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Antimicrobial agents in aquaculture: Practice, needs and issues

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Abstract. Aquaculture is a fast-growing food production sector and the need for antimicrobial agents varies markedly between countries. Intensification of aquaculture has led to the promotion of conditions that favour the use of a wide range of chemicals, including antibiotics, pesticides, hormones, anaesthetics and various pigments. The quantities used and the usage patterns also vary between countries and individual aquaculture operations. However, the market for aquaculture antimicrobials is small and the approval process for new compounds is expensive. Antibiotics can be used in fish to effectively treat bacterial diseases but are not for growth promotion as in other food-producing industries. Antifoulants that contain biocides and pesticides used for treatment of certain parasites are also used in aquaculture operations. The provision of increased selection pressure leading to antibiotic resistance can occur by overuse or misuse in human medicine, livestock, agriculture, horticulture and aquaculture. There are virtually no antimicrobial agents available for treatment of molluscan or crustacean diseases and alternative control measures are therefore required. Alternatives to the use of antimicrobial agents include good husbandry, adequate feed composition, vaccines, biological control and movement restrictions through legislation. Further research is required in areas such as vaccine development, immunostimulants and the use of probiotics.

Keywords. Antimicrobial agents – Application methods – Aquaculture – Control measures – Fish diseases.

Agents antimicrobiens en aquaculture: Pratique, besoins et problématique

Résumé. *L'aquaculture est un secteur de production d'aliments en expansion rapide, et les besoins en agents antimicrobiens varient de façon notable selon les pays. L'intensification de l'aquaculture a conduit à favoriser des conditions menant à l'utilisation d'une vaste gamme de produits chimiques, y compris les antibiotiques, pesticides, hormones, anesthésiants et plusieurs pigments. Les quantités utilisées et les modes d'utilisation varient également selon les pays et le fonctionnement individuel des unités aquacoles. Toutefois, le marché des antimicrobiens pour aquaculture est réduit et le processus d'approbation de nouveaux composés est onéreux. Les antibiotiques peuvent être utilisés chez les poissons pour traiter efficacement les maladies bactériennes mais ce ne sont pas des promoteurs de croissance comme dans d'autres industries productrices d'aliments. Les anti-algues contenant des biocides et des pesticides utilisés pour le traitement de certains parasites sont aussi employés pour le fonctionnement de l'aquaculture. La mise en place d'une pression de sélection accrue menant à la résistance aux antibiotiques peut survenir par surutilisation ou mauvaise utilisation dans les domaines de la médecine humaine, l'élevage, l'agriculture, l'horticulture et l'aquaculture. Il n'y a virtuellement pas d'agents antimicrobiens disponibles pour le traitement des maladies des mollusques ou des crustacés et des mesures de contrôle alternatives sont donc nécessaires. Parmi les alternatives à l'utilisation d'agents antimicrobiens se trouvent les bonnes pratiques d'élevage, une composition adéquate de l'aliment, les vaccins, le contrôle biologique et les restrictions des mouvements à travers la législation. Des recherches ultérieures sont nécessaires dans des domaines tels que le développement de vaccins, l'utilisation d'immunostimulants et de probiotiques.*

Most-clés. *Agents antimicrobiens – Méthodes d'application – Aquaculture – Mesures de contrôle – Maladies des poissons.*

I – Introduction

The phrase "prevention is better than cure" is often used when dealing with the use of antimicrobial agents in aquaculture but it is perhaps easier to say than put into practice. If prevention was better than cure and also worked in 100% of cases then there would probably be no need to write this article. According to the Concise Oxford Dictionary "prevention" means hinder or avoid and "cure" means remedy through a course of treatment, or restore to health.

Nevertheless, a simple understanding of prevention or cure also requires a consideration of the causes of disease and an agreement on a definition. Unfortunately, a definition of disease is not as easy as it sounds, even for fish pathologists, because ever since the beginning of medicine, physicians have also sought to define the same term and they have not so far been very successful. In fact, although it has been defined as *"An Undefined Word*" (IHMMR, 1973), there have been many different attempts *at a definition, including the following:*

> *"...any departure from a state of health, or an illness, or a sickness...may affect the whole body or any of its parts ... its etiology, pathology, and prognosis may be known or unknown*..." (Dorland's Medical Dictionary; Dorland, 2000).

> *"... illness, sickness. An interruption or perversion of functions of any of the organs, a morbid change of any of the tissues or an abnormal state of the body as a whole, continuing for a longer or shorter period..."* (Stedman's Medical Dictionary; Stedman, 2000).

The US Food and Drug Administration has also attempted to define disease as:

> *"...any deviation from, impairment of, or interruption of the normal structure or function..."*

or, more simply,

> *"...any deviation from normal..."* (FDA, 2000),

but the latter loose definition created a certain amount of heated industry and political debate because it was related to the use of dietary supplements and the FDA have subsequently back tracked to the previous definition.

Unfortunately, these are not operational definitions and suffer from what in science is called circular logic, which actually makes them of little value (Wilken, 2002). Consequently, one could think that it might be easier to define the concept of health rather than disease. However, this is even more problematic and, in fact, the difficulty of defining health has led to most attention being given to defining disease instead (Lewis, 2001).

Nevertheless, there is another definition in Blakiston's New Gould Medical Dictionary that defines disease as:

> *"The failure of the adaptive mechanism of an organism to counteract adequately the stimuli or stressors to which it is subject, resulting in a disturbance in function or structure of any part, organ, or system of the body"* (Gennaro, 1979).

This is not a circular definition of disease but rather an explicit operational definition that also introduces another important word, namely the concept of "stressors". An easy and simple definition of stress is:

> *"The non-specific response of the body to any demand."* (Selye, 1978)

Stress-related disease, however, results from excessive and prolonged demands on an organism's resources and, certainly, in a medical context, it is now believed that 80-90% of all disease is stress-related (Ford-Martin, 2001).

It is also relevant to consider the meaning of certain similar terms used when discussing treatment of diseases:

(i) Antimicrobial: any agent that destroys or inhibits the growth of microorganisms (Miriam-Webster, http://www.m-w.com); a drug used to treat a microbial infection, which is a general term referring to antibiotics, antifungals, antiprotozoals and antivirals (Medilexicon, http://www.pharma-lexicon.com); any of a large variety of chemical compounds and physical agents that are used to destroy microorganisms or to prevent their development (Encyclopaedia Britannica; http://britannica.com).

(ii) Drug: a substance used as a medication intended for use in the treatment or prevention of disease (Miriam-Webster; http://www.m-w.com); any chemical substance that affects the functioning of living things and the organisms (such as bacteria, fungi, and protozoans) that infect them (Encyclopaedia Britannica; http://www.britannica.com).

(iii) Chemotherapeutant: in its broadest sense, chemotherapy refers to the treatment of disease with chemicals (Encyclopaedia Britannica; http://www.britannica.com).

(iv) Antibiotic: chemical substance produced by a living organism, generally a microorganism, that is detrimental to other microorganisms (Encyclopaedia Britannica; http://www.britannica.com). It should be noted, however, that the original definition of an antibiotic was a substance produced by one microorganism that selectively inhibits the growth of another microorganism. However, wholly synthetic antibiotics (usually chemically related to natural antibiotics) have since been produced that accomplish comparable tasks (Medilexicon, http://www.pharma-lexicon.com).

II – Aquaculture

Aquaculture is still a fast-growing food production sector because it is an increasingly important source of protein available for human consumption. According to the FAO, the supplies of fish, crustaceans and molluscs from aquaculture increased from 3.9% of total production by weight in 1970 to 27.3% in 2000 (FAO, 2002) and aquaculture is growing more rapidly than all other animal food producing sectors. The rate of increase has been 9.2% per year since 1970, compared with only 1.4% for capture fisheries and 2.8% for terrestrial farmed meat production systems. There were more than 210 different farmed aquatic animal and plant species reported for 2000 of which more than half were finfish, in contrast to terrestrial farming systems, where the majority of production is based on a limited number of species. However, the majority of aquaculture production is composed of only 29 species that account for 78% of the production. Currently, the top four countries for aquaculture production (2000 figures) are China (32.4 million tonnes/year), India (2.1 mt), Japan (1.3 mt) and Philippines (1.0 mt). By way of contrast, the largest European producers in 2000 (EC, 2003) were Spain (0.31 mt), France (0.27 mt), Italy (0.22 mt) and the UK (0.15 mt). However, if Mediterranean countries are also considered, then Egypt would displace the UK, since it has similar production figures to Italy. Europe as a whole had an aquaculture production of 1.3 mt in 2000 (FAO, 2002). Nevertheless, the proportion of finfish, shellfish, crustaceans and aquatic plants, and hence the need for antimicrobial agents, varies markedly between countries. Aquaculture production at the world level is forecast to continue increasing and will be led by Chinese production, followed by South Asia, Latin America, the Caribbean and Europe that will contribute smaller increases, with freshwater species and molluscs dominating aquaculture production for the foreseeable future (FAO, 2002).

In Europe itself, it is estimated that aquaculture production will exceed 2.5 million tonnes by 2015 and reach 4 million tonnes in 2030 (FAO, 2002). In 1998, the main species consumed in Europe were mussels (7% of all consumption), cod (7%), tuna (6%), herring (6%), cephalopods (squid, octopus and cuttlefish – 5%), sardines (5%), salmon (4%), shrimps (4%) and trout (3%). The European market with more than 480 million consumers (370 million in EC member countries) is one of three important markets for fish products. The other major markets for fish are Japan and the USA.

III – Antimicrobial agents used in aquaculture

The discovery and development of antimicrobial agents to treat systemic bacterial infections is one of the most fascinating stories in the history of microbiology, since early workers examined the property of azo dyes for possible antimicrobial action. Chemical modification of a synthesized compound called prontosil led directly to another series of compounds, the sulphonamides, which were soon recognized as having early potential for treating furunculosis in trout (Gutsell, 1946). Penicillin was the first natural antibacterial agent that was clinically successful in the medical field. Although Fleming observed the inhibition of *Staphylococcus aureus* on an agar plate by a contaminating *Penicillium* it was some years later when other workers purified enough penicillin for clinical trials. Since this early work in the 1940's, there have been many reported studies on chemotherapeutic agents, with the result that there are now antibiotics available for virtually all bacterial infections. However, because of developing resistance problems and newly emerging or re-emerging diseases this development work remains on-going and is likely to remain so in the future.

Intensification of aquaculture has led to the promotion of conditions that favour the development of a number of diseases and problems related to biofouling. Consequently, a wide range of chemicals is used in aquaculture, including antibiotics, pesticides, hormones, anaesthetics, various pigments, minerals and vitamins, although not all of them are antimicrobial agents. The use varies between different types of operation, with, for instance, finfish farms and hatcheries using many more chemicals than shellfish farms, which hardly ever use chemicals. Usage patterns also vary between countries and between individual aquaculture operations within the same country.

IV – Antimicrobial approval

Compared to agricultural use and medicinal use the market for aquaculture antimicrobials is fairly small and the approval process can be expensive. In fact, if we take the USA as an example, only five drugs have been approved by the US Food and Drug Administration (FDA) for disease-treatment (Schnick, 2000). Although there are only limited data regarding quantities of antimicrobial usage, there are concerns connected with the use of such agents in relation to the persistence and potential effects of chemicals. These include pesticides, disinfectants, antibiotics, and chemotherapeutants, which are used for prevention and treatment of disease and control of aquatic pests. The FDA also maintains a list of aquaculture drugs which have undergone review and are classed as new animal drugs of low regulatory priority. These include compounds such as acetic acid (a parasiticide dip for for fish), carbon dioxide gas (for anesthetic purposes), hydrogen peroxide (for fungi control on fish and eggs), sodium chloride (as an osmoregulatory aid and parasiticide) or even garlic (used for control of helminth and sea lice infestations of marine salmonids), onion (used to treat external crustacean parasites) and the ice used to reduce the metabolic rate of fish during transport.

In Europe, fish oral drugs, as well as external antibacterial and antiparasitic compounds, are considered as Veterinary Medicinal Products (VMP) and the Salmonidae are classed as major animal food producing species. The availability of antimicrobial agents for aquaculture use is affected by the setting of maximum residue limits (MRLs), which was adopted by a Regulation in 1990 (EC, 1990). However, the setting of an MRL is only a preliminary step towards achievement of full marketing authorisation, although the European Medicine Agency (EMEA) recently extrapolated the MRLs of twelve antibiotics to all food producing animal species (Daniel, 2002). Nevertheless, the list of fully authorised licensed pharmaceuticals for aquaculture is still quite small (Table 1). In addition, although Europe has taken steps to harmonise the availability of antimicrobial agents there is still large variation between individual European countries (Daniel, 2002).

V – Regulation

The use of antimicrobial agents in food animal species, including fish, is controlled by regulations, particularly in Europe and the USA. Stringent safety and efficacy standards, including residue testing, are required before approval of any such agent. This rigorous approval process is very costly and time consuming though 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 behalf of pharmaceutical companies for developing new antimicrobials and registering them.

Table 1. Number of drugs approved for aquaculture in the world (adapted from: Schnick *et al*., 1997 and Daniel, 2002*)

Drug type	Australia	Canada	Europe*	Japan	USA
Antimicrobials	--	4	7†	27	3
Microbicides	--	4	6††	3	1
Anaesthetics	1	2	1†††	2	1
Hormones	3	--	--	--	--

†Amoxicillin, florfenicol, flumequine, oxolinic acid, oxytetracycline, sarafloxacin and sulfadiazine-trimethoprim.

††Azamethiphos, bronopol, cypermethrin, emamectin benzoate, hydrogen peroxide and teflubenzuron.

†††Tricaine methane sulphonate (MS222).

Follow-up requirements insist that residues must be below a tolerable level before fish or fish products enter into the food chain. In fact, on-going testing related to these levels can result in product withdrawal, particularly for compounds specifically prohibited for use in food animals, such as chloramphenicol or the nitrofurans. This situation can affect trade quite severely, as was the case in 2002 when the European Union (EU) detected unacceptable levels of chloramphenicol in shipments of shrimps from South East Asia. Product withdrawal affecting trade in this way is a powerful argument for ensuring that no prohibited compounds are used on fish destined for the market or that excessive use of permitted compounds leads to detectable residues.

VI – Antimicrobial practice

1. Use of chemicals

The types of chemicals not only include chemotherapeutants but also pesticides, oxidants, disinfectants, algicides, herbicides and biocides in general. These compounds are used to prevent and treat disease, control aquatic pests and protect farm infrastructures from fouling. As in other agricultural sectors, antibiotics can be used in fish to effectively treat bacterial diseases. Antibiotics used in aquaculture are usually administered to fish in feed to treat disease and not to promote growth as in some other food-producing industries. As such, all antibiotics require veterinary prescription. Nevertheless, concerns exist regarding the persistence and effects of antibiotics and the other chemicals used in the aquaculture industry. Antifoulants that contain biocides, which are released slowly over a period of time, can be considered to be antimicrobial agents since they are designed by their very nature to be toxic to aquatic organisms, and therefore they will have an impact on non-target organisms.

However, establishing the exact level of drug use and the nature of any potential dangers is difficult due to lack of sufficient data, fragmented laws, regulations, jurisdictions and interpretations of reporting guidelines (OTA, 1995). It is also thought that the large quantities of aquaculture products imported from countries with both legal and illegal drug use may escape documentation (Benbrook, 2002). Nevertheless, there have been some attempts to estimate usage for major aquaculture species:

(i) In US aquaculture, the total antibiotic use for catfish production has been estimated to be between 126,000 and 252,000 pounds (approx. 57,100 to 114,300 kg) per annum to treat enteric septicaemia of catfish. Trout and salmon production uses an estimated 63,000 to 104,600 pounds (approx. 28,500 to 47,400 kg) and other species 15,200 to 76,000 pounds (approx. 6,900 to 34,500 kg) annually. Using these estimates, Benbrook (2002) indicated that total aquaculture industry use in the USA is between 204,000 and 433,000 pounds (approx. 92,500 to 196,400 kg). It is considered that this represents about 2% of the non-medical use in beef, swine, and poultry production, and about half the level of use in companion animals, as estimated recently by Mellon *et al*. (2001).

(ii) In Canadian aquaculture, official data indicates approximately 2.5% of all milled feeds are medicated each year in British Colombia for salmon production in fish of less than 2 kg (non-food fish size). Figures for 1999 indicated an antibiotic usage of approximately 323 kg (based on 263 g/metric tonne). However, although the usage was much lower (165 g/mt) in 2001, the actual percentage of medicated feed is not available. Despite a reduction is use though, production rose by approximately 36% (MAFF, 2003). British Colombia produces approximately half of the total finfish production in Canada, therefore the total antibiotic usage could be double this estimated figure and be situated around 650 kg/year, assuming the same parameters. There were also small quantities of ivermectin (0.0001 g/mt) and emamectin (0.0635 g/mt) used in 2001.

(iii) It is very difficult to estimate antibiotic usage in Asia but it is known that shrimp farmers use antibiotics in large quantities. However, Moriarty (2000) indicates, from 1994 feed usage and production data, that for Thailand alone it could be as high as 500-600 tonnes/year (excluding hatcheries for fry production) based on 10% medicated feed.

(iv) In the UK, official data shows that 2 metric tonnes of therapeutic antimicrobials (e.g. mainly tetracyclines and potentiated sulphonamides) were sold for fish (i.e. salmon and trout) during 2000 at a time when production remained almost constant at around 134,000 tonnes. This compares to the figures for 1993, when 10 tonnes of antimicrobials were sold for a production of 55,000 tonnes of fish (Veterinary Medicines Directorate, 2002).

VII – Application methods

Antimicrobial agents are used in aquaculture to control systemic disease, external parasites, fungal outbreaks, as well as to correct water quality problems, disinfect eggs and equipment, and control aquatic weeds and free-living molluscs (Table 2). The balance between success and failure depends on correct diagnosis, correct dose rate and the most appropriate administration method (Bruno and Munro, 1991).

Table 2. Types of antimicrobial agents, target use and application method (adapted from Goldburg *et al*., 2001)

Type of agent	Usage	Method of application
Chemotherapeutants	Treatment of bacterial fish diseases	Oral –medicated feed; injection; topical; bath
Parasiticides	Control of sea lice on salmon; treatment of parasites in ornamental fish ponds; control of protozoa and trematodes on finfish	Oral –medicated feed; bath; dip; flush
Oxidants	To kill disease organisms and phytoplankton in pond systems	Direct; flush
Biocides, algicides and herbicides	Reduce plant growth in pond systems; antifouling treatment for fish farm cage netting	Direct; flush

Dry powder commercial formulations of antimicrobial agents frequently contain substances other than the active ingredient. The "non-active" proportion is normally added as a stabiliser, carrier or diluent agent, such as corn or rice derivatives and calcium carbonate. Consequently, it is necessary to take the presence of these additives into account when calculating the quantity of the active ingredient required for any treatment. Similarly, some antimicrobials are supplied as liquid formulations that contain a stated weight of active ingredient per unit volume (e.g. 0.4 kg/l) and these need diluting accordingly (Bruno and Munro, 1991).

The application methods for fish can be divided into six different groups (Austin and Austin, 1999) comprised of oral (via medicated feed), bath, dip, flush, injection and topical application. Each method has its advantages and disadvantages depending on the targeted use, as well as potential environmental impact. For instance, the use of medicated feed relies on the fact that fish want to eat, which is always a problem in disease outbreaks, whereas bath treatment may give rise to disposal problems and flush treatment could lead to unwanted environmental impacts. However, in general, many of the chemicals used in aquaculture are applied directly to water.

1. Medicated feed

In food fish or ornamental aquaculture, many bacterial diseases of fish can be successfully treated with medicated feeds and it is usually the preferred method of treatment. Recently, some parasitic treatments, such as for sea lice, have also been administered in feed. Care must be taken though because some of the causes of disease, such as stress, can lead to treatment failures or the recrudescence of disease after completion of treatment. Typical stress conditions include increased fish density, poor or inadequate nutrition, poor water quality (e.g. low dissolved oxygen, high ammonia and nitrite content), parasite infestation and handling (Durborow and Francis-Floyd, 1996).

More often than not medicated feeds are commercially prepared, either as sinking or floating pellets, although such feeds have a limited shelf life. As already mentioned, one problem with the use of medicated feeds is that diseased fish stop eating and this may be compounded by unpalatable feed caused by the presence of the drug itself, which makes the problem worse. Consequently, good diagnosis that includes an antibiotic sensitivity test plays an important role in the use of the correct early treatment.

The incorporation of an antibiotic in the feed is usually via a powdered premix in conjunction with a binder, such as gelatin (up to 5%), fish or vegetable oil. One of the important considerations is that the feed and the drug have to be mixed thoroughly to give an even distribution of the drug and coating of the pellets. The dosage required for treatment with a medicated feed depends on the original level of active ingredient/kg fish body weight. The feed is then administered for a recommended treatment period, according to the specific disease to be treated and the instructions of a veterinary practitioner. It is also important that treated fish must not be harvested for food use until a specified withdrawal period has elapsed. Medicated feed needs to be kept under adequate storage conditions, such as in a cool dry place kept separate from other feeds, to avoid any deterioration of the feed quality and drug efficacy.

The dosage rates used in medicated feed will vary according to the specific antibiotic used but usually the rate is based on a number of grams per 100 kg of fish per day (Table 3). The exact dosage will also require the number and average weight of fish to be treated, as well as a daily feeding rate and a consideration of whether the fish are marine or fresh water species.

One problem for the treatment of marine species is that antibiotics have been shown to be less effective in seawater, which is related to their reduced bioavailability, due to binding with the Mg^{2+} and Ca^{2+} divalent cations that occur in seawater (Lunestad and Goksøyr, 1990; Smith *et al*., 1996). This has major implications for the use of certain antibiotics because their minimum inhibitory concentrations (MIC) may be much higher than in fresh water. For instance, the quinolone oxolinic acid has been shown to have a MIC 40- to 60-fold higher in seawater against the bacterial fish pathogen *Aeromonas salmonicida* (Barnes *et al*., 1995). The bioavailability of some aquaculture drugs in salmon held in seawater is shown in Table 4.

Table 3. Examples of possible dosage rates for some common antibiotics or drugs

Antibiotic/drug	Dosage rate (g/100 kg fish/day)
Potentiated sulphonamide	7.5 g for 5-7 days
Oxolinic acid	1 g for 10 days
Oxytetracycline	7.5 g for 5-0 days

Table 4. Examples of reduced bioavailability for some aquaculture drugs in seawater (Adapted from Martinsen and Horsberg, 1995; Horsberg, 2002)

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

2. Injection

Injection of antibiotics can be a more effective treatment for bacterial infections than using a medicated feed, particularly for advanced infections. However, it is usually only practical for valuable individual fish, such as broodstock or ornamental fish (e.g. koi carp), rather than fish in large scale production facilities. Injection quickly leads to high blood and tissue levels of antibiotic. Normally, an individual fish will also need to be anaesthetized before treatment. Typical injection sites include the intraperitoneal cavity and the intramuscular route.

The volume required for injection of antibiotics is based on the weight of fish to be treated, the recommended dosage for the antibiotic being used and its supplied concentration. This is usually expressed as:

$$\text{Volume of antibiotic required} = \frac{\text{recommended dosage (mg/kg) x weight of fish (kg)}}{\text{supplied solution concentration (mg/ml)}}$$

3. Topical

Topical treatments are usually only necessary for more valuable individual fish, such as ornamental varieties or broodstock. Open sores or ulcers can be treated with a topical antiseptic microbicide, such as an iodine-based solution, followed if necessary by a topical antibiotic. Nevertheless, it is possible that ulcers can heal themselves with improved water quality and the elimination of parasites.

4. Baths and dips

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 antibiotic being used. The method of application can therefore be used for surface infections such as fin rot, bacterial gill disease, superficial fungal infections and ectoparasitic infestations. Another disadvantage with bath type treatments is that a lot more antibiotic is required when compared with oral (feed) treatments or injections. Bath treatments are also not recommended for recirculation systems or aquarium systems using biological filters. Consequently, fish treatment by bath usually uses a separate container, tank or simply with the flow stopped. Additional aeration may also be required for bath treatments. Accurate calculation of the volume of water in the tank, pond or cage is also required (Bruno and Munro, 1991).

VIII – Fin fish

In Europe, the main aquaculture products are finfish and molluscs. The farmed marine finfish species are salmon, sea bass, sea bream, trout, turbot, tuna, sole, halibut, European eel and cod. Sea cage fish farming is largely confined to salmon in Norway, Scotland and Ireland, as well as sea bass and sea bream in Greece, Italy and Spain. Also worthy of note in recent years is the rise in the production of tilapia and mullet largely from countries such as Egypt.

The main fish diseases requiring treatment in the Mediterranean region are bacterial and parasitic (Rodgers and Furones, 1998; Toranzo and Barja, 2002). Examples include vibriosis, pasteurellosis, flexibacteriosis, ectoparasitic protozoa (flagellates: *Amyloodinium ocellatum*, *Ichthyobodo* sp.; ciliates: *Crytocaryon irritans*, *Trichodina* spp.), endoparasitic protozoa (*Eimeria* spp., *Ceratomyxa* spp.), ectoparasitic metazoa and crustacea (*Dactylogyrus* spp., *Diplectanum* spp., *Caligus* sp.) and epitheliocystis (*Chlamydia* sp.). Table 5 shows main aquaculture drugs and their use by species and treatment.

Table 5. Examples of aquaculture drugs and their use by species and treatment

Aquaculture drug examples	Species	Treatment/control
Chemotherapeutants		
Tetracyclines	Finfish and lobster	Bacterial diseases
Sulphonamides (incl. potentiated)	Finfish	Bacterial diseases
Quinolones	Finfish	Bacterial diseases
Parasiticides/fungicides		
Acetic acid	Finfish	Parasites
Cypermethrin/dichlorvos/emamectin benzoate/pyrethrum	Salmonids	Sea lice
Formalin	Finfish, finfish eggs and crustaceans	Protozoa, monogenean trematodes and fungi
Hydrogen peroxide	Finfish and eggs	Fungi
Iodine	Finfish eggs	Surface disinfectant
Sodium chloride	Finfish	Parasites and osmoregulatory aid
Hormones†		
Chorionic gonadotropin	Finfish broodstock	Spawning aid
Anaesthetics†		
Tricaine methanesulfonate (MS-222)	Finfish	Anaesthesia
Carbon dioxide	Finfish	Anaesthesia
Flesh colour enhancers†		
Astaxanthin/canthaxanthin	Salmonids	Enhancement of flesh pink colour

†These compounds are usually defined as drugs for approval purposes although they are not all strictly antimicrobial agents.

1. Antibacterials

As in other animal production sectors, antibiotics are used in aquaculture during both production and processing, mainly to prevent (prophylactic use) and treat (therapeutic use) bacterial diseases (FAO, 2002). The three main groups are the tetracyclines, sulphonamides and quinolones, although others, such as florfenicol (similar to chloramphenicol) and amoxycillin, are also available. Nevertheless, authorisation of these compounds varies between countries.

2. Antiparasitics

Due to the large-scale production and economic importance of salmon in certain countries, such as Canada and Scotland, there has been a need to find suitable treatments for diseases caused, for instance, by parasites. One of the common problems to the industry is infestation of salmon by sea lice parasitic copepods (e.g. *Lepeophtheirus salmonis* and *Caligus elongatus*), which occur naturally and feed on salmon mucus, skin and blood of salmon (DFO, 2000). They cause lesions, reduced growth and mortalities. Infestations have been treated by a variety of bath treatments, including hydrogen peroxide, pyrethrins, dichlorvos or azamethiphos (Haya *et al*., 1999). Ivermectin, administered through fish feed, has also been used to deal with sea lice (DFO, 2000). Other compounds are also used in Scotland, which include azamethiphos and cypermethrin as bath treatments, and emamectin benzoate and teflubenzuron as in-feed treatments (SECRU, 2002).

IX – Molluscs

There are no antimicrobial agents available for treatment of molluscan diseases. The majority of diseases affecting molluscs are viral or parasitic and it is not viable to use any antimicrobial agents in open water culture (Table 6). In addition, the molluscan parasitic diseases are often intracellular (e.g. *Bonamia ostreae*) and there are simply no treatments available. It may be possible to observe behavioural changes in some stocks, particularly broodstock and larvae in hatcheries, and detailed record keeping (e.g. water parameters such as temperature and salinity, etc.) may also be helpful for manipulating local environmental conditions. This is really only feasible though under controlled hatchery conditions, since disease can break out very quickly in susceptible stock. Feeding behaviour of larval stages may also give early indication of health problems. Signs of weakening (e.g. gaping shells) in juvenile or adult stages can also be used to predict potential problems, as can decreased movement in motile species (e.g. scallops, clams).

Specific alternative control measures are therefore required for molluscs. These generally include reduced stocking density, altered salinities and lowered water temperatures, as well as prevention of introduction or prohibited transfer of shellfish from known enzootic areas. The development of resistant stocks of oysters, particularly for the potential control of *Perkinsus* spp. or *Haplosporidium* spp., has also been suggested but the possibility of creating sub-clinical carriers of the pathogens could be an additional problem with such stocks.

X – Crustaceans

Apart from only two or three exceptions, virtually all major crustacean diseases have a viral aetiology. Consequently, despite being reared largely in ponds, there are no known antimicrobial agents available for treatment of such diseases. Although gross observations of clinical signs can be made they may not be sufficient for diagnosis. However, they can be useful for management of disease outbreaks designed to reduce losses or disease spread. The options available include destruction or isolation of infected stocks and modification of on-farm husbandry procedures (Table 7).

The lack of treatments for viral shrimp infections means that preventative measures are the most useful for reducing disease spread. These include broodstock screening programmes and disinfection of farm equipment or pond facilities. However, stock destruction using excess chlorine and pond disinfection with quick lime may be the only solution in some cases. As with molluscs, prevention of introduction or prohibited transfer of crustaceans from known enzootic areas may also be required.

Treatment of shrimp with oxytetracycline in medicated feed following early diagnosis of necrotising hepatopancreatitis (NHP) is a minor example of a specific treatment for a bacterial disease in crustaceans. However, *Vibrio* spp., especially the luminous *V. harveyi*, are implicated as the main bacterial pathogens of shrimps. Although antibiotics have been used to try and control the Vibriosis problems, there has been developing resistance and the efficacy of treatments is now generally low. In many areas though, the *Vibrio* species are resistant to antibiotics such as chloramphenicol, furazolidone, oxytetracycline and streptomycin.

Oxytetracycline has been indicated as a treatment for the bacterial disease gaffkaemia caused by *Aerococcus viridans* in the lobsters *Homarus americanus* and *H. gammarus*.

Table 6. Some molluscan diseases with their host species and treatment or control measures

Molluscan disease	Causal agent	Host range	Treatment/control[†]
Viral			
Iridovirosis (Oyster Velar Virus Disease)	*Iridoviridae*	*Crassostrea gigas*	No treatment – reduced stocking densities, improved water exchange, general hatchery disinfection
Herpesvirosis (Oyster Herpes-like Virus Disease)	*Herpesviridae*	*Ostrea edulis* and *Crassostrea gigas*	No treatment or control
Bacterial			
Brown Ring Disease	*Vibrio tapetis*	*Tapes philippinarum*	No treatment – raised salinity; experimental treatment with nitrofurans
Parasitic			
Bonamiosis	*Bonamia ostreae* and *Bonamia* sp.	*Ostrea edulis* and *Ostrea* spp.	No treatment – reduced stocking densities, lower water temperature, prevention of introduction
Marteiliosis	*Marteilia refringens*; *M. sydneyi*; *M. maurini*	*Ostrea edulis*, *Ostrea* spp., *Crassostrea gigas* and *Crassostrea* spp.; *Saccostrea glomerata* and *Saccostrea* spp.; *Mytilus edulis* and *Mytilus* spp.	No treatment – high salinity, prevention of introduction
Perkinsosis	*Perkinsus marinus*; *P. olseni*; *Perkinsus* spp.	*Crassostrea virginica*; *Haliotis rubra, Haliotis* spp. and *Tapes* spp.	No treatment – development of resistant oyster stocks, filtration and sterilization of inflow water
Haplosporidiosis	*Haplosporidium costale* and *H. nelsoni*	*Crassostrea virginica*	No treatment – development of resistant oyster stocks, filtration and sterilization of inflow water

[†]Source: FAO, Asia Diagnostic Guide to Aquatic Animal Diseases (2001).

Table 7. Some crustacean diseases with their host species and treatment or control measures

Crustacean disease	Causal agent	Host range	Treatment/control†
Viral			
Yellowhead Disease (YHD)	Yellowhead virus (YHV) Coronaviridae	*Penaeus monodon* (other *Penaeus* spp. by experimental infection; other shrimp species can be carriers)	No treatment – broodstock screening, equipment and water disinfection, stock destruction and pond disinfection
Infectious Hypodermal and Haematopoietic Necrosis (IHHN)	Infectious Hypodermal and Haematopoietic Necrosis virus (IHHNV) Parvoviridae	*Penaeus vannemei* and other *Penaeus* spp. (both natural and experimental and infections)	No treatment – stock destruction and pond disinfection
Taura Syndrome (TS)	Taura Syndrome virus Picornaviridae	*Penaeus vannemei* and other *Penaeus* spp. (both natural and experimental and infections)	No treatment – use of wild caught shrimp, selective breeding for resistant stocks, stock destruction and pond disinfection
Bacterial			
Bacterial White Spot Syndrome (BWSS)	?*Bacillus subtilis* but no causal relationship	*Penaeus monodon*	No treatment – frequent water change, avoid indiscriminate use of probiotics containing *Bacillus* spp., pond disinfection
Necrotising Hepatopancreatitis (NHP)	?Intracellular Proteobacteria	*Penaeus vannemei*, *Penaeus stylirostris* and other *Penaeus* spp.	Oxytetracycline in medicated feed and pond disinfection
Vibriosis	Luminous *Vibrio* spp. (*V. harveyi*)	*Penaeus* spp.	Oxytetracycline or quinolones in medicated feed
Gaffkaemia	*Aerococcus viridans*	*Homarus* spp.	Oxytetracycline in medicated feed or by injection
Fungal			
Crayfish plague	*Aphanomyces astaci*	*Astacus astacus*, *Astacus* spp. and *Austropotamobius* spp.	No treatment – prevention of movement of infected stock and escapees, disinfection or drying of equipment

†Source: FAO, Asia Diagnostic Guide to Aquatic Animal Diseases (2001).

XI – Needs and issues

From being seen as a "green" industry with great potential to alleviate some of the pressure on capture fisheries whilst at the same time provide a relatively cheap source of protein, aquaculture in recent years has had to endure an increasing amount of criticism. Much of this criticism has come from the environmental lobby and concerns the potential for increased pollution associated with certain types of larger scale production facilities. However, criticism has also been directed at the use of antimicrobial agents and the possible creation of antibiotic resistance and residues in fish products. Consequently, the potential hazards and risks that may affect food safety represent an important issue for aquaculture as well as other food production sectors. Food-borne trematode infections, food-borne diseases associated with pathogenic bacteria and viruses, residues of agro-chemicals, veterinary drugs and heavy-metal organic or inorganic contamination have been identified as possible hazards in aquaculture products (Garrett *et al*., 1997; Reilly *et al*., 1997). These hazards are usually associated with the aquaculture habitat, the species being farmed, the general condition of the local environment, and cultural habits of food preparation and consumption (Howgate *et al*., 1997).

In general, aquaculture in inland waters carries a greater risk of contamination from agro-chemicals, while aquaculture in estuaries is more susceptible to contamination from industrial pollutants. Two classes of industrial pollutants are of concern with respect to human health, namely, heavy metals and chlorinated hydrocarbons.

The use of chemotherapeutants to treat fish for control of diseases and parasites is of concern, as is the use of biocides for reduction of pests and biofouling, particularly from the point of view of the potential creation of food residues and the effects of these residues on human health. Antibiotic resistance is also an issue because of the potential for mutation or the acquisition of resistance genes that could transfer between bacteria and different ecosystems. Unfortunately, the problem of such resistance has been known for almost as long as the occurrence of antimicrobial agents and it has been recognized as a severe limitation to their use. The creation of increased selection pressure leading to antibiotic resistance can occur by overuse or misuse in human medicine, livestock, agriculture, horticulture and aquaculture.

1. Overuse of antibiotics

There is no doubt that the overuse and/or misuse of antibiotics in humans are contributing factors to the development of antibiotic resistance (EMEA, 1999; WHO, 2000; Levin, 2001; UCS, 2002), which are related to the prescribing practices of health workers and to medication-taking practices of patients (WHO, 1997).

Antimicrobials have been increasingly used in efforts to control disease and promote growth of livestock. Nevertheless, in recent years there has been a move away from the use of antibiotics as growth promotants in countries such as the UK, Sweden and Denmark. The European Union has also made moves to ban antibiotics for growth promotion in food animals and the WHO also recommend that use of antimicrobial growth promoters should be terminated or rapidly phased-out (WHO, 2001). Antibiotics can be used in livestock production in sub-therapeutic doses to favour growth or in low-level doses for prophylactic purposes, as well as in therapeutic doses to treat infections. Unfortunately, the non-therapeutic use of antimicrobial agents is also considered to have led to an increase in resistant bacteria. Antibiotics are also used to treat fruit trees and fruit for prevention of bacterial diseases.

In some areas there is misuse of antibiotics in aquaculture to control diseases, which is directly related to increasing or maintaining production. For instance, the illegal use of chloramphenicol or nitrofurans in some Asian countries for controlling shrimp diseases has been highlighted recently by the EU through the bans placed on imported shrimps containing unacceptable residues from China (EC, 2002a), as well as Thailand, Vietnam and Myanmar (EC, 2002b).

Chemicals such as tributyl tin used to control pond organisms (e.g. snails) may also be a problem that could lead to residues.

XII – Avoiding antimicrobial agents

Is it possible to manage diseases without the use of antimicrobial agents? The methods used to combat diseases in aquaculture enterprises should not rely solely on antimicrobial agents but they should also incorporate alternative control measures. There are instances, of course, where the use of an antimicrobial agent is not practical or indeed where they are simply unavailable, particularly for viral diseases and the treatment of molluscan or most crustacean diseases. Apart from the control measures outlined in Tables 6 and 7 (molluscs and crustaceans, respectively), there are other ways of controlling fish diseases, particularly those with a bacterial aetiology. These include good husbandry, adequate feed composition, vaccines, water treatment, fallowing marine cage sites, applying integrated pest management, movement restrictions and biological control (e.g. probiotics). Such alternative strategies for disease control have already been used successfully in some areas of aquaculture. For instance, Norway reduced its reliance on antimicrobial agents by almost 98% (from 50 metric tons per year to 746 kg) in the ten year period from 1987-1997, whilst at the same time increasing farmed fish production by approximately 14%. Such a decrease was achieved through the development and use of effective vaccines (Markestad and Grave, 1997; Brekke, 1998). The UK has also shown a decline in the sales of antimicrobials used in aquaculture in combination with increased production. It has been suggested that this reduction is due mainly to improved husbandry techniques and the use of vaccines (Veterinary Medicines Directorate, 2002).

There is a hypothesis of disease propagation that demonstrates that the cause of all disease is stressor overload (Wilken, 2002). Consequently, the reduction of unnecessary stress is the main aim of any satisfactory programme of improved health, whether it be human, animal or fish. However, elimination of all stressors is virtually impossible and, therefore, reduction of only the unnecessary stressors, the so-called destructive stressors, should be attempted.

In general, the FAO have defined the development of affordable vaccines, the use of immunostimulants and non-specific immune-enhancers, and the use of probiotics and bioaugmentation for the improvement of aquatic environmental quality as major areas for further research in disease control in aquaculture (Subasinghe, 1997), which would help to reduce the use of antimicrobial agents.

1. Husbandry practices

Fish need to be reared under pristine conditions to avoid any problems with disease. Unfortunately, modern production cycles relying on intensive techniques often lead to lowered water quality and raised stocking densities. This combination is known as a prerequisite for disease (Austin and Austin, 1999). Consequently, management practices have to consider the optimum conditions for parameters such as feed rates, water dissolved oxygen, stocking densities and even controlled temperature, where this is feasible. In fact, warmer water marine cage culture probably benefits greatest from health management through good husbandry rather than reliance solely on therapy. Early diagnosis of problems is also important, since the main disease problems for Mediterranean aquaculture producers are monogenean parasites, streptococcosis, pasteurellosis, mycobacteriosis and viral nervous necrosis, which can be difficult to control if they become established. In addition, these diseases are neither notifiable nor are they specified by the OIE guidelines or current EU legislation.

2. Feed composition

The formulation of fish diets is fundamental for the provision of proteins that are used to produce maximum growth, and the feed cost can represent up to 50% of the total operational costs of a fish farm. There is evidence that fish health can be related to diet and many studies have shown the potential importance of dietary factors such as vitamins and trace elements for controlling infections or avoiding signs of nutritional deficiency (e.g. bone deformation or cataracts). Therefore, it is clear that feed composition plays an important role in fish health maintenance. Nutritional status is considered one of the important factors that determines the ability of fish to resist diseases, since nutritional and physical characteristics of diets can modulate susceptibility of fish to infectious diseases (Lall, 2000). However, it is a complex issue and a better understanding of the mechanisms through which nutrition influences the immune system is still necessary. Immunomodulators that enhance the function of phagocytic cells have potential application for those cases where vaccines have been shown to give only partial protection. Examples include glucans, chitin, levamisole, peptidoglycans, algal-derived polysaccharides and some vitamins (e.g. vitamin C).

3. Vaccination

The development of fish vaccines can be realistically traced back for more than 60 years to the work of Duff (1942), who inactivated *Aeromonas salmonicida* with chloroform. Development since then has led to commercial availability of vaccines against other bacterial fish pathogens and protection against diseases such as furunculosis, enteric redmouth, vibriosis, edwardsiellosis and pasteurellosis, as well as promising preparations against streptococcosis. It is probably also true to say that it is possible to produce "autovaccines" against almost any other bacterial fish pathogen. However, there is still some work needed to improve vaccines, particularly to increase protection levels and optimise delivery methods, especially for warmer water or tropical species. Nevertheless, currently, vaccination has a major prophylactic role in protection of fish against diseases. Enhancing the non-specific defence mechanisms of the host by immunostimulants using an adjuvant effect is also a promising area, possibly in combination with vaccines, although administration methods can still be problematic.

The development of vaccines for protection against viral fish vaccines has met with less success and, in fact, there are few preparations currently available.

4. Water treatment

Application of disinfectants to water can be used in certain circumstances for disease control or sterilization. Chemicals or derivatives used include chlorine, formalin, iodophors, ozone and the application of ultraviolet light.

5. Movement restrictions

Historically, in many cases, the spread of diseases has been related directly to the movement of infected stocks. Prevention of disease spread can therefore be avoided by the application of movement restrictions, which are usually enforced by legislation in the case of notifiable diseases. This takes in the concepts of health certification programmes and disease zoning as it relates to facilitation of trade. Different diseases may, of course, have different means of spreading and therefore the delineation of zones (e.g. free zones, surveillance zones and infected zones) can differ depending on the disease concerned. The standard setting organization for aquatic animal health is the Office International des Epizooties (OIE) and the guidelines for zoning are contained in the OIE International Aquatic Animal Health Code (OIE, 2002). By way of example, the European Union has translated these concepts into legislation through Council Directive 91/67/EEC, which is concerned with the animal health conditions governing the placing on the market of aquaculture animals and products. A more graphic example can be seen in the OIE International Database on Aquatic Animal Disease that contains information on EU approved zones by country, farm and disease (http://www.collabcen.net/toweb/aq2.asp). However, none of the diseases that affect Mediterranean aquaculture are notifiable and the concept of movement restrictions cannot therefore be easily applied in this important production region.

6. Biological control

There is some evidence that natural extracts of seaweed have anti-tumour activity in mammals, and that polysaccharides (Fujiki *et al*., 1997) or other substances (Fujiki and Yano, 1997; Skjermo *et al*., 1995) obtained from seaweed can enhance disease protection in fish.

Integrated pest management is also a good example of using biological controls. One example has been the use of wrasse to reduce sea lice populations in salmon cages.

The use of beneficial bacteria (probiotics) to displace pathogens by competitive processes is being used in the animal industry as a better remedy than administering antibiotics and is now gaining acceptance for the control of pathogens in aquaculture (Havenaar *et al*., 1992; Moriarty, 2000) or for improving water quality. Their mechanism of action is unclear but may include competitive exclusion of pathogenic bacteria, production of inhibitory substances, provision of essential nutrients for the cultured animal, provision of digestive enzymes and direct uptake or decomposition of water-borne organic matter. If the application of probiotics can decrease the use of antimicrobial agents they will have excellent potential application in improving aquaculture in the future. *Vibrio alginolyticus* has been employed as a probiont in many Ecuadorian shrimp hatcheries since late 1992 (Griffith, 1995) and the addition of probiotics is now also a common practice in commercial shrimp hatcheries in Mexico (Rico-Mora *et al*., 1998). Evidence is accumulating that the health and zootechnical performances of many cultured aquatic species can be improved by the prophylactic use of probiotics, although there is a scarcity of data about their effective practical implementation and their exact modes of action (Verschuere *et al*., 2000). Disturbances in the normal microflora can be caused by several things, one being the administration of antimicrobial agents, particularly in the intestine. Probiotic microorganisms are thought to counteract such disturbances and thereby reduce the risk of colonization by pathogenic bacteria (Tannock, 1999).

XIII – Conclusion

In general, at least for the USA and Europe, there are a limited number of antimicrobial agents in common veterinary usage. Those that are available are controlled by legislation but they are mainly for therapeutic use and are not, as in the agricultural field, for prophylactic use or growth promotion. There is good availability of vaccines for bacterial diseases and they have resulted in reduced use of antimicrobial agents, although there is still a need for improvements in delivery methods and efficacy, as well as the development of viral vaccines. Additional control measures such as good husbandry, adequate feed composition, movement restrictions, immunostimulants and biological control could contribute to reduced antimicrobial usage throughout the aquaculture industry.

Many disincentives also exist to curtail antibiotic usage in aquaculture, including the increasing marginal cost-effectiveness of their use in disease treatment, the increasing need to be HACCP compliant, as well as the growing concern over the negative impact that some seafood products have had recently in connection with antibiotic residues. If these factors are taken into account and the potential problems related to the use of antimicrobial agents can be overcome, then a positive outlook could be generated for reduced reliance on antibiotic-based management strategies in the future.

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