

# Antibacterial Chemotherapy in Aquaculture: Review of Practice, Associated Risks and Need for Action

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## ABSTRACT

This paper briefly reviews the use of chemicals to prevent and treat bacterial diseases in aquaculture, and provides a detailed summary of the current state of knowledge on the development of bacterial resistance to antimicrobial agents in fish and shellfish. The topics covered include mechanisms of resistance, resistance of bacterial fish pathogens, resistance to antibacterial agents associated with use in aquaculture, and factors causing selection of resistant variants. Emphasis is placed on avoiding and solving problems related to bacterial resistance in aquaculture, and recommendations on antibiotic usage in aquaculture are made.

## INTRODUCTION

Interactions leading to bacterial disease in fish depend on the availability of the pathogen, the quality of the environment and the general health status of the fish. Balance of these conditions can ensure fish health without the use of antibacterial agents.

Health is promoted by ensuring good water quality, optimizing stocking densities and providing balanced nutrition. It is very important to eliminate highly specific pathogens from the stock and system (e.g., *Renibacterium salmoninarum*) or, in the case of opportunistic pathogens such as vibrios or motile aeromonads, to reduce the bacterial load. Resistance to disease can be enhanced by use of specific vaccines, where they are available, or more generally, by non-specific stimulation of the innate defenses.

However, aquaculture is driven by commercial forces, and stocking densities and rearing conditions are adjusted to maximize returns within the limits of acceptable risk. Within this scheme antibacterial agents are used widely. They are used both prophylactically, at times of heightened risk of disease, and therapeutically, when an outbreak of disease occurs in the system.

## ANTIBACTERIAL CHEMOTHERAPY IN AQUACULTURE

### History of Use

Antibacterial chemotherapy has been applied in aquaculture for over 50 years, with early attempts to use sulphonamides in the treatment of furunculosis in trout and the tetracyclines against a range of Gram-negative pathogens. However, they didn't come into general use until the 1970s when the sulphonamides were used, potentiated with trimethoprim. Since then, their use has grown, both in numbers and quantity, as the problem of bacterial disease has increased.

The potential of most veterinary antibacterials for use in aquaculture has been considered, and now countries vary widely in the drugs they use in their aquaculture systems. More details of their use throughout Asian countries are given by other contributors to this volume.

## Methods of Application

Antibiotic usage in aquaculture is predominantly by three methods of administration, namely:

- a) oral therapy (in feed)
- b) immersion therapy (bath, dip, flow or flush) or
- c) injection.

Topical therapy, by ointments, sprays or brush, is also used for valuable individual fish or broodstock, but the most commonly used methods are those given above. Combination therapy i.e., oral and bath, is used in some situations.

Medicated feed is usually prepared on site by mixing the drug with pelleted feed and surface coating with an agent such as oil, gelatin or whole egg, or simply mixing with trash fish. Alternatively, the drug may be incorporated by the feed mill where commercial diets are prepared. The main advantage of oral therapy is that it does not stress the fish. The disadvantages are that this route is unavailable when fish are anorexic, a clinical sign often present when fish are sick and, moreover, leaching of the drug from the feed may occur prior to ingestion.

Immersion therapy, commonly used for problems involving ectoparasites, is used less often to treat bacterial disease; however, land-based hatcheries and tank systems, especially marine finfish hatcheries, do use antibiotic baths. These usually last 1-2 h, but more prolonged baths are not uncommon. This method is employed where the biomass is small, such as with fry, and when adequate oral therapy is impractical, as with larvae. Tank water volume is usually reduced, and consequently, the amount of drug required is reduced. The discharge of such treated water, however, poses an environmental threat that should not be dismissed.

Injection of antibiotics, usually by interperitoneal or intramuscular routes, has been utilized historically for individual fish or valuable broodstock. Recently, there has been increased interest in this method as an effective means of clearing bacterial infections from carrier fish or in conjunction with vaccination to confer protection before the immune response is mounted (Inglis *et al.* 1996).

## Concerns About the Use of Antibacterials in Aquaculture

Antibacterial chemotherapy has been a cornerstone upon which the aquaculture industry has been built. In the growing industry there were many outbreaks of disease, as wild species were first kept in captivity and before the full significance of environmental aspects of health control was appreciated. Initially, field developments outstripped the rate at which the body of scientific knowledge underpinning applied chemotherapy was being gathered, and the use of antibiotics was essential in preventing the commercial collapse of many aquaculture enterprises. At first antibacterial chemotherapy was highly successful, possibly to the extent that drugs were relied upon to increase yields and obviate more costly disease control strategies. Unfortunately, this has led to problems, and concern is now centred on treatment failures, environmental impacts and risks to human health.

Antibacterials may disturb the balance of the environmental microflora, and this is the subject of a later paper (see Weston, this volume). The risk posed to human health by disturbance of the gastrointestinal flora, selection of resistant strains and allergies is also addressed elsewhere (see Sinhaseni *et al.*, this volume).

Treatments may fail for several reasons, but probably the most consistent and fundamental cause of their failure is the emergence of resistant bacteria. This paper will deal with antibacterial chemotherapy and associated resistance.

## RESISTANCE

### Mechanisms of Resistance

An improved understanding of how resistance emerges and is selected for among bacteria is essential in evaluating the impact of aquacultural use of antibacterial agents, identifying the high risk procedures and designing ways to reduce these effects. Bacteria acquire resistance by acquisition of foreign DNA or by modification of the chromosomal DNA. Examples of both are found among bacterial fish pathogens, and these are well illustrated in relation to the tetracyclines and the quinolones. A brief consideration of these compounds is helpful in elucidating the causal relationship of drug use and emergence of resistance and in planning intervention strategies to reduce negative effects.

*Tetracycline resistance.* Firstly, there is evidence that in evolutionary terms, the origins of tetracycline resistance is remote. Tetracyclines are produced by species of *Streptomyces* (which produce numerous other groups of antibiotics) possessing tetracycline resistance determinants. A popular theory is that resistance determinants originate in such organisms and were then disseminated by interspecies transfer by a variety of routes (Chopra 1985). DNA encoding resistance may be transferred by plasmids, conjugative transposons, and bacteriophages, as well as free DNA. Plasmid-mediated resistance can occur with a high transfer frequency. It may be expressed by extrachromosomal replication of the plasmid with subsequent opportunities for spread within the species or to other genera; or it may be transferred to the chromosome where it becomes integrated. Particularly in the latter case, when the selection pressure is removed, the potential for expressing resistance remains.

It has been suggested (Levy 1989) that tetracycline resistance has been evolving for a very long time (millions of years), perhaps in response to competition with organisms producing tetracycline-like substances. The use of tetracyclines in human and veterinary medicine has been relatively recent, yet resistance factors were found before use was widespread and from remote locations.

*Quinolone resistance.* A major group of antimicrobial agents used in aquaculture, but which has been synthetically produced, is the quinolones. Transferable resistance of the type described has not yet been recorded (Courvalin 1990). Nevertheless, resistance to these agents does arise and increases rapidly under pressure of their use. Quinolones kill bacteria by interrupting the DNA supercoiling (Hooper and Wolfson 1989). The DNA repair mechanisms may then cause mutations coding for resistance (Lewin *et al.* 1990). Laboratory evidence suggests that these mutations are stable. The mutants survive well and may grow to produce a dominant sub-group. In this case, use of the drug has been the cause of resistance developing where it had not been before. The long-term effect on the environmental microflora is not known, and the full implications of this are not yet realized.

*Expression of resistance.* Laboratory studies have shown that expression of resistance is selected against in the absence of the drug (Lee and Edlin 1985, Modi *et al.* 1991). The term "persistence" has been used (Bryan 1989) to describe the form of resistance only manifested in the presence of the antibiotic. "Persistent" strains are detected only during and shortly after therapy. They then recede but remain in the environment until they emerge under positive selection pressure. This is of particular importance when sensitivity is determined at the outset of an epizootic and therapy introduced to which the infecting strain rapidly becomes resistant. This mechanism may also affect findings with laboratory collections that have been cultured for some time *in vitro* before

minimum inhibitory concentrations are determined (Smith *et al.* 1994).

Tetracycline resistance has been found to survive in the microbial flora of farm animals years after tetracycline has stopped being used in the feed (Smith 1975). Such persistence within an ecosystem suggests either the continuing presence of the tetracycline or else that the intrinsic deleterious effect of encoding the resistance has been attenuated.

The use of antibacterial agents may result in mutations to resistance among bacteria as well as the selection of resistant variants that are already present in the environment. Under positive selection pressure of drug use, they will increase in relative proportion. When the drug is withdrawn they may recede but are unlikely to disappear.

## Resistance of Bacterial Fish Pathogens

*Interpretation of results.* Resistance is a relative term allowing comparison of variants within a strain or between species. It is determined for fish pathogens, as more generally, *in vitro*, and the numerical value of a zone size in a disk diffusion test or end-point in a serial dilution test translated into resistant, moderately resistant or sensitive. Many other methods are available (see Piddock 1990) and include measurement of a range of bacterial activities such as pH change, bioluminescence, electrical conductivity or impedance. Results are affected by between-laboratory variation in techniques, but more importantly, by variation in interpretations of results. With some drugs (e.g., oxytetracycline), many groups of bacteria display a clear bimodal distribution of sensitivity, and classification into sensitive or resistant is easy. Problems arise with strains designated of intermediate sensitivity, as happens when resistance is increased in small steps (Inglis and Richards 1991). The problem of differences in the interpretation of results has been well exemplified in a between-laboratory study involving six countries in Europe. However, while it should be possible to overcome this source of error within a laboratory or groups of co-ordinated workers by use of standardized techniques, and to achieve comparable results and classification of the same group of bacteria, this alone would be insufficient to predict clinical efficacy. Culture conditions also must be considered.

*Cultural conditions in determining sensitivity.* The environment of a pathogen in artificial culture conditions and in clinical use differs, and as a consequence, the concentrations required to inhibit or kill in the two situations may differ. Laboratory media, especially those designed for antimicrobial sensitivity testing, do not simulate *in vivo* conditions. The biological activity of oxolinic acid and oxytetracycline is reduced in the presence of  $Mg^{2+}$  and  $Ca^{2+}$  so that the efficacy of these agents in fish in sea water is much lower than in fresh water (Barnes *et al.* 1995). The presence of buffers, availability of iron and incubation temperature may all be different from the *in vivo* situation, and have an effect on the outcome (Inglis *et al.* 1991, Martinsen *et al.* 1992). The condition of the bacterial inoculum, which has been grown in the laboratory on artificial media, subjected to centrifugation etc., is also different from that *in vivo*.

*Clinical relevance.* Determination of *in vitro* sensitivity is required to be reliable, not only to allow detection of resistance changes, but also to be a good predictor of clinical efficacy. In medicine, prediction of efficacy is based on the minimum inhibitory concentration (MIC), pharmacokinetics and clinical experience. If the lowest MICs of sensitive strains are low, the prediction of a good clinical outcome can be made with considerable confidence. With intermediate resistance, the laboratory prediction of the outcome of a clinical efficacy is less reliable.

A major factor affecting clinical outcome is the concentration of the antibacterial agent, in its active form, that is achieved at the site of infection. This is further influenced by the terminal half-life of the agent and the total amount present during the dosing period. While many sites within the animal can become infected, in the case of fish the window of opportunity for treatment may be restricted to the pre-clinical stage, when it is still possible to deliver an effective dose by feeding.

## Resistance to Antibacterial Agents Associated with Use in Aquaculture

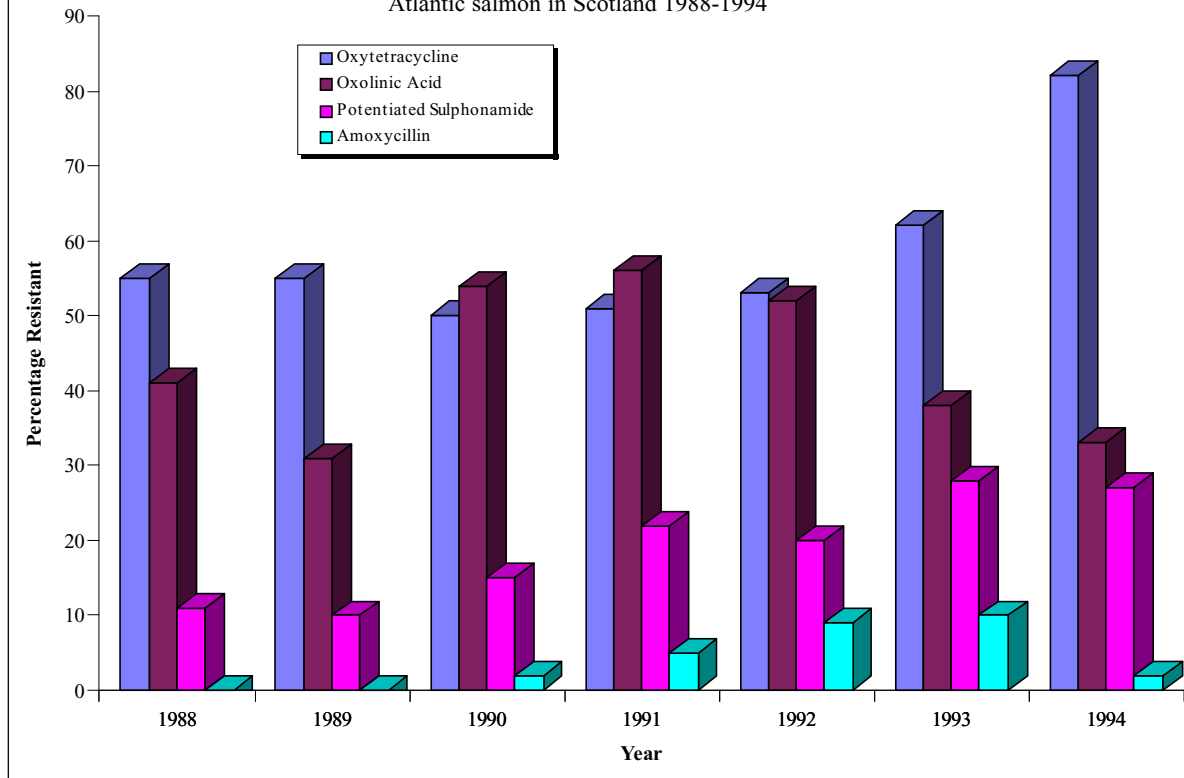
*Frequency of drug use and emergence of resistance.* There is widespread concern that the use of antibacterial agents in aquaculture has led to the emergence and selection of resistant bacteria. In general terms, it is agreed that antimicrobial resistance is associated with frequency of use in an environment, and there are several studies that illustrate this (Hamilton-Miller 1990, Kruse 1994). In food-producing animals kept under intensive conditions, common pathogens emerged with resistance against commonly used drugs. Increased frequencies of resistance to penicillin of *Staphylococcus aureus* causing mastitis on dairy farms (Prescott and Baggot 1988) and resistance of *Escherichia coli* from pigs to sulphonamides, streptomycin and tetracycline has been reported. The response in these industries has been to move from one drug to another as resistance catches up. In aquaculture, it is reasonable to assume that a similar thing has happened: that the increased use of antibacterial agents has led to an increase in the incidence of resistance among relevant pathogens.

*Emergence of resistance to new antibiotics.* A causal relationship between use of drugs and selection of resistant mutants can be inferred from first reports of resistance to drugs newly introduced to aquaculture. The history of the use of quinoline, oxolinic acid, in Europe is well documented. It was identified as being very useful in the control of furunculosis in salmonids in Europe in 1983 (Austin *et al.* 1983), although it had been used earlier in Japan. Initially it was very effective in treating furunculosis in Scotland, but in 1987 the first outbreaks occurred which failed to respond to therapy, and resistant strains were isolated (Hastings and McKay 1987). By the 1990s, 40-50% of isolates of *Aeromonas salmonicida* in Scotland were resistant (Inglis *et al.* 1991). Similarly, amoxycillin was not used in aquaculture in the UK before 1990. It had been used earlier in Japan, where initially the treatment of pasteurellosis in yellowtail was very successful; but resistance started to emerge in 1982 and is now widespread. In the UK, however, isolates of *A. salmonicida* taken between 1988-90 were all sensitive (Inglis *et al.* 1991, Barnes *et al.* 1994). However, three years after the introduction of the drug in 1990, Inglis *et al.* (1993a) reported a furunculosis outbreak from which resistant variants were isolated.

*Surveys of resistance.* Survey data upon which to assess the extent of the problem or upon which to evaluate intervention strategies are poor. At present, it is not possible to make direct comparisons between published information on resistance of bacterial fish pathogens because of the lack of standardization of procedures and systems to interpret results. Moreover, the composition of the sample sets of bacteria tested is often ill-defined and subject to numerous biases. Ideally, they should be statistically representative of a defined aqua-system, reflecting the geographical spread of aquaculture sites and species of fish cultured and collected with information on local drug use. More often, the set is a collection from a diagnostic laboratory where pathogens subjected to frequent antimicrobial treatments are likely to be over-represented and repeat isolates from the same site or same outbreak may be included. Awareness of these sources of error can improve, but not eliminate, bias. Other sample sets reported appear to be little more than random collections, sometimes assembled initially for some other reason, such as antigen analysis or phenotyping. With these reservations in mind, the records of established diagnostic laboratories provide useful information which gives some insight into antibiotic usage and antibiotic resistance patterns over a longer time span e.g., for Switzerland (Meier *et al.* 1992) and Germany (Schlotfeldt 1992).

A survey of the resistance of *Aeromonas salmonicida*, isolated in Scotland from Atlantic salmon with furunculosis, has been conducted, along with monitoring of the antibiotics in use in that country. Bacteria in this study came from 36 geographically separate seawater or freshwater sites distributed throughout the Scottish salmon farming industry. Between 1988 and 1992, oxtetracycline resistance was 50-55%, but more recently has risen to greater than 80%. Resistance to oxolinic acid was 50% between 1990 and 1992 but has fallen in recent years (Richards *et al.* 1992, and see also Fig. 1). There has been a slow gradual increase in resistance to potentiated sulphonamide, and some resistance to amoxycillin, but with a very low incidence.

**Figure 1:** Antibiotic resistance of *Aeromonas salmonicida* isolated from Atlantic salmon in Scotland 1988-1994



Since 1991, management practices in Atlantic salmon farming have greatly improved; effective furunculosis vaccines have been introduced for the first time and disease outbreaks and drug usage have been much reduced. Reduction in the amounts of drugs used in Norway preceded that in Scotland. By 1993, a decline in the resistance of *A. salmonicida* to oxytetracycline had already been recorded in Norway (Høie *et al.* 1992). Decline in resistance of *Vibrio anguillarum* associated with reduced drug use had previously been seen in Japan (Aoki *et al.* 1985). This appears to provide only temporary respite, however, because levels of resistance have been found to rise again when the drugs were re-introduced to the system (M. Endo pers. comm.).

Some efforts have been made to relate frequency of resistance to exposure to antibacterial treatments, but available data are not satisfactory. It has often been observed that patterns of resistance reflect patterns of use, but the choice of agents tested usually is a reflection of the agents available for use in the area the samples came from (Aoki *et al.* 1981, Takashima *et al.* 1985).

To evaluate the relationship between drug use in aquaculture and the development of resistance, it is essential that the sets of bacteria used are representative collections and that the surveys are repeated to analyze trends. In an attempt to measure the present situation in South East Asia, a project was set up with participants from five countries in the region. The aim was to assemble a representative collection of aquatic bacterial pathogens from each country. Initially, this was restricted to *Vibrio* and *Aeromonas* species. Samples were drawn from a wide range of aquaculture facilities in each country and the antibiograms of each isolate determined. Information on environmental conditions and drug use was collected simultaneously. The bacterial collection and records are

held by the Aquatic Animal Health Research Institute, Bangkok (Inglis *et al.* 1997). This provides a baseline against which changes can be measured, either on a regional basis, in which case repeat surveys will be needed each 5 or 10 years, or in local studies to evaluate the effectiveness of controlled drug withdrawals.

Although there is an insufficient database to prove a causal effect or evaluate interventions, it is generally felt among the community of scientific aquaculturists that there is a need to modify practice. While there can be no justification for delaying action while epidemiological data are being collected, it is of value to review the information available and identify what more is needed to demonstrate and quantify the causal effect, identify high risk practices and design modifications, and allow efficacy of interventions to be analyzed.

### **Selection of Resistant Variants in Aquaculture**

*Predisposing conditions.* Resistance is either caused or selected by the presence of antibacterial agents in concentrations insufficient to kill the bacteria treated, and evidence is beginning to accumulate to allow identification of the high risk practices in aquaculture.

Whether by chromosomal mutation, by DNA transfer or by selection, outgrowth of resistant variants is strongly favored by prolonged exposure to sub-inhibitory concentrations of antibacterial drug. This opportunity may arise within the fish, in the water or in the sediments in fish ponds, or below fish cages. Antibacterials may reach the aquatic environment and be deposited in sediments as a result of leaching from feed, inappetence in the fish and excretion of active metabolites. This is particularly important in relation to ubiquitous opportunistic pathogens. Sub-inhibitory levels may also occur in fish, due to insufficient dose delivery and during the elimination period. Efficacy is heavily dependent upon a sufficient concentration of the drug reaching the relevant site within the host. Little work of this kind has been done on fish. The general assumption is that a tissue concentration of 3-4 times the MIC is required to eradicate the pathogen (Stamm 1989). Since blood is easy to collect, most reported studies measure serum concentrations of drug. This may be a good indicator in generalized septicemias, but is less useful in localized infections. In effect, most treatments are prophylactic. Treatment is usually started at the first signs of disease in the population but while the majority are still unaffected and, therefore, still feeding actively. At this stage the infection may not yet be systemic, and in many diseases (e.g., furunculosis in salmonids), it is unclear where the initial site of infection is and, therefore, what is the relevant tissue for an inhibitory drug concentration.

The duration for which effective concentrations are maintained is critical in determining outcome. Little is known about drug levels attained in key tissues throughout therapy as a basis for planning the most effective dosing strategy. Two approaches have been used: pharmacokinetic studies, either following single dose administration by the intravenous, intramuscular, intraperitoneal or oral routes, or during and after a course of medicated feed. Pronounced species differences have been found and a marked effect of temperature on drug elimination and metabolism. These studies have shown that bioavailability of oxytetracycline is very low, being 0.38% for carp and 1.25% for trout after a single oral dose of 60 mg/kg, while plasma concentrations of 0.65 and 0.37 µg/mL were achieved in trout at 10 °C and 19 °C and 0.15 and 0.81 µg/mL in carp at 8 °C and 20 °C, respectively (Nouws *et al.* 1992). Very little has been done to measure drug levels during oral therapy. In a study on serum and liver concentrations of oxolinic acid in Atlantic salmon during and after a 10-d treatment with oxolinic acid at 10 mg/kg (see Tables 1 and 2), up to four fold variation was found between individuals taken at the same sampling point. Drug concentrations were highest immediately on completion of the 10-d course; the mid-course levels were similar to those after 3-d withdrawal. The effect of temperature was clearly demonstrated, in that fish treated at 15 °C achieved higher concentrations than those treated at 8 °C, but the residues persisted longer at lower temperatures (unpublished results). The effects of temperature are discussed further

**Table 1.** Oxolinic acid concentration in serum of Atlantic salmon following oral therapy at 10 mg/kg in fresh water at 8 °C.

Concentration of Oxolinic Acid in µg/mL Serum							
During Treatment (2.5 d)	Days after treatment						
	0.8	2.8	4.7	7.7	10.7	14.8	21.8
3.2	3.3	2.9	1.6	1.4	1.0	0.2	ND <sup>1</sup>
3.0	5.4	2.4	3.3	1.1	1.5	0.2	0.1
3.1	3.1	3.6	2.1	1.4	0.8	ND	ND
3.1	6.1	2.4	2.0	1.7	0.6	0.2	ND
2.7	4.8	3.7	1.2	1.8	0.6	ND	ND
	4.5	3.7	3.1	1.5	1.2	ND	ND
	4.8	3.5	3.7	1.7	0.3	0.3	ND
	6.5	3.8	3.2	0.6	1.3	0.3	ND
	11.5	3.8	2.5	1.1	0.7	0.2	ND
	7.6	4.3	4.2	1.2	0.2	0.3	ND
Mean: 3.0	5.7	3.4	2.7	1.3	0.8	0.2	
SD: 0.2	2.3	0.6	0.9	0.4	0.4	0.1	

<sup>1</sup>ND = not detected.**Table 2.** Oxolinic acid concentrations in the liver of Atlantic salmon following oral therapy at 10 mg/kg in fresh water at 8°C.

Concentrations of Oxolinic Acid in µg/gm Liver							
During treatment (2.5 d)	Days after treatment						
	0.8	2.8	4.7	7.7	10.7	14.8	21.8
7.1	6.0	4.5	2.4	1.5	0.7	ND <sup>1</sup>	ND
5.1	6.1	4.9	4.6	2.1	1.6	ND	ND
5.7	7.5	7.2	3.3	1.8	1.2	ND	ND
5.7	5.5	4.6	3.1	3.0	1.3	ND	ND
7.1	9.5	5.0	1.8	3.2	0.6	ND	ND
	9.9	8.0	5.9	3.6	1.4	ND	ND
	9.9	6.2	6.8	3.4	0.5	0.6	ND
	6.0	10.7	5.4	1.1	1.5	1.0	ND
	11.1	7.2	4.4	1.5	0.7	ND	ND
	9.7	6.6	4.9	1.4	0	0.3	ND
Mean: 6.1	6.1	8.1	6.5	4.3	2.2	0.9	
SD: 0.8	0.8	2.0	1.8	1.5	0.9	0.5	

<sup>1</sup>ND = not detected.

later, however, there is a shortage of information on tissue concentrations achieved in different species and the relationship between the dose delivered, regimens and variations within fish populations.

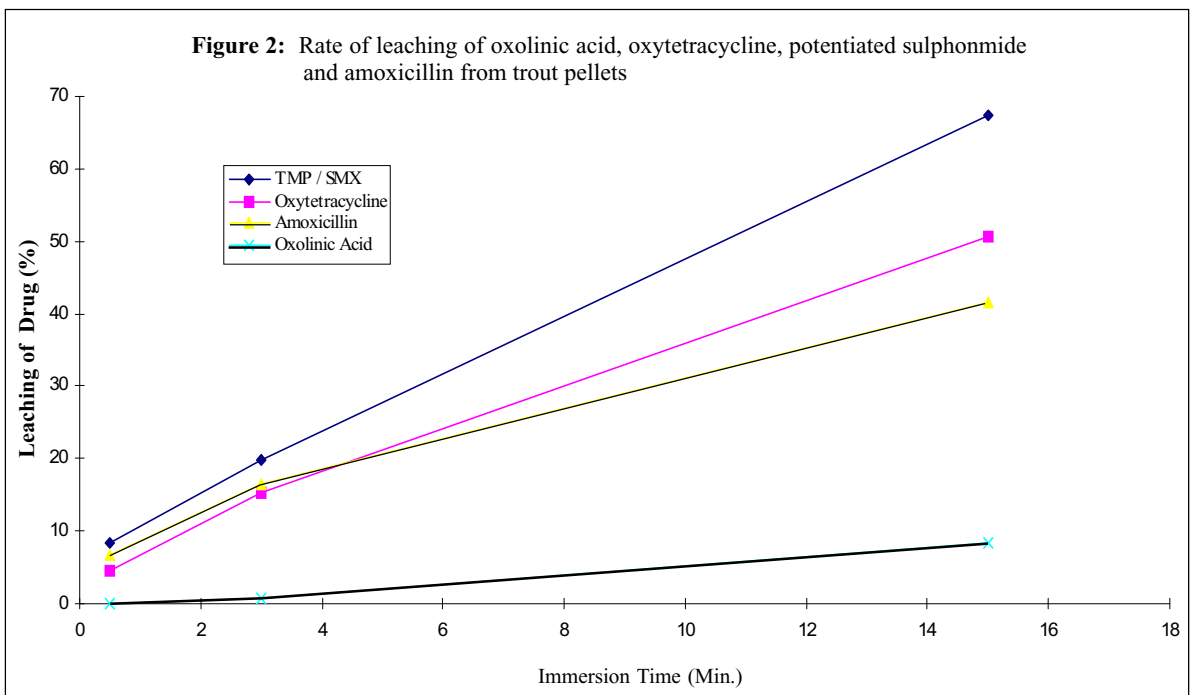


Clinical experience, added to pharmacokinetics studies and MIC data, together provide a powerful predictor of outcome. In most areas of aquaculture, information from all three sources is unavailable.

*Dose delivery.* Delivering an effective dose depends on selection of a drug to which the pathogen is sensitive and a medication system that is capable of achieving levels in the fish, for a prolonged period, in excess of the MIC. With increasingly good diagnostic laboratory services supporting aquaculture, the choice of an appropriate drug is unlikely to be a major problem provided that the national regulations allow an adequate range to choose from. With ever tightening regulations for drug registration and strict residue testing requirements in some countries, this may become difficult.

Delivery systems are, at present, a greater source of difficulty. Although dips, flushes and injections are all possible (Austin and Austin 1993), overwhelmingly, the method of choice is *per os* with drug attached to the feed (Rae 1992). Usually this is achieved by simply mixing the drug with the feed, often with a coating, such as oil, also applied. Compared with bath treatment, the amounts of drug required are much smaller and the impact on the environment much reduced. Nevertheless, using this method, drug may be lost by leaching from the surface-coated pellet as it passes through the water column. This is particularly important in species such as shrimp, which feed slowly (Goldblatt *et al.* 1980), but it is significant for all feed which remains in the water for even a few minutes before it is eaten (Fribourgh *et al.* 1969, Duis *et al.* 1995a). The leaching rate is dependent on many variables, including size of food particle, water temperature and turbulence. It varies also with the solubility of the drug. Rates of leaching of oxolinic acid, oxytetracycline, potentiated sulphonamide and amoxicillin from trout pellets are shown in Figure 2. The effect of pellet size is shown in Figure 3, where the greater surface area to volume ratio in smaller pellets results in relatively more drug loss by leaching.

More sophisticated presentation systems have been suggested to reduce these losses. The incorporation of antibiotics and chemotherapeutants into live food organisms has been suggested as an economic and environmentally friendly way of delivering drugs in larviculture (Cherel and Nin 1992, Verpraet *et al.* 1992). Bio-encapsulation in *Artemia* has been achieved with potentiated



sulphonamides and chloramphenicol (Mohney *et al.* 1990) and with quinolones (Duis *et al.* 1995b). Although there was little loss of drug from the *Artemia* before it was taken up by the fish, there was a very high level of wastage in preparing the medicated *Artemia*. This method has potential only in specialized situations, such as treatment of valuable fry. Alternative binding agents to replace oil have also been considered, for example, the efficacy of an algininate, commonly used as a thickening and gelling agent in the food industry, to surface coat antibiotics to fish food pellets was investigated (Duis *et al.* 1995a). This was effective in reducing leaching losses by up to 50% depending on the drug, in an immersion time of 15 min (see Fig. 4).

Another effective modification which reduced losses from medicated shrimp feed was a simple change to using an aqueous solution of oxytetracycline instead of a drug in the powder form to apply to the feed (Pearson and Chanratchakool 1993). While the additional steps in preparing medicated feed are often rejected *prima facie* because they involve cost and extra effort, they may well be highly cost effective. Tightly controlled field trials are necessary to develop this aspect.

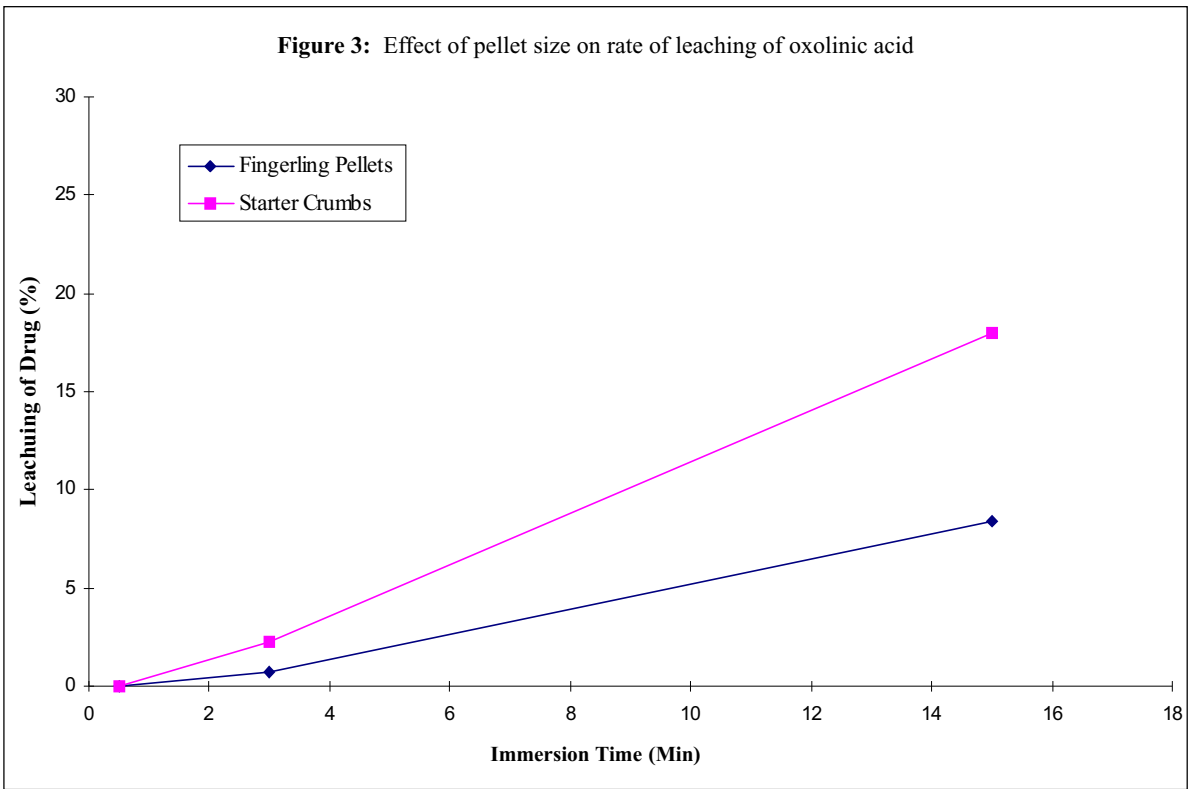
*Feeding behavior and medication.* Another factor in effective eradication of a pathogen is the quantity of medicated feed taken by individual fish. It is well accepted that feeding rates differ greatly between individual fish in a population, even when these are initially matched for size (McCarthy *et al.* 1993). A social hierarchy develops, with aggressive individuals affecting the behavior of others. This has an important bearing on the receipt of medicated feed. In studies on drug palatability, we have often observed dominant fish which restricted the feeding behavior of others in the group and, thereby, the dose of drug reaching the individual.

In the tightly controlled trial referred to earlier, there was wide variation in intake of oxolinic acid between healthy individuals. Unevenness of uptake of drug is a major factor in field treatments. In studies on amoxycillin treatment of furunculosis in Atlantic salmon, there were great differences between drug serum levels in populations of fish nominally receiving the same treatment. There were also big differences between members of the same population (Inglis *et al.* 1993b). An important factor contributing to this is the health status of the population. Inappetence is one of the first clinical signs of disease, and if treatment is not started very early in an epizootic, uptake of drug may be very low.

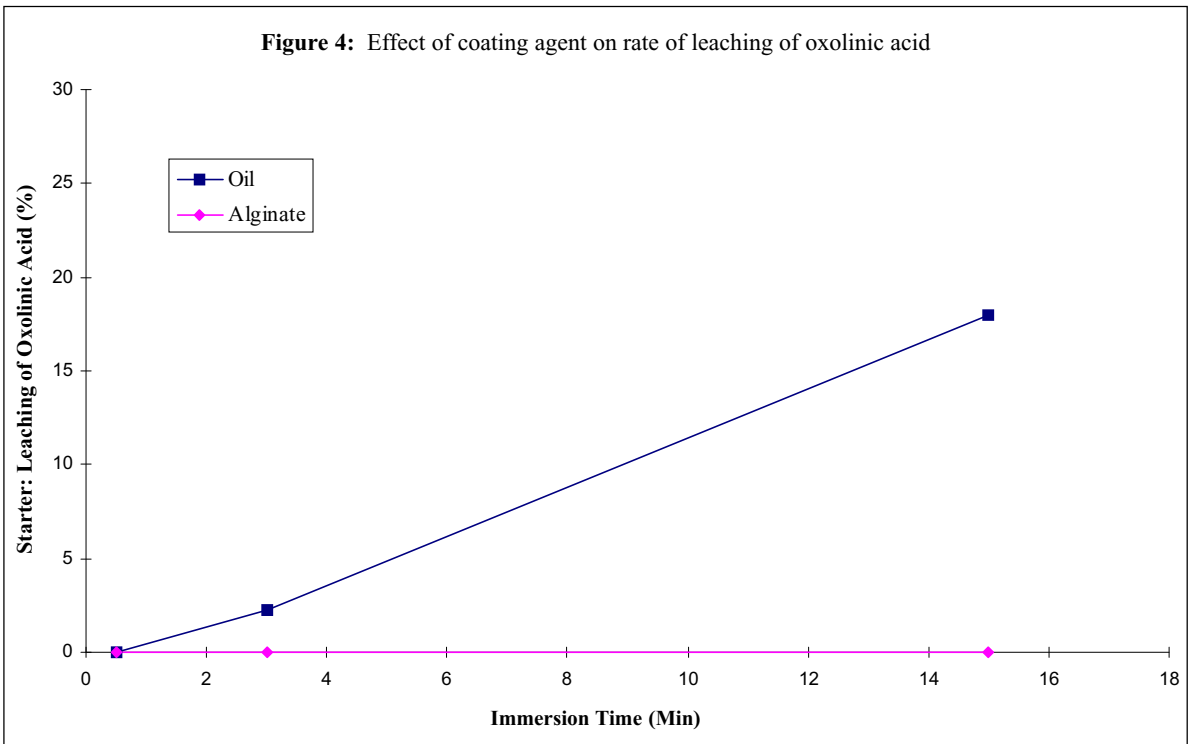
*Drug excretion.* Temperature has a marked influence on the elimination of drugs from fish, excretion being more rapid at higher rather than at lower temperature, within the range of tolerance. A widely held convention describes elimination time in degree days, this being the product of temperature in degrees Celsius and the withdrawal period in days since the cessation of treatment. Within this range, it is proposed that a 10% increase in metabolic rate should be allowed for every 1 °C rise in temperature. However, adequate data sets are unavailable for many drugs and fish species, and some studies simply give elimination rates for specified temperature ranges (e.g., Jacobsen 1989). There are distinct differences in the elimination periods required by different species; oxytetracycline residues were cleared from rainbow trout (*Oncorhynchus mykiss*) in 348 degree days (at 12 °C) and from African catfish (*Clarias gariepinus*) in 775 degree days (at 25 °C). Elimination of various antibiotics from fish has been reviewed by Ellis (1991). In conclusion, available data indicate that elimination is faster at higher temperatures, but the relationship is not always linear and moreover, differs between species. However, excretion can be very slow at lower temperatures and in Figure 5 it can be seen that, in the oxolinic acid study, excretion was slower at the lower temperature, and detectable residues of drug were present in blood and liver for 10 d. Slow excretion provides an opportunity for selection of resistant bacteria among the normal gut flora and in pathogens persisting in the host. This is a potential hazard in cold water temperature. Even at 15 °C, excretion was much faster, suggesting that this risk may be only minimal in warm-water systems.

Antibacterial agents are widely used in aquaculture, and this is likely to remain so in the foreseeable

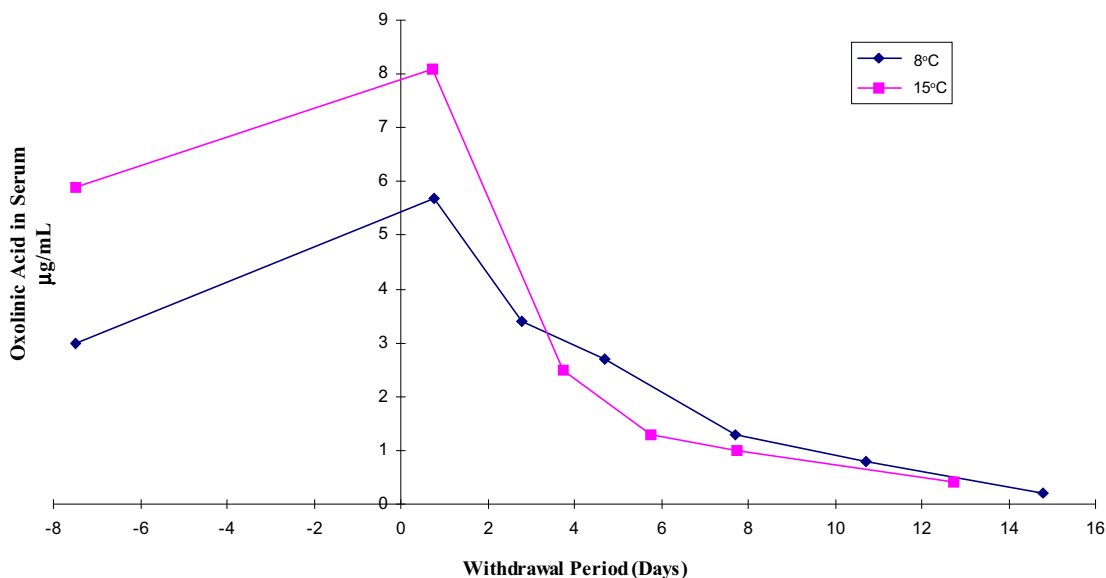
**Figure 3:** Effect of pellet size on rate of leaching of oxolinic acid



**Figure 4:** Effect of coating agent on rate of leaching of oxolinic acid



**Figure 5:** Oxolinic acid residues in the sera of Atlantic salmon in fresh water, after oral treatment with 10 µg/kg for 10 d at 8 °C and 15 °C



future. The task facing aquacultural scientists is to develop and support implementation of a code of practice to ensure the greatest benefits in efficacy and commercial terms with minimal environmental impact and damage to health.

Actual use of antibacterial agents is influenced strongly by commercial forces of cost and supply, services of veterinarians and diagnostic laboratories, available guidance on good procedure and local practice. Agreement and adoption of a scheme for antibacterial chemotherapy in aquaculture must be reached following wide-ranging discussion and negotiation for each country within the Asian Region. The following highlights the points that must be included in this discussion.

## RECOMMENDATIONS FOR ANTIBIOTIC USAGE IN AQUACULTURE

### 1. Establish the cause of the disease condition.

Can the disease be treated with antibiotics, and if it can, should they be used? The clinician responsible must decide if therapy will be worthwhile and consider what, if any, impact may result on the local environment.

### 2. Establish an antibiogram or sensitivity pattern for the pathogen.

This will ensure that an effective antibiotic is utilized. In the absence of such, the clinician must decide on the drug of choice based on previous applications, history of antibiograms in any previous isolations, drugs available and economics.

### 3. Use the correct dosage for the recommended duration.

Suboptimal dosage or duration of therapy can result in rapidly developing antibiotic resistance.

Even if a positive result is obtained before the full course of treatment is complete, the total duration of therapy should be undertaken.

**4. Adhere to careful storage of antibiotics.**

Antibiotics are subject to degradation and should be stored in a cool, dark, secure, rodent-free facility. The quality of the chemicals should be ensured by utilizing only licensed products obtained from reputable wholesalers. A high standard of hygiene should be maintained to prevent cross contamination. All antibiotics have expiry dates and out-of-date drugs should not be used.

**5. Use as narrow a spectrum of antibiotic as possible and avoid indiscriminate use of drugs, especially with live feeds (rotifers and *Artemia*).**

**6. Avoid oral therapy if fish are inappetent.**

Treatment should, however, be instituted as soon as possible.

**7. Avoid repeated use of the same antibiotic and blanket treatment for prophylactic use.**

Rotation of available antibiotics should reduce the chances of resistant organisms being selected.

**8. Antibiotic resistance patterns should be monitored as a routine.**

**9. Avoid polypharmacy.**

The only exclusion to this is if synergism is likely, as with trimethoprim and the sulphonamides.

**10. Whenever possible use products licensed for the species.**

If no licensed product is available, then use that licensed for another food-producing species. In cross-species prescription, an application of a minimal withdrawal period of at least 500 °C d is recommended.

**11. For all treatments, the prescribing clinician should record the date of examination, the clients, the number of fish treated, the diagnosis, product prescribed, dosage, duration of treatment and withdrawal period recommended.**

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