium or of its susceptibility to the range of available antibacterials (Smith et al., 2008). There is also a need for assurance that the usage will not harm animals or humans (Phillips et al., 2004). With an increase in consumers' recognition of the health benefits associated with seafood consumption, the volume of fisheries and aquaculture products consumed is expected to rise (Storey, 2005). There is little doubt that aquaculture production will continue to grow (Asche et al., 2008). The world food supply will probably have to double in quantity and increase in quality over the next 30–50 years as populations and incomes rise. The demand for fish as food will probably double and could even more than double (Pullin et al., 2007). Consequently, an increase in the number of problems associated with aquaculture production may be expected.

5.1 Toxicity to the host

Antibacterials that are sufficiently non-toxic to the host are used as chemotherapeutic agents in the treatment of the infectious diseases of humans, animals and plants. Direct host toxicity is the most important factor limiting drug dosage. Tolerance studies must be carried out to determine the safety of the product to the target fish species (Alderman & Hastings, 2009). Also, it is important for the clinician to report adverse drug events to legal authorities. Antibacterial agents can have a wide variety of damaging effects on the host, including: (1) direct host toxicity; (2) adverse interactions with other drugs; (3) interference with the protective effect of normal host microflora or the disturbance of the metabolic function of microbial flora in the digestive tract of herbivores; (4) the selection or promotion of antibacterial resistance; (5) tissue necrosis at injection sites; (6) drug residues in animal products that are intended for human consumption; (7) impairment of the host's immune or defence mechanisms; and (8) damage to foetal or neonatal tissues (Guardabassi & Kruse, 2008; Mulcahy, 2011). Nonetheless, the most used aquaculture antibacterial agent oxytetracycline may have genotoxic and ecotoxic effects in aquatic ecosystems (Zounkova et al., 2011). The selective toxicity of antibacterials is variable. Some agents, such as beta-lactams, are generally considered to be safe, whereas others, such as the aminoglycosides, are potentially toxic (Guardabassi & Kruse, 2008).

5.2 Resistance of aquatic bacteria

The capacity of bacteria to adapt to changes in their environment and thus survive is called resistance. Drug choices for the treatment of common infectious diseases are becoming increasingly limited and expensive and, in some cases, unavailable due to the emergence of drug resistance in bacteria (FAO, 2005). In general, aquatic bacteria are not different from other bacteria in their responses to exposure to antibacterial agents, and they are capable of transferring antibacterial resistance genes to other bacteria (Heuer et al., 2009). The WHO has long recognised that antibacterial use in food animals – which seems to outweigh antibacterial use for human therapy in many countries – contributes importantly to the public health problem of antibacterial resistance (WHO, 2011). The resistance of pathogenic bacteria to antibacterials is a growing problem in human and veterinary medicine, and antibacterial use in fish – especially in aquaculture – is an area of increasing concern over health risks (Kemper, 2008; Mulcahy, 2011). The fact that some of the bacteria that cause infections in fish belong to the same genera as the bacteria causing infections in humans is likely to increase the probability of the spread of antibacterial resistance from aquaculture to humans (Heuer et al., 2009). The continued use of subtherapeutic levels of antibacterials to prevent disease increases the likelihood of establishing populations of multiply resistant strains of pathogenic bacteria. These may ultimately result in outbreaks of disease which cannot be controlled by antibacterial therapy (Mulcahy, 2011; Roberts, 2004). Also, the selection and use of inappropriate antibacterials, and the use of insufficient dosages, incorrect routes of application, incorrect dosing frequencies and administering antibacterials for an insufficient time period, are ways to select for antibacterial-resistant bacteria (Mulcahy, 2011). The excessive use of antibacterials in fish aquaculture is increasing the resistance in bacteria that can infect both humans and animals (Burridge et al., 2010; Kümmerer, 2010; Defoirdt et al., 2011). It is not only the direct therapeutic use of antibacterials, but also their indirect contact with them which might enhance the resistance of bacteria: not taking into account the bacteria's origins,

resistance genes have been isolated from human pathogens, bacteria of animal origin and even environmental bacteria (Kemper, 2008; Martinez & Silley, 2010; Martinez, 2009). The consequences of increasing resistance in bacteria and the diminishing impact of therapeutic drugs reach far beyond the geographic origins of antibacterial compounds and are, therefore, of global concern (Kemper, 2008).

Antibacterials exhibit different activity spectra and mechanisms of action. It has been recognised for some time that susceptibility to antibacterials varies markedly both between different groups of organisms and within these groups (Kümmerer, 2008). A large variety of antibacterial resistance mechanisms have been identified in bacteria and several different mechanisms may be responsible for resistance to a single antibacterial agent in a given bacterial species. Antibacterial resistance mechanisms can be classified into four major categories (shown in Table 3.1 by the asterisk): (1) the antibacterial agent can be prevented from reaching its target by reducing its penetration into the bacterial cell; (2) general or specific efflux pumps may expel antibacterial agents from the cell; (3) the antibacterial agent can be deactivated by modification or degradation, either before or after penetrating the cell; or (4) the antibacterial target may be modified so that the antibacterial cannot act on it anymore, or else the microorganism's acquisition or activation of an alternate pathway may render the target dispensable (see Table 8) (Boerlin & White, 2008; Nikaido, 2009).

Drug resistance may be natural or acquired (Roberts, 2004; Douet et al., 2009). Some organisms have always been resistant to a particular agent by the nature of their physiology or biochemistry (i.e., inherent or intrinsic resistance); others have acquired resistance as a result of the application of antibacterials by humans (i.e., acquired resistance) (Kümmerer, 2008). Resistance to antibacterials may be acquired by the mutation of a chromosomal gene which modifies the structure of the ribosomal target or by the infection of the cell with a resistant R-factor plasmid. Plasmids are extrachromosomal circular DNA molecules capable of autonomous replication (Alderman & Hastings, 1998; Boerlin & White, 2008; Defoirdt et al., 2011). Once they are integrated in successful gene-transmission elements, antibacterial resistance genes can persist and spread even in the absence of antibacterials (Martinez, 2009). Multidrug resistance in bacteria occurs with the accumulation – on resistant R plasmids or transposons – of genes, with each coding for resistance to a specific agent, and/or by the action of multidrug efflux pumps, each of which can pump out more than one drug-type (Nikaido, 2009). The demonstration of R-factor transfer to fish pathogens was first shown with certain strains of *Aeromonas salmonidae*. Also, transferable R-factor plasmids in drug-resistant strains were shown with *Aeromonas hydrophila, Vibrio anguillarum,* marine *Vibrio sp., Edwardsiella tarda* and *Patteurella piscicida* (Alderman & Hastings, 1998). Tetracycline-resistance genes are found even in small farms which rarely use antibacterials. The copy numbers of tetA, tetC, tetH, and tetM genes (tetR reported by Seyfried et al., (2010)) remain elevated at farms over the surveillance period of four years in the absence of any selection pressure from tetracycline or even other antibacterials (Schmitt & Römbke, 2008; Tamminen et al., 2010). The continued introduction of tetracycline-resistant organisms from the hatchery to the stream, even after a significant time period had elapsed since the use of antibacterials, indicates the presence of reservoirs of organisms or unknown sources of resistance (Stachowiak et al., 2010) as well as other aquatic bacteria, and also illustrates that these bacteria can act as reservoirs of resistance genes that can be further disseminated. Ultimately, resistance genes in the aquatic environment may reach human pathogens and thereby add to the burden of antibacterial resistance in human medicine (Heuer et al., 2009).

Antibacterial agent	**Resistance mechanism**	*	**Examples of genetic determinant**
Tetracycline	Inducible efflux of tetracycline in *E. coli* and other *Enterobacteriaceae*	2	tetA, tetB, tetC
	Ribosomal protection in Gram-positive bacteria	4	tetO, tetM
Chloramphenicol	Efflux in *Enterobacteriaceae*	2	cmlA, floR
	Acetylation in *Enterobacteriaceae*	3	catA
ß-lactams	ß-lactamases in *Enterobacteriaceae, Staphylococcus aureus*	3	$bla_{TEM,}$ $bla_{SHV,}bla_{CMY-2,}bla_{Z}$
Oxacillin, methicillin	Alternate penicillin-binding proteins in *Staphylococcus aureus*	4	mecA
Imipenem	Decreased porin formation in *Enterobacter aerogenes* and *Klebsiella spp.*	1	Mutations
Aminoglycosides	Phosphorylation, adenylation, and acetylation of aminoglycosides in Gram-negative and -positive bacteria	3	Numerous genes with a broad variety of specificities
Streptomycin	Modification of ribosomal proteins or of 16s rRNA in *Mycobacterium spp.*	4	Mutations
Macrolides, lincosamides, streptogramins	Methylation of ribosomal RNA in Gram-positive organisms	4	ermA, ermB, ermC
Macrolides, streptogramins	*Staphylococcus spp.*	2	vga(A), msr(A)
Fluoroquinolones	DNA topoisomerases with low affinity to quinolones	4	Mutations in gyrA, gyrB, parC, parE
Sulphonamides	Bypass of blocked pathways through additional resistant dihydropteroate synthase in Gram-negative bacteria	4	Sul1, sul2, sul3
Trimethoprim	Bypass of blocked pathways through additional resistant dihydrofolate reductase	4	Diverse dfr genes

Note: This is by no means a comprehensive list of all the resistance mechanisms for each category of antibacterials listed. *: Numbers 1, 2, 3 and 4 refer to mechanisms listed in the text.

Table 8. Examples of resistance mechanisms (Boerlin & White, 2008).

Aquaculture is thought to stimulate the spread and stability of antibacterial resistance in the environment (Sapkota et al., 2008). Commercial fish production facilities could be a source of antibacterial-resistant microorganisms to receiving waters at times when there is no active use of antibacterials as a result of cross-resistance induced by biocides (Stachowiak et al., 2010). It has been shown that antibacterial-resistant bacteria are more likely to occur in the water and sediment associated with aquaculture. Already, in several areas of the world, this is beginning to take place. The comparison of predicted antibacterial concentrations to published minimum inhibitory concentrations suggests that antibacterials in wastewater – but probably not antifungals – may select for low-level antibacterial resistance (Kostich & Lazorchak, 2008). Also, the presence of R-factor-infected populations of bacteria in aquaculture systems may lead to the transfer of antibacterial resistance to other micro-

organisms, including potential human pathogens (Roberts, 2004; Cabello, 2006). Both the percentage and level of bacterial resistance to drugs was higher when drugs were administered as medicated feed. In addition, the duration of the resistance was longer when medicated feed was the mode of administration. The presence of feed residue in the aquatic system would have an important effect for the generation and maintenance of the drug resistance of bacteria in sediment (Yu et al., 2009).

These results call for the development of better management strategies for fish farming so as to prevent the emergence of resistant gene pools in the sediments of aquaculture facilities, and to promote the disappearance of established resistant gene pools (Tamminen et al., 2010). Principles for the prudent use of antibacterials should be developed and awareness of the problem of antibacterial resistance should be raised by informing the public (FAO, 2005). The most effective and direct approach is thought to be the reasonable use of antibacterials in health protection and agriculture production (Zhang et al., 2009). Without a doubt, a promising approach for proper risk-assessment and management would be the reduction of the emission of antibacterials into the environment, whether of human or veterinary medical origin. In either case, it may not be appropriate to assume that terminating the use of antibacterials will lead to a rapid decrease in resistant organisms (Stachowiak et al., 2010). The appropriate use of antibacterials in livestock production will preserve the long-term efficacy of existing antibacterials, support animal health and welfare, and limit the risk factors of transferring antibacterial resistance to animals and humans (Kemper, 2008). Whatever is done, the competent surveillance of disease and antibacterial resistance, as well as the repeated refinement of risk analyses, are a necessity if we are to concentrate our efforts to limit the effects of antibacterial resistance on what is shown to work in practice (Phillips et al., 2004; Sanders, 2005). In general, the emergence of resistance to antibacterials is a highly complex process, which is not yet fully understood with respect to the significance of the interaction of bacterial populations and antibacterials, even in a medicinal environment (Kümmerer, 2010). In the EU, the EMA works for the development of a harmonised approach to the surveillance of antibacterial usage in animals and the collection of data from EU Member States (WHO, 2011). Also, research projects should be encouraged which aim at the better understanding of the mechanisms of the emergence and spread of resistance within a species, and from animal to human and the environment (FAO, 2005).

5.3 Aquatic food residues

The case of the residues of antibacterial substances in fish and fish products represents, in practice, a complex problem for society and regulators, and particularly in developing countries where regulations and the possibilities for enforcing them are scarce (Cabello, 2006; Lupin, 2009). In addition to selecting for antibacterial resistance, the heavy prophylactic and therapeutic use of antibacterials in aquaculture environments can lead to elevated antibacterial residues in ponds, marine sediments, aquaculture products, wild fish and other natural aquatic environments that are impacted by aquaculture facilities (Sapkota et al., 2008). Also, the use of large amounts of antibacterials that have to be mixed with fish food creates problems for industrial health and increases the opportunities for the presence of residual antibacterials in fish meat and fish products (Cabello, 2006). Withdrawal times are recommended, and in many countries they are legally enforced for some drugs, and especially antibacterials. The Food Animal Drug Avoidance Databank (FARAD) assists veterinarians in estimating residue-depletion times for antibacterial agents that are

administered at doses in excess of label recommendations (Walker & Giguére, 2008). A good rule of thumb for the withdrawal time is 500 degree days. Thus, if the mean daily water temperature after treatment is 10°C, the withdrawal period should be at least 50 days (10 x 50 = 500), while at 25° C, the withdrawal period would be 20 days (Noga, 2010). Obviously, this can only be a rough estimate of the elimination-rate because temperatures fluctuate diurnally and from day-to-day and other factors besides temperature affect elimination-rates. Note also that 500 degree days might not be sufficient in some cases (Treves - Brown, 2001). Therefore, the accurate and sensitive determination of antibacterial residues is now a necessity. In order to protect human health, the EU and other regulatory authorities worldwide have established MRLs for antibacterial residues in animal products entering the human food chain (Cañada-Cañada & Pena, 2009; Lupin, 2009). Research projects should be promoted on pharmacology and the PK of antibacterials in aquatic species in order to provide a more exact approach to establishing MRLs' values (Table 7) (FAO, 2005).

5.4 Environmental impact of antibacterial use in aquaculture

Aquaculture is so integrally linked to the surrounding environment that if sustainable practices are not employed, the degradation of the surrounding environment will ultimately lead to the degradation of the industry itself (Bergh, 2007). The wellbeing of the environment – in cases of disease and treatment – is related to two aspects of biota conservation; the transmission of microbial pathogens to wild populations and the pollution from chemotherapeutics (Grigorakis, 2010). The extensive use of veterinary pharmaceuticals is supposed to represent a daunting public health risk, resulting not only in the emergence and spread of resistant bacteria, but also in other human, animal and environmental impairments (Kemper, 2008). The input of resistant bacteria into the environment from different sources seems to be the most important basis of resistance in the environment. The possible impact of resistant bacteria on the environment is not yet known and the health risks of active pharmaceutical ingredients remain poorly understood (Kümmerer, 2010). The physicochemical fate and environmental concentrations of antibacterials in soil has been the subject of a number of recent studies. During recent years, significant attention has been paid to the occurrence of drugs in the environment. Several classes of antibacterials have been detected in field soils, and their sorption behaviour and degradation have been studied to a large extent (Schmitt & Römbke, 2008; Zounkova et al., 2011). In general, farmed fish is as safe and nutritious as wild-caught species, but there are public health hazards associated with ignorance, abuse and the neglect of aquaculture technology. Numerous small fish ponds increase the shoreline of ponds, causing higher densities of mosquito larvae and cercaria, which can increase the incidence and prevalence of lymphatic filariasis and schistosomiasis (Lessenger, 2006). Fish production can generate considerable amounts of dissolved effluents, which potentially affect water quality in the vicinity of the farms and, due to rapid dilution, also at larger scales (km-scale) (Costanzo et al., 2005; Holmer et al., 2008). High antibacterial load in sediments and in concentrations potent enough to inhibit the growth of bacteria have been reported for aquaculture (Kümmerer, 2008).

Tetracycline has a low bioavailability in fish (< 10%), due to binding with sea-water-borne divalent cations such as Mg^{+2} and Ca^{+2}. It is noteworthy that non-bioavailable tetracyclines contaminate the environment (Rigos & Troisi, 2005). However, it has been

shown that residues of oxytetracycline in marine sediments were very stable over a period of months (Toutain et al., 2010). Often, the existing data used to assess the environmental effects of antibacterials is not adequate for the establishment of how long bacteria maintain antibacterial resistance in the absence of continued selective pressure for that resistance (Kümmerer, 2008). Also, in order to minimise the possible risks of antibacterials in dust, the use of antibacterials in livestock farming should be strictly reduced to therapeutic use (Hamscher & Hartung, 2008). In one study (Wei et al., 2011) conducted in China (Jiangsu Province) – the biggest aquaculture producer (FAO, 2011) – contamination with antibacterials indicated that ten veterinary antibacterials around farms were found in animal wastewaters, eight antibacterials were detected in pond waters, and animal farm-effluents and river water samples were contaminated by nine antibacterials. The most frequently detected antibacterials were sulphamethazine (75%), oxytetracycline (64%), tetracycline (60%), sulfadiazine (55%) and sulphamethoxazole (51%). This research has demonstrated that animal wastewater is a major source of pollution of veterinary antibacterials. By applying the animal wastewater to agricultural soils, the antibacterials might contaminate the soils and surrounding water systems, thus posing a serious threat to humans and wildlife (Figure 2) (Boxall et al., 2004; Wei et al., 2011). Antibacterials may be detected in effluent entering receiving waters and be detectable 500m from the source (Costanzo et al., 2005). There is very little information about the chronic toxicity or the bioaccumulation potential of pharmaceuticals in biota and food chains (Christen et al., 2010). Not much is known about the occurrence, fate and activity of metabolites (Kümmerer, 2010). Another study showed that more than 30 antibacterial substances have been found in sewage influent and effluent samples, in surface waters and even in ground and drinking water (Kemper, 2008). At the same time, with antibacterials, disinfectants, and heavy metals being released into water, they might exert selective activities as well as ecological damage in water communities, resulting in antibacterial resistance (Baquero et al., 2008). For example, the exposure of eels to pollution during their development is inducing changes on the biomarkers involved in physiological functions that are determinants for the survival and performance of the eels, namely biotransformation enzymes and antioxidative stress defences, and these alterations may have negative effects on sexual development. In addition, the mechanisms used to face chemical stress need energy which is probably allocated from other functions, such as tissue repair, growth and weight increase, and which are determinants for a successful migration into the reproduction area (Gravato et al., 2008).

Ideally, aquaculture operations would be planned with background knowledge of the ecosystems in which the facilities will operate as well as knowledge of the potential environmental, social, and economic effects (both positive and negative) that could be incurred, and the cost:benefit ratio associated with operating, given knowledge of that background (Bergh, 2007). Environmental observations and models can then be combined with effective aquaculture husbandry practices so as to manage environmental risks from all sources (Hargrave et al., 2005). Hopefully, research on aquaculture-environment interactions has progressed remarkably during recent years, particularly in the framework of EU-funded projects, which have provided useful information for the understanding of various ecosystem processes affected by the presence and operation of fish farms (Holmer et al., 2008).

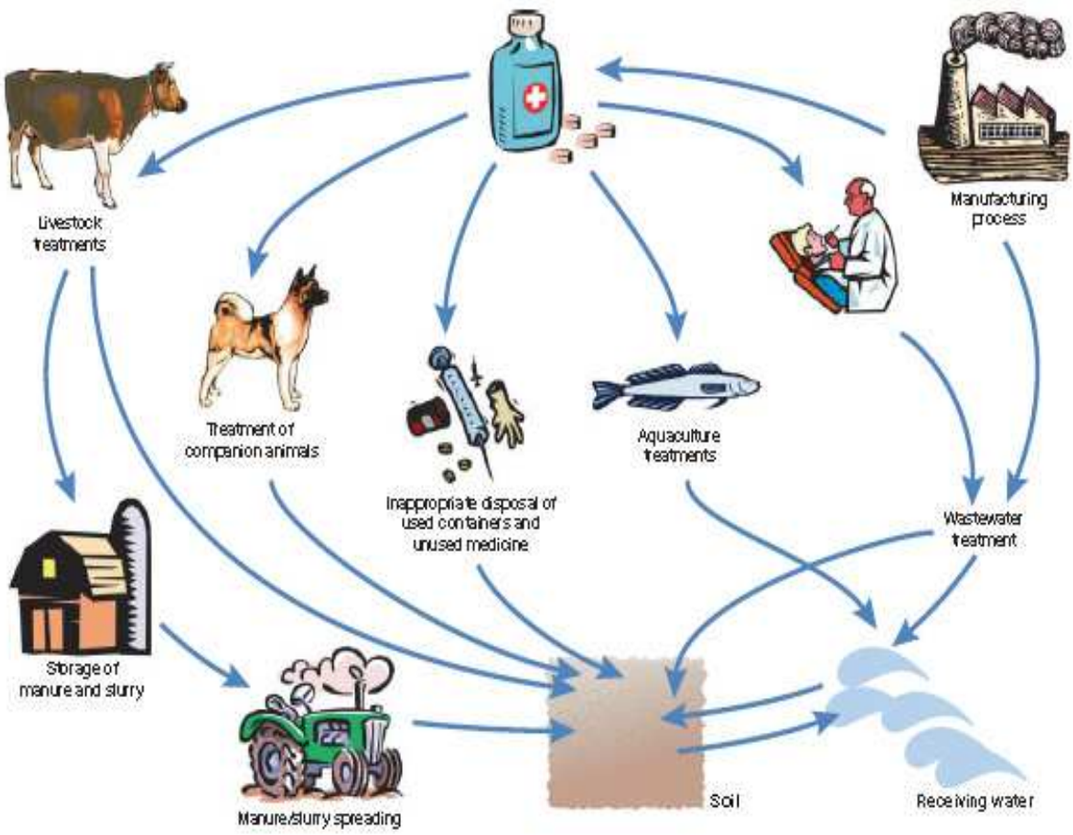


Fig. 2. Routes of pharmaceuticals entering the environment (Boxall et al., 2004).

6. Antibacterial usage suggestions in aquaculture

When it is apparent that a treatment is necessary, the following check-lists may be useful (Winton, 2001):

Before treating:

1. Accurately determine the water-volume, flow-rate, and temperature.
2. Accurately determine the number and total weight of fish in the rearing unit.
3. Confirm the identity, expiration date, and active ingredient concentration of the regulated product to be applied.
4. Double-check treatment calculations. Beware of confusion from mixing metric and standard units.
5. Have aeration devices ready for use if needed.
6. If treated water is to be discharged, make sure all appropriate permits are in place and regulatory authorities have been notified.
7. If possible, conduct a bioassay on a small group of fish before treating the entire population in the rearing unit.

When treating:

1. Dilute the regulated product with rearing water before applying it (or follow product directions).
2. Ensure that the regulated product is well-mixed and evenly applied in the rearing units.
3. Observe the fish closely and frequently during treatment for signs of distress.
4. Monitor the temperature and dissolved-oxygen levels in the rearing unit during treatment.
5. Except for oral treatments, discontinue feeding during treatment. Fish are unlikely to feed during treatment, and uneaten feed will foul the system and may reduce the efficacy of some treatments.
6. Discontinue treatment and restore normal culture conditions if the fish become distressed.

After treating:

1. Observe the fish frequently for at least 24 hours following the treatment.
2. Do not stress the treated fish for at least 48 hours.
3. Recheck the fish to determine the efficacy of the treatment

Judicious antibacterial use principles for veterinarians are discussed and concluded in Table 9 (FDA, 2009).

The food fish veterinarian should:

1. Accept responsibility for helping clients design management, immunization, production unit and nutritional programmes that will reduce the incidence of disease and the need for antibacterial treatment.
2. Use antibacterial drugs only within the confines of a valid veterinarian-client-patient relationship, including both the dispensing and issuing of prescriptions and veterinary feed directives. Extra-label usage should be consistent with regulatory agency laws, regulations and policies.
3. Properly select and use antibacterial drugs. Veterinarians should participate in continuing education programmes that include therapeutics and the emergence and/or development of antibacterial resistance.
4. Have strong clinical evidence of the identity of the disease's aetiology, based upon history, clinical signs, necropsy, laboratory data, and/or past experience before recommending an antibacterial drug treatment.
5. Treat food fish with antibacterial drugs according to the product label recommendations (including indication, dosage, duration, fish species and environmental conditions).
6. Choose an antibacterial drug and treatment regimen based on the available laboratory and label (including package insert) information, additional data in the literature, and consideration of the pharmacokinetics, spectrum of activity and pharmacodynamics of the drug.
7. Use antibacterial drugs with a specific clinical outcome(s) in mind, including a specific target for population morbidity and/or mortality-rate reduction.
8. Specific outcome criteria will prevent an unnecessarily long therapy and indicate when the current therapy is no longer effective.

9. Determine the production population pathogen susceptibility at the first indication of increasing morbidity or mortality, and monitor the therapeutic response so as to detect changes in microbial susceptibility and in order to evaluate antibacterial selections.
10. Routine necropsy examination of fish populations should be periodically performed, including antibacterial susceptibility testing and update historical information for developing treatment and control protocols.
11. Use products that have the narrowest spectrum of activity and known effectiveness *in vivo* against the pathogen causing the disease problem.
12. Choose antibacterial drugs of lesser importance in human medicine, if these receive future food fish use approval, and do not choose an antibacterial for which the emergence of resistance is expected in an advanced stage.
13. Use, whenever possible, an antibacterial drug labelled to treat the condition diagnosed.
14. Do not use combination antibacterial drug therapy unless there is information to show that this decreases or suppresses the target organism resistance development.
15. Do not compound antibacterial drug formulations.
16. Do not use antibacterial drugs to treat cases with a poor chance of recovery.
17. Do not use antibacterial drugs prophylactically.
18. Ensure proper on-farm drug use and protect antibacterial drug integrity through proper handling, storage and observation of the expiration date.
19. Prescribe, dispense or write a Veterinary Feed Directive for drug quantities appropriate to the production-unit size and expected need using the approved formulation.
20. Work with producers and/or facility fish health management personnel so as to ensure that farm personnel receive adequate training on the use of antibacterial drugs, including indications, diagnoses, dosages, withdrawal times, the route of administration, storage, handling and accurate record-keeping.
21. Work closely with all other fish health experts involved in fish population health management at the fish production facility.

Table 9. Judicious antibacterial use principles for veterinarians (resumed from (FDA, 2009)).

7. Conclusion

The presence of disease in farmed fish populations has severe welfare implications for the affected fish, and poses a threat to the welfare of unaffected fish. Large quantities of antibacterials are used in aquaculture in some countries, often without professional consultation or supervision. Consequently, many problems are associated with the use of antibacterials in aquaculture. More research is needed in order to determine the consequences of the application of large quantities of antibacterials. Considering the rapid growth and importance of the aquaculture industry in many regions of the world and the widespread, intensive, and often unregulated use of antibacterial agents for animal production, additional efforts are required to prevent the development and spread of

antibacterial resistance in aquaculture. Also, safer, more effective medicines are necessary, along with improvements in husbandry and management which will reduce the need for those medicaments. However, without the use of veterinary medicines, aquaculture food production would be impaired. Furthermore, fish farmers and their veterinary surgeons must confirm that fish are kept in the best state of health and welfare. Governments, farmers and veterinary surgeons all have a shared responsibility to ensure that medicines are used judiciously.

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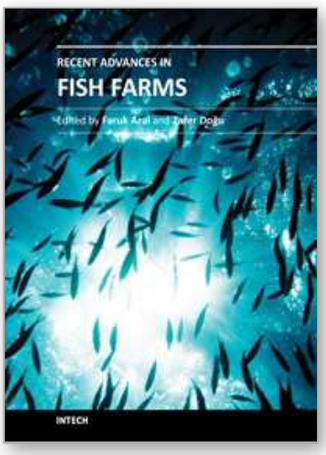
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The world keeps changing. There are always risks associated with change. To make careful risk assessment it is always needed to re-evaluate the information according to new findings in research. Scientific knowledge is essential in determining the strategy for fish farming. This information should be updated and brought into line with the required conditions of the farm. Therefore, books are one of the indispensable tools for following the results in research and sources to draw information from. The chapters in this book include photos and figures based on scientific literature. Each section is labeled with references for readers to understand, figures, tables and text. Another advantage of the book is the "systematic writing" style of each chapter. There are several existing scientific volumes that focus specially on fish farms. The book consists of twelve distinct chapters. A wide variety of scientists, researchers and other will benefit from this book.

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