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The risks associated with the use of veterinary drugs and chemicals in aquaculture: Assessment and control

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Abstract. The use of antimicrobial agents in aquaculture can lead to both direct and indirect effects, as a result of the response of an organism to the presence of the agent. These effects can be considered in terms of the risks associated with toxicity linked to residues in human food, the development of resistance following antimicrobial therapy or the potential for environmental consequences. There is a current lack of data concerning the conditions required for selection of resistance and the movement of bacteria capable of transferring resistance between specific ecosystem compartments. The contribution of the aquatic-based use of antimicrobial agents and the corresponding non-human use of antimicrobials are compared to use in humans. The quantities of antimicrobial agents used in the agri-food sector and human medicine are not known exactly and there are problems with data standardization and validation. Some risk reduction and control measures are outlined based on the responsible use of antimicrobial agents. It is concluded that the main factors leading to the emergence of antimicrobial resistance relate to the adaptive ability of bacteria, compounded by the use and misuse of antimicrobial agents. The contribution of aquaculture to the global problem of resistance is suggested as minimal.

Keywords. Antimicrobial agents – Aquaculture – Bacterial resistance – Control methods – Residues – Risk analysis – Risks – Veterinary drugs.

Les risques liés à l'utilisation de produits pharmaceutiques vétérinaires et chimiques en aquaculture : Évaluation et contrôle

Résumé. *L'utilisation d'agents antimicrobiens en aquaculture peut mener à des effets directs et indirects, comme résultat de la réponse d'un organisme à la présence de l'agent. Ces effets peuvent être considérés en termes des risques associés à la toxicité liée aux résidus dans les aliments pour l'homme, du développement de résistance suite à une thérapie antimicrobienne ou des conséquences environnementales potentielles. Il existe un manque actuel de données concernant les conditions nécessaires pour la sélection de la résistance et le mouvement des bactéries capables de transférer la résistance entre compartiments spécifiques des écosystèmes. La contribution de l'utilisation aquacole d'agents antimicrobiens et l'utilisation correspondante d'antimicrobiens en dehors de l'homme sont comparés à l'utilisation humaine. Les quantités d'agents antimicrobiens utilisés dans le secteur agroalimentaire et celui de la médecine humaine ne sont pas connues avec exactitude et il y a des problèmes quant à la standardisation et la validation des données. Quelques mesures de réduction et de contrôle des risques sont soulignées, basées sur une utilisation responsable d'agents antimicrobiens. Il est conclu que les principaux facteurs menant à l'apparition de résistance antimicrobienne sont liés à la capacité adaptative des bactéries, aggravée par l'utilisation et le mauvais usage d'agents antimicrobiens. La contribution de l'aquaculture au problème global de la résistance est suggéré comme étant minime.*

Most-clés. *Agents antimicrobiens – Aquaculture – Résistance bactérienne – Méthodes de contrôle – Résidus – Analyse de risques – Risques – Produits pharmaceutiques vétérinaires.*

I – Introduction

What are risks? In the terminology adopted by the Office International des Epizooties (OIE, 2001), risks are equated with a process of hazard identification, which is designed to answer the question of What can go wrong? However, this refers to trade and it is defined as "the process of identifying any pathogenic agents which could potentially be introduced in the commodity considered for importation". Nevertheless, this process of hazard identification is the first step in performing a risk analysis and risk analysis is equally applicable to other areas of decision making. In other words, hazard identification is merely the step of identifying what it is that might go wrong in whatever activity being considered (MacDiarmid, 2001). The essential first step in any risk analysis is to provide a definition of the hazard or risk being considered and in the case of the potential risks associated with the use of veterinary drugs and chemicals in aquaculture it would be prudent to consider two general effect categories, although other minor risks also exist. As Smith (2001) has already pointed out, with respect to the use of antimicrobials in aquaculture, it is possible to recognise both direct effects, resulting from the presence of the agent itself, and indirect effects, resulting from the responses of organisms to the presence of the agent. Consequently, the risks associated with the use of antimicrobial agents could be defined by a dominant direct effect, such as toxicity resulting from their residues in human food or action on aquatic organisms, whereas any indirect effects would occur in the context of antimicrobial therapy of infectious disease related to the development of resistance.

In general, the potential risks are related to the type of agent being considered (Table 1). For instance, the use of chemotherapeutants may lead to the development of resistant bacteria or food residues, whereas parasiticides, oxidants, algicides, biocides and herbicides can result in toxicity for aquatic organisms.

Table 1. Potential risks associated with the use of drugs and chemicals in aquaculture

Type of agent	Potential Risks
Chemotherapeutants (e.g. antibiotics)	Development of resistant bacteria; residues in food
Parasiticides (e.g. pesticides)	Acute toxicity to marine organisms; irritation to handlers; residues in food; development of resistance
Oxidants	Explosive; toxicity; irritation to handlers
Algicides, biocides and herbicides	Toxic to aquatic life at high dosages; irritation to handlers; liver, kidney and thyroid effects in humans; carcinogenic; blood, liver and kidney effects in animals

Incentives for the use of drugs in aquatic animal species include the need to: (i) treat and prevent disease; (ii) control parasites; (iii) affect reproduction and growth; and (iv) tranquilization (e.g. during transit). In general, relatively few drugs have actually been approved for aquaculture. Consequently, in certain circumstances, this may lead aquaculture operations to use non-approved drugs, general purpose chemicals that are not labelled for drug use, or approved drugs in a manner that deviates from the labelled instructions (Price and Tom, 1997). However, as a means to control any identified risks in food animals, all antimicrobial agents, whether for direct medication or for addition to feed, should be approved by the relevant national authority. In the case of fish, there is the added potential for causing environmental problems since the use of fish feed as a delivery vehicle for antimicrobials may inevitably lead to a certain amount of leaching into the surrounding water. Moreover, antiparasitic treatments are often added directly to the water column and may have direct toxicity effects on other aquatic organisms.

II – Risk characterization

1. Chemotherapeutants

A. Development of resistant bacteria

It is probably true to say that the current main risk concern is the development of antibiotic resistance related to the use of antimicrobials in aquaculture, as well as in other food animal producing sectors and humans. Consequently, any risk has to be considered in terms of the extent to which it contributes to the incidence of this particular hazard. For more than 30 years, the use of antimicrobials in food animals, including farmed fish (Watanabe *et al*., 1971), has been considered as a potential risk. This follows a conference held by the New York Academy of Sciences in 1971 to discuss the risks to the future success of human disease therapy represented by the recently discovered phenomenon of transferable drug resistance in bacteria. It is considered that progress since then has been slow at best or, at worst, there has been no progress at all in quantifying the risk because the conclusions of several reports have indicated that the risk is simply greater than zero (Smith, 2001). It is though thought to be significant but that it might be incalculable (NRC, 1999). Nevertheless, examination of major multi-centre studies performed over many years, such as those of O'Brien (1986), suggests that rises in resistance have not been experienced in all pathogens nor in all geographical areas (Smith *et al*., 1994).

From all the studies on drug resistance in fish pathogenic bacteria in the last three decades there seems to be a clear link between use of antibacterial drugs in aquaculture and the development of antibiotic resistance in fish pathogenic bacteria. At the same time there also seems to have been an impact on the environmental bacterial flora surrounding fish farms where antibacterial drugs are being used (Smith *et al*., 1995; Kerry *et al*., 1996; Sørum, 1999). However, the data regarding antibiotic resistance in the vicinity of aquaculture facilities is difficult to interpret. For instance, it has not only been shown that the sediments near some fish farms that have used a large amount of antibiotics harbour a higher frequency of antimicrobial resistance than surrounding farms using a smaller amount of antibiotics, but also that some farmed fish carry a larger number of bacteria with individual and multiple antibiotic resistance (Herwig *et al*., 1997; Schmidt *et al*., 2000). On the other hand, aquaculture facilities can use antibiotics judiciously and rarely encounter resistant organisms (Herwig *et al*., 1997) and there are also examples of fish farms that use very little or no antibiotics and still have resistant bacteria. In addition, with samples collected from the wild, there have also been reports of multiple resistant bacteria isolated from wild fish and in sea- and fresh water (Sabry *et al*., 1997; González *et al*., 1999; Ash *et al*., 2002).

In animals, the normal bacterial flora contains antibiotic resistance genes to various degrees, even in individuals with no history of exposure to commercially prepared antibiotics (Sørum and Sunde, 2001). Apart from exposure of the intestinal flora to antibacterial drugs used as feed additives, other factors such as stress from temperature, crowding, and management also seem to contribute to the occurrence of antibiotic resistance in normal flora bacteria (Sørum and Sunde, 2001).

Antibiotic resistance has also been found in non-agricultural animals, such as US Navy dolphin blowholes (http://www.marineconnection.org/latest_news/news/news_dolphins_show_antibiotic_resistance.htm), wild rodents (Gilliver *et al*., 1999), wild baboons (Rolland *et al*., 1985), wild geese (Eichorst *et al*., 1999), wild birds from the Brazilian Atlantic forest (Nascimento *et al*., 2003) and domestic and pet animals (Schwarz *et al*., 1998; Schroeder *et al*., 2002). It has also been found in rural ground water supplies (McKeon *et al*., 1995), treated waste water (Raloff, 1998), bottled mineral water (Mary *et al*., 2000), naturally occurring soil organisms (Waters and Davies, 1997) and antibiotic resistance genes have even been shown to transfer from transgenic plants to bacteria, possibly via the intestine of insects (Smalla *et al*., 2000). The evidence indicates that it is not a small problem waiting to go away.

Consequently, there is no doubt that there has been an increase in bacterial resistance to some antibiotics and a concomitant increase in the ability to find it, although this is most evident for certain human pathogens. For instance, there is concern about the spread of resistance from the closed hospital environments into open communities, which is seen as a threat to public health (Acar and Röstel, 2001). Resistance is a natural phenomenon that develops through the selection pressure exerted by the use of an antibiotic and such potential resistance can occur following any use of antibiotics, irrespective of whether they are required for fish treatment, human health care, companion animals, agriculture, horticulture or even food processing. One calculation estimates that the antibiotic market worldwide consumes between 100 and 200 x 10^6 kg, which when combined with the estimated global number of bacteria associated with man and animals, possibly in the region of 10^{28}, gives an idea of the potential selection pressure (Wise, 2002).

However, although the various ecosystems can be considered as separate compartments within the environment, there is little data available on the conditions required for selection of resistance or, perhaps more importantly, the movement of bacteria and the transfer of resistance between discrete compartments. What is certain though is that resistance, once developed, is not bound to the borders of different ecological environments or countries (Acar and Röstel, 2001), although the contribution of aquaculture is not expected to be a significant percentage of the non-human use of antimicrobials (IARPS Committees, 2002). Nevertheless, the exact quantities of antimicrobial use in the agri-food sector are not known, although there are some international data available that have been based on estimates. In the USA, for instance, it is estimated that 50% of the 22.7 million kg of all antimicrobials prescribed annually are for humans and 50% are for animal, agriculture and aquaculture use (Levy, 1997). Also, Mellon *et al*. (2001) calculated antimicrobial use in agriculture from publicly available information including total herd size, approved drug lists, and dosages. They conservatively estimated that US livestock producers use approximately 11.2 million kg of antimicrobials for non-therapeutic purposes – primarily for growth promotion of cattle, pigs and poultry. On the other hand, an Animal Health Institute survey in 1999 indicated that 9.3 million kg of antimicrobials were marketed for both food and companion animals in the USA. Of the antibiotics used in animals, 8.1 million kg were to prevent and treat diseases, whereas 13% (1.3 million kg) were to enhance growth or improve feed efficiency (Animal Health Institute; http://www.ahi.org/).

In the EU, although consistent sources of information are difficult to find, the European Federation for Animal Health (FEDESA) provided data which showed that from a total volume of 10,493 tons of active ingredient antibiotic sold during 1997, approximately 5400 tm (52%) was for human medicine use, 3494 tm (33%) for veterinary animal health, and 1599 tm (15%) for animal production (growth promotion). They further estimated that 90% of antimicrobials for animal use were administered in feed; 60% were used in pigs, 20% in poultry and rabbits, 18% in ruminants, and 1% each in pets and fish. Within the animal health category (therapy, prevention and control), 66% of antimicrobials were tetracycline, 12% macrolide, 9% penicillin, and 12% other drugs (Schwarz and Chaslus-Dancla, 2001).

In the UK, a House of Lords (1998) report suggested that up to 95% of antimicrobial use is within the community (as opposed to hospitals), although the US National Academy suggests it is closer to 75% (http//www.cdc.gov/drugresistance/actionplans).

In Asia, shrimp farmers use antibiotics in large quantities. Warehouses supplying the industry in all the major centres sell a range of antibiotics in containers of 500 g or more in size. The antibiotics in current use include fluoroquinolones but especially norfloxacin and enrofloxacin, furazolidone, oxolinic acid, oxytetracycline, trimethoprim and sulphadiazine. It is difficult to determine just how much antibiotic use there is in the industry, but Moriarty (2000) has made an estimate from feed usage and production. In 1994, Thailand produced about 250,000 tonnes (a quarter of the world production) of farmed shrimps, which consumed 500,000-600,000 tonnes of feed. For each crop at semi-intensive to intensive scales of production, farmers used 5-10 g antibiotics per kg feed at least once per day at weekly intervals, although it is known that some used them for more extensive periods. Thus antibiotics would be used in about 10% of feed. It is possible, therefore, that the antibiotic usage in shrimp farm production in Thailand in 1994 was as much as 500-600 tonnes, assuming all farmers used them, but not including that used in hatcheries for fry production (Moriarty, 2000).

According to Benbrook (2002), given the lack of attention to data collection, as well as current disease reporting and aquaculture product quality surveillance systems, it would be likely that short-term spikes in antibiotic use would not be detected by government regulatory officials or public health experts. This blind-spot in knowledge of antibiotic drug use in aquaculture is serious because the odds of resistant bacteria emerging and spreading beyond farm production sites are greatest during periods of intensive use (Benbrook, 2002). A further confusion when considering antimicrobial resistance is that it has more than one definition according to the scientific discipline considered. Such definitions can be clinical, pharmacological, microbiological and molecular or epidemiological (Acar and Röstel, 2001).

The Union of Concerned Scientists (UCS) is forthright in claiming that antibiotic-resistant bacteria are on the rise (http://www.ucsusa.org/). They state that patients once effectively treated for pneumonia, tuberculosis, or ear infections may now have to "try three or more antibiotics before they find one that works". They consider that the reason for antibiotic resistance is overuse of antibiotics through overprescription by physicians and hospitals. Veterinarians, too, are thought to have overprescribed drugs to treat sick animals, and livestock producers use "massive amounts" to promote animal growth and make their business more efficient and profitable. Growers also spray antibiotics on crops to control bacteria that damage vegetables and kill trees. The UCS considers that medicine must act to slow the emergence of resistant bacteria and that it is equally important to eliminate uses, primarily agricultural, whose benefits are economic, not therapeutic (UCS, 2002).

On the other hand, although the leading pharmaceutical company Pfizer agrees that "Antibiotic resistance is a growing public health problem, resulting in increasing morbidity and mortality around the world", their chairman and chief executive officer believes "It is critical that we continue to find new anti-infectives that work against drug-resistant bacteria" (McKinnell, 2003). Pfizer's total revenues for the first quarter of 2003 were $8.525 billion (with human pharmaceutical operations having revenues of $7.548 billion, whereas animal health sales in the same period were $269 million; http://www.pfizer.com/main.html).

2. Movement of resistance

There are two major but separate routes through which a resistance risk could occur (Smith, 2001). The first involves the movement of resistant bacteria from one environmental compartment to another (e.g. aquaculture to humans or animals to humans or animals to aquaculture, etc.) and the second involves the movement of resistance genes. However, the emergence of antibiotic resistance and its spread or transfer between different ecosystems is a complex subject.

A. Resistant bacteria

Intercompartmental spread of resistance leading to treatment failures in animals or humans is dependent on the emergence of antibiotic-resistant bacteria as a result of selection pressure related to antibiotic use, which as already indicated can vary tremendously.

The movement of bacteria from the aquaculture compartment is dependent on the very presence of bacteria that could be capable of infecting animals or humans in another compartment and by default has to be considered as a two way process. The bacteria also need to be exposed to an antimicrobial agent and this process should result in selection of bacteria with reduced susceptibility to antimicrobials. Finally, these resistant bacteria need to move from one environment to another and initiate infection requiring further antimicrobial therapy. According to Smith (2001), it is important to note that the only events of significance are the changes in susceptibility to the antimicrobials used in the therapy of infectious diseases and there is a limited range of infectious agents for which any particular antimicrobial is recommended.

In order to study the movement of resistant bacteria between compartments a certain amount of quantitative data is required, although one important consideration is that data should be collected with standard methods and interpreted using validated criteria. The type of data relates to:

(i) The infectious diseases for which therapy with a specific antibacterial is indicated.

(ii) The bacteria associated with such diseases.

(iii) The frequency of occurrence of these bacterial groups in each compartment and subcompartment.

(iv) The frequency of occurrence of resistant variants of these bacterial groups in each compartment and subcompartment.

(v) The incidence of bacterial infections associated with commodities or products from other compartments (list adapted from Smith, 2001).

B. Resistance genes

The movement of resistance genes is concerned only with the movement of genes encoding resistance to antimicrobial agents and depends on a bacterial host carrying such genes to another environmental compartment. The process depends on the creation of resistance genes following selection pressure and the ability of the gene or genes to be transferred. By way of example, it is generally accepted that tetracycline resistance is readily transferable (Adams *et al*., 1998; Schmidt *et al*., 2001) but that genes encoding quinolone resistance are non-transferable (Courvalin, 1990; Martinez *et al*., 1998). The remainder of the process relies on the transfer of the gene encoding resistance from its original host to another bacterium capable of infecting another animal or human and finally the initiation of an infection requiring antimicrobial therapy by the pathogen which has acquired the resistance gene (Smith, 2001).

Several published studies document the presence of genes encoding resistance to antimicrobial agents in the vicinity of aquaculture facilities (Sandaa *et al*., 1992; Aoki, 1997; Herwig *et al*., 1997). However, transferable resistance genes have also been isolated from a number of natural environments that have a minimum of human disturbance, such as upland tarns (Jones *et al*., 1986).

The issue of the frequency of the movement of bacteria containing transferable resistance genes from one environmental compartment to another is very complex. Adaptation of a pathogen to one environment does not necessarily mean it will adapt easily in another, or even that a bacteria-plasmid-gene complex could survive the transfer. In addition, the studies of plasmid stability under laboratory conditions may not be relevant for the range of environments encountered in the movement, for instance, from aquatic operations to humans, even in the absence of antimicrobial agents (Smith, 2001).

There is no doubt that plasmid encoded specific resistance genes encountered in the vicinity of aquatic operations can, under laboratory conditions, transfer to bacteria capable of infecting humans (Hayashi *et al*., 1982; Nakajima *et al*., 1983) and can also transfer under environmental conditions (Kruse and Sørum, 1994). On the other hand, Guardabassi *et al.* (2000) indicated that clinical strains of *Acinetobacter* spp. were not capable of transferring tetracycline resistance in vitro to aquatic strains and they considered that there was no important flow of resistance genes between such populations. However, although these, and other studies, indicate that movement of specific resistance genes between different compartments is possible, they do not indicate the direction of movement.

According to Smith (2001), given the propensity of humans to dump their wastes deliberately into the living environment of fish, there are grounds for considering fish-associated bacteria to be more at risk from human bacteria than vice versa. He further poses the question of "From where did the original resistant human pathogen obtain its resistance gene?" and considers that it must have come from another bacterium whose presence is itself a function of selection and enrichment in an area of antimicrobial use.

There are various areas where the use of antimicrobials can be considered as possible sources of resistance genes. These include human medicine, land-based horticulture, land-based veterinary medicine and aquatic-based veterinary medicine.

It is known that antibiotics are present in many fresh water sources throughout the USA and that four of the most frequently detected antibiotics are used to treat humans for pneumonias, "strep" throat, and middle ear, urinary tract, respiratory tract and HIV-opportunistic infections (Kolpin *et al*., 2002). In the same study, tylosin, used in beef cattle and swine production, was the fifth most frequently detected antibiotic. Although the antibiotic levels were generally low (less than 2 µg/l), it was considered that even low-level concentrations in the environment could increase the rate at which pathogenic bacteria develop resistance to these compounds. The results supported the conclusion that antibiotics are not being effectively removed from urban wastewater effluent and that antibiotics used in livestock production can occur in waterways. In addition, the data mentioned earlier indicated that around 52% of antibiotics are destined for human medicine use in the EU (50% in the USA) and 33% for veterinary animal health. Of this latter figure approximately 1% is for fish health use, which means that an estimated 0.33% of total sales is destined for aquaculture operations. Although there is always a danger of extrapolating this type of information, it is likely that this estimate is representative at least for Europe and the USA. No recent accurate attempt has been made to estimate the antimicrobial usage in other regions, such as Asia. These considerations suggest that human use > land-based animal use > water-based use (Smith, 2001) is the most likely direction of movement for specific bacterial resistance genes. Nevertheless, a survey of 13 fish hatcheries in the USA has shown that a small percentage (14%) of randomly collected water samples from five hatcheries contained detectable trace concentrations (mainly 0.10-to 2.0 µg/l) of either oxytetracycline or sulfadimethoxine (Thurman *et al*., 2002). Consequently, the contribution of antimicrobial use in aquaculture to the selection pressure and potential appearance of resistance genes in some waterways cannot be ruled out, albeit at a low level.

The incidence of infection by bacteria that have acquired resistance genes is governed by factors relevant to human disease epidemiology (Smith, 2001), which has little relevance to the use of antimicrobials in aquaculture. In fact, the World Health Organization (WHO) have stated that "in general, there is little doubt that treatment problems in humans due to resistant bacteria are primarily related to the prescribing practices of health workers and to medication-taking practices of patients" (WHO, 1997). Nevertheless, establishing a quantitative relationship between the frequency of resistance and volume of drug use has proved difficult, although there is a critical level of drug consumption required to trigger the emergence of resistance to significant levels (Austin *et al*., 1999). However, it has been shown that in Europe (Bronzwaer *et al*., 2002) antimicrobial resistance of *Streptococcus pneumoniae* to penicillin is correlated with use of beta-lactam antibiotics (e.g. penicillin) and macrolides (e.g. erythromycin), which have practically no use in aquaculture. In addition, a recent study suggests that human population density is an important factor in the development of antibiotic resistance and warrants special attention as a factor in resistance epidemiology (Bruinsma *et al*., 2003).

As with the movement of resistant bacteria there are certain key data requirements needed for the movement of resistance genes between compartments. The type of data relates to:

(i) Characterisation of the resistance genes, at the sequence level, which are causing concern in human medicine and the other compartments.

(ii) The frequency of resistance gene sequences in aquatic environments and the other compartments (list adapted from Smith, 2001).

C. Residues in food

The detection of antimicrobial residues in food tends to favour a zero tolerance (no detectable residue) approach because the toxicity data for antibiotics such as chloramphenicol are largely based on their historical therapeutic drug doses before it was illegal to use them for human treatment or for food animals. The subsequent reduction in the sensitivity of analytical techniques for detecting antibiotic residues in food samples has led to variable interpretation of the term zero tolerance. For instance, in Japan, the zero tolerance threshold for chloramphenicol is 50 ppb, whereas in the EU it is based on the minimum detectable limit and application of the precautionary principle. In the case of the EU, it is claimed that this is a bit of a moving target because continual improvements in analytical technology mean that the limit keeps getting smaller. Some Asian shrimp imports, for instance, have been rejected recently at chloramphenicol levels of 1 ppb and lower. In the USA, the zero tolerance threshold is now similar. Product rejection means a ban on imports and possible destruction of positive shipments which in turn leads to a knock-on effect that causes economic loss for other producers.

Although it is not an antimicrobial agent, the pigment canthaxanthin is used as a cosmetic feed additive to colour food for adding a reddish colour to salmon, egg yolks and poultry products. The European Commission has been concerned about its use following scientific assessments that seemed to establish a link between high canthaxanthin intake and accumulation of pigments in the retina leading to eyesight problems. As a result, an EC Directive has been adopted to reduce the authorised level of canthaxanthin in animal feed, which for salmonids will mean a decrease from 80 mg canthaxanthin/kg of feed to 25 mg/kg. It was envisaged the Directive to be implemented by the end of 2003 (EC, 2003a). Some other food and colour additives can cause an allergic-type reaction in sensitive individuals. However, these compounds tend to be applied to fishery products at the processing stage and can include sulphiting agents to prevent the formation of "black spot" (e.g. in shrimp and lobster). Such additives are generally accepted providing their presence is declared by labelling (Price and Tom, 1997).

3. Parasiticides

A. Toxicity

There has been concern about the use of antiparasitic treatments (e.g. sea lice control in salmon) because the agents are applied directly in large closed bags, but often the antimicrobial finds its way into the water column when the treatment is finished. Such pesticides (e.g. cypermethrin, dichlorvos, emamectin benzoate and pyrethrum), along with ivermectin, have been shown to have different environmental effects. For instance, dichlorvos (now banned in the UK; DEFRA, 2002) disperses quite rapidly, although its half-life can be from 1-30 days depending on parameters such as temperature, pH, and salinity (Ross, 1989). Ivermectin is not as easily dispersed as dichlorvos and may have much greater persistence (Grant and Briggs, 1998). Cypermethrin as a bath treatment and emamectin benzoate as an in-feed treatment are most widely used in Scotland and are considered to present the greatest environmental risk. Bath treatments involve the discharge of dissolved medicine into the water column after the treatment period, whereas in-feed treatments are ingested by the fish and then excreted over a period of time with most of the losses occurring to the sediments rather than the water column (SECRU, 2002).

It has been shown by dispersion modelling that cypermethrin released following a single bath treatment is rapidly diluted in the receiving environment, with the majority being adsorbed onto particulate material that settles to the seabed. However, absorption takes several hours and leads to a discharge plume that may retain its toxicity to certain aquatic life for this perod of time. On the other hand, emamectin has low water solubility with a high potential to be adsorbed to suspended particulate material (e.g. fish faeces and uneaten fish food). Consequently, sediment organisms are most likely to be affected by emamectin benzoate use since it can remain in sediments for a long time, and it has a half life of approximately 175 days (SECRU, 2002). The aquatic species most likely to be affected include benthic amphipods in the sediment, polychaetes, planktonic organisms and crustacean invertebrates, although any potential toxicity will depend on the nature of the individual compound, its mode of action and the dilution factor. Nevertheless, sea lice are crustaceans and there is concern, as reported in the press, that the treatments designed to kill them may also have effects on the sensitive larval stages of other species, such as lobsters, crabs, mussels, oysters and scallops.

B. Residues in food

Environmental chemical contaminants (e.g. hydrocarbons or heavy metals) and pesticides in fish or shellfish may pose a potential human health hazard if they are harvested from waters that are exposed to varying amounts of industrial chemicals and toxic elements (Price and Tom, 1997). Although accumulation can occur in fish and shellfish, any risk is usually associated with long-term exposure. Such risks occur in fish from capture fisheries of fresh water, estuaries and near-shore coastal waters or in shellfish from harvesting areas. However, the pesticides used in aquaculture operations as antiparasitic treatments have the potential to contaminate food fish (FDA, 2001).

Potential chemical contamination in molluscan shellfish is usually considered as part of a programme deigned for classification of harvesting waters. Depending on the results from a testing regime, any unacceptable contamination will be sufficient to close harvesting areas for certain periods of time under certain conditions.

4. Biocides, algicides and herbicides

The use of pesticides in marine aquaculture is generally small in comparison with terrestrial agriculture. However, more than a dozen types of herbicides are approved for use in US aquaculture facilities to control aquatic weeds, algal blooms and fouling organisms. Most herbicides are used in pond and tank-based aquaculture systems, although netpen operators often treat their nets with paints that contain copper-based algae killers. The antifoulants that contain such 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, water column concentrations of copper (the usual active ingredient) have been found not to be significant between the environment inside and outside of fish farm nets, providing the tidal exchange and dilution factor are sufficient to minimise any problems. Consequently, although copper is toxic to many aquatic organisms, the copper compounds used in aquaculture are thought to be relatively safe when applied in approved dosages (Eisler, 1998; Boyd and Massaut, 1999).

The cumulative organometal antifoulant tributyltin (TBT), on the other hand, has been shown to have adverse effects on some oyster and dog whelk populations and has been banned for small sailing craft in some countries, such as France, UK, USA and Japan. The use of TBT was even shown to be responsible for the collapse of the large shellfish industry in Arcachon Bay (Atlantic coast of France) in the 1970s and 1980s (Santillo *et al*., 2002). However, current regulations seem to have now successfully limited the environmental impacts of TBT in coastal areas, except in areas close to ship yards that still use TBT on large ocean going ships.

III – Data gathering and interpretation

There are many potential problems with data gathering, particularly for quantitative data. For instance, in the case of antimicrobial resistance, these problems are related to the lack of standard methods for the determination of the frequencies of bacteria with resistant phenotypes and the frequency of resistance genes. In addition, it is difficult to interpret the various mechanisms involved in resistance, as well as the effect of antimicrobial usage on resistance itself. The use of different methods, media and break-point concentrations in different studies means that little or no comparison can be made between the available data (Smith, 1998).

Nevertheless, in researching this article and reviewing the available data, no documented case was found where antibiotic use in fish has caused treatment failure in humans. On the other hand, there is evidence for the spread of resistant animal bacteria to humans, the transfer of antibiotic resistance genes from animal to human pathogens and resistant strains of animal pathogens causing human disease (JETACAR, 1999). However, this evidence has also been refuted by the Animal Health Institute, which is the US trade association that represents manufacturers of animal health care products, and it is considered that the benefits of antibiotic use in food animals outweigh the risks (AHI; http://www.ahi.org/).

IV – Risk analysis

Risk analysis is a tool intended to provide decision-makers with an objective, repeatable and documented assessment of the risks posed by a particular course of action (MacDiarmid, 1997). It is important as a tool to aid decision-making for disease surveillance, disease control, international trade and other risk-based evaluations. Risk analysis is intended to answer the following questions:

(i) What can go wrong?

(ii) How likely is it to go wrong?

(iii) What would be the consequences of its going wrong?

(iv) What can be done to reduce either the likelihood or the consequences of its going wrong?

The Office International des Epizooties (OIE) International Aquatic Animal Health Code (OIE, 2001) describes the four components of risk analysis as hazard identification, risk assessment, risk management and risk communication. The risk assessment phase of a risk analysis is further divided into a release assessment, exposure assessment and consequence assessment.

Risk analysis is a complex discipline and it is a team effort comprised of numerous different skills and expertise. To ensure the technical robustness of a risk analysis, so that decision-makers can be sure that it will withstand close scrutiny by stakeholders, it should also be subject to a process of scientific review by experts of both aquatic animal diseases and risk analysis itself. Nevertheless, despite these in-built safeguards, one of the most difficult problems faced by decision-makers is that of deciding what constitutes an "acceptable risk". In addition, the acquisition of the necessary data for risk analysis is expensive and time-consuming and the analysis itself will consume significant intellectual resources (Smith, 2001). It has also been suggested that some sort of prioritisation should therefore be undertaken and, in the case of aquaculture, this could firstly consider the relationship of aquatic enterprises to human sewage (Smith, 2001).

Risk assessment of microbiological hazards in foods has been identified as a priority area of work for the Codex Alimentarius Commission (CAC). In addition, the World Health Organization (WHO) has called for a risk-based evaluation of the potential human health effects to be conducted for all uses of antimicrobial drugs in food producing animals, including currently approved products. Characterization of the risk should include consideration of the importance of the drug or members of the same class of drug to human medicine, the potential exposure to humans from antimicrobial-resistant bacteria and their resistance genes from food animals. If a risk assessment demonstrates an antimicrobial to be unacceptable, it's withdrawal from the market for veterinary use should be considered (WHO, 2001).

The WHO also recommends that use of antimicrobial growth promoters belonging to classes of antimicrobial agents used (or submitted for approval) in humans and animals should be terminated or rapidly phased-out in the absence of risk-based evaluations. The termination or phasing-out should be accomplished preferably by voluntary programmes of food animal producers, but by legislation if necessary. Characterization of the risk may include consideration of the present and potential future importance of the drug to human medicine, its selection of resistance and the potential exposure to humans of resistant bacteria from food animals (WHO, 2001).

The OIE *Ad hoc* Group of experts on antimicrobial resistance also developed an objective, transparent and defensible risk analysis process for antimicrobial resistance. The Group recommended independent risk assessment based on scientific data, an iterative risk analysis process, a qualitative risk assessment systematically undertaken before considering a quantitative approach, the establishment of a risk assessment policy and the availability of technical assistance for developing countries (Vose *et al*., 2001).

V – Risk reduction and control management

According to modern public health concepts of prevention and control of food-borne diseases, consumer hazards derived from food intake should be considered under a HACCP (hazard analysis and critical control point) scheme. The HACCP system is applied from production to consumption and offers a systematic sequential approach to the control of food-borne hazards, avoiding the many weaknesses inherent in the traditional inspection approach (Howgate *et al*., 1997).

The reason why antimicrobials are applied in aquatic enterprises is to control disease (Smith, 2001). For each application there are routinely used treatment regimes which normally specify the dose and duration of any therapy. However, one of the problems is that there are few empirical data underpinning any of the recommended durations of therapy and this applies both to human medicine (Lambert, 1999) and fish diseases (Smith, 2001).

What is required is a concerted strategy for containment and reduction of antimicrobial resistance through prudent and responsible use of antimicrobials, in conjunction with the development of risk analysis methodology already mentioned, designed to assess and manage the risks to animal and human health. In addition to these measures, there is a need for harmonisation of surveillance systems and laboratory methodologies, which should be complemented by information gathering exercises for improving knowledge on antimicrobial resistance world-wide (Acar and Röstel, 2001). Nevertheless, it has been estimated by using mathematical models of the epidemiology and population genetics of antibiotic treatment and resistance in open communities and in hospitals that it will take years or even decades to see substantial reductions in the frequency of antibiotic resistance solely as a result of more prudent (reduced) use of antibiotics (Levin, 2001).

In the Office International des Epizooties (OIE) Guidelines on antimicrobial resistance, the OIE *Ad hoc* Group of experts made the following recommendations as a means of dealing with the problem (Acar and Röstel, 2001):

(i) Risk analysis methodology for the potential impact on public health of antimicrobial resistant bacteria of animal origin.

(ii) Responsible and prudent use of antimicrobial agents in veterinary medicine.

(iii) Monitoring the quantities of antimicrobials used in animal husbandry.

(iv) Standardization and harmonization of laboratory methodologies for the detection and quantification of antimicrobial resistance.

(v) Harmonization of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food.

There are also responsibilities for the regulatory authorities, veterinary pharmaceutical industry, pharmacists, veterinarians and the producers, as summarized in detail by Anthony *et al*. (2001). In general, these consist of the support role and marketing authorisation provided by the national regulatory authorities, as well as the submission of relevant data by the pharmaceutical industry. Additional requirements include the supply of appropriately labelled prescription only antimicrobials by distributing pharmacists, veterinarian encouragement of good farming practice to minimise the need for antimicrobial use and the use of health plans by producers to outline preventative measures that regard therapeutic antimicrobial products as complementing good management, vaccination and farm hygiene. Additional control measures for aquaculture drugs can also include product traceability schemes, on-farm visits to review drug usage, drug residue testing and certification that a producer operates under an audited quality assurance programme for aquaculture drug use (Price and Tom, 1997).

Legislation also plays a role in control since before a product can be authorised for use in food producing animals an assessment of residue safety and the establishment of maximum residue limits (MRLs) need to be undertaken. In Europe, this is controlled by legislation that establishes the MRLs for veterinary medicinal products in foodstuffs of animal origin (EC, 1990), although other countries have also taken steps to reduce the problem of antimicrobial resistance in food animals through legislation. In 1986, Sweden banned the use of all animal growth promoters, even those which are not used in human medicine. In 1997, the European Union banned all antimicrobial animal growth promoters that were also used in human medicine. In 1999, Denmark voluntarily suspended the use of all animal growth promoters and Switzerland did the same in 2000. Studies in Denmark have shown that voluntary suspension resulted in an overall reduction of antimicrobial use in Danish livestock of more than 60% with no significant economic impact or negative change in animal health status and food safety (WHO, 2002).

It may also be necessary to limit the use of antibiotics for specific and identified uses in aquaculture, such as already occurs in some countries. Regulation of their commercial availability is one of the ways to ensure that they are used responsibly in aquaculture (FAO, 2002). In addition, rational dosing of antimicrobial drugs should be designed to optimize efficacy and minimize opportunities for the development of antimicrobial resistance. This depends on knowledge of physiology, anatomy and pathology, including disease condition, and in major respects these differ between animals and humans and between species of animal, which leads to species variation in drug pharmacokinetics (Lees and Shojaee Aliabadi, 2002).

One methodological control tool for studying resistance in populations is surveillance, although few countries have active surveillance for antimicrobial resistance in bacteria from food animals and food of animal origin. The development of surveillance tools, as well as the development of effective surveillance strategies and mechanisms to fund surveillance studies are viewed as important components to stem the rise in antibiotic resistance, although the critical establishment of baseline antibiotic resistance levels is essential for any surveillance study (Isaacson and Torrence, 2002).

Efficient control of residues is an essential contribution to the maintenance of a high level of consumer protection in the EU. European Commission Decision 2002/657/EC implementing Council Directive 96/23/EC established criteria and procedures for the validation of analytical methods to ensure the quality and comparability of analytical results generated by official laboratories (EC, 2002). In addition, the Decision established common criteria for the interpretation of test results and introduced a procedure to establish progressively minimum required performance limits (MRPL) for analytical methods employed to detect substances for which no permitted limit (maximum limit) has been established. In particular, this is important for substances whose use is not authorised or is specifically prohibited in the EU.

The Codex Alimentarius Committee on Residues of Veterinary Drugs in Foods is concerned with determining priorities for the consideration of residues, recommending maximum levels of such substances, developing codes of practice as may be required and considering methods of sampling and analysis. The Committee also produces official standards for MRLs of pesticides and veterinary drugs in foods.

A product withdrawal time is another measure designed to prevent unacceptable residues in fish and other food animals. A withdrawal time must be observed after the use of antimicrobial agents to ensure that any product used on an aquatic site or on animals does not exceed legal tolerance levels in the animal tissue. Using proper withdrawal times helps to ensure that products reaching consumers are safe and wholesome. Withdrawal times are usually reported as a specific number of days after exposure to a drug or pesticide and they are often temperature dependent.

The World Health Organization (WHO) issues global principles for the containment of antimicrobial resistance in animals intended for food, which include recommendations designed for use by governments, veterinary and other professional societies, industry and academia (WHO, 2001). Some of the most important measures stipulated are:

(i) Obligatory prescriptions for all antimicrobials used for disease control in food animals.

(ii) Termination or rapid phasing-out of the use of antimicrobials for growth promotion if they are also used for treatment of humans in the absence of a public health safety evaluation.

(iii) Creation of national systems to monitor antimicrobial usage in food animals.

(iv) Pre-licensing safety evaluation of antimicrobials with consideration of potential resistance to human drugs.

(v) Monitoring of resistance to identify emerging health problems and timely corrective actions to protect human health.

(vi) Guidelines for veterinarians to reduce overuse and misuse of antimicrobials in food animals.

VI – Aquaculture in context

One problem in considering risks is that most studies are often of a qualitative nature and there is a tendency to report potential risks rather than to assess their importance objectively (Smith *et al.*, 1995). Aquaculture covers a very diverse set of operations conducted in very diverse environments in very diverse cultures (Smith, 2001). Aquaculture facilities range from large industrial units with massive capital investment to small family units and they also form part of peasant subsistence food production. Their products are consumed locally and are traded internationally. The two countries with the largest aquaculture production figures (FAO, 2002) are China (32.4 million tonnes/year) and India (2.1 million tonnes), whereas the largest European producer (EC, 2003b) is Spain (0.31 million tonnes). The gross national income (GNI) and the 2000 total population of these countries are shown in Table 2. Also included are the corresponding comparative figures for Europe (European Monetary Union; EMU).

Table 2. Estimates of population, gross national income and aquaculture production for several countries†

Country	Population in 2000 (millions)[1]	Gross National Income/capita[1] ($)	Gross National Income/capita[1] (Rank)	Aquaculture Production in 2000 (10^6 tonnes)
China	1,262	840	141	32.4[2]
India	1,016	450	159	2.1[2]
Spain	39	15,080	38	0.31[3]
Europe (EMU)	304	21,730	–	1.3††

†Figures from: [1] the World Bank Group; [2] FAO; [3] EC.

††The European Community countries.

Essentially, a total of approximately 2.3 billion people (2000 figures), accounting for almost 40% of the world population, live in the two largest aquaculture producing countries and they have a combined GNI per capita of less than 1500 USD (World Bank Group, 2002). This compares with Europe (EMU zone) that has a population of 0.3 billion and a GNI per capita of 21,730 USD. It is a known fact that most of the world's people are poor, in fact Milanovic (1999) found that the richest 25% of the world's population receives 75% of the world's income. This occurs because a large proportion of the world's population lives in the poorest countries, and within the poorest regions of those countries, particularly in the rural areas of China, as well as rural and urban areas of India and Africa. The largest killer in the world is not genetically modified soya, pesticide residues, tobacco or even the use of veterinary drugs and chemicals in aquaculture. It is something that is given the code Z59.5 in the WHO's International Classification of Disease Handbook and accounts for more deaths worldwide than any other single factor. It is defined as "Extreme Poverty".

In Asia, aquaculture has developed mainly as a rural activity integrated into existing farming systems. According to the FAO (2002), it has made significant contributions to the alleviation of poverty. This has been directly through small-scale household farming of aquatic organisms for domestic consumption or income, and indirectly by providing employment for the poor or low-cost fish for poor rural and urban consumers. In China, specifically, significant expansion and intensification of aquaculture are taking place and small-scale farms account for 60% of production, but with intensive systems being based increasingly on formulated feeds, instead of the more traditional manuring system. In India, rural aquaculture using extensive to semi-intensive modes of production in ponds and tanks also contributes significantly to rural household incomes.

VII – Conclusions

It is evident, therefore, that a small fraction of aquaculture is conducted in the richest, most technologically developed countries but the majority occurs in countries with limited wealth and technological infrastructure. It is extremely unlikely that any single analysis or set of recommendations regarding the potential risks associated with the use of veterinary drugs and chemicals could be formulated which was both relevant and practicable in all areas of aquaculture (Smith, 2001).

The perceived risks of using antibiotics in fish are mainly related to the ability of bacteria to develop resistance to antibiotics. It has also been shown that it is possible to find antibiotic resistance in all compartmental ecosystems and that there are links between the compartments. The main two common links are water and the food chain, which could be used as the means to transfer antibiotic resistance either into or out of each compartment to one degree or another. However, in the opinion of Smith (2001), even if all aquacultural use of antimicrobials were stopped immediately, it is unlikely that any consequent changes in the frequency of resistance in human pathogens would be detected. Furthermore, it is possible that any antimicrobial resistance created in one environmental compartment could remain largely within its own compartment, except for the existence of only minor transient intercompartmental links, which would have only a small impact on the possibility of resistance transfer, particularly out of the aquatic compartment. In other words, to reduce the impact of resistance in aquaculture, veterinarians and producers should concentrate their attention on good aquaculture practice and preventative measures within this particular compartment. The same would be true for resistance in food animals or human medicine where efforts should concentrate on the sales techniques and usage patterns that govern the use of antibacterials in these compartments (Smith, 2001).

It is also obvious that data on antimicrobial resistance is restricted both in quantity and quality, which leads to a parallel need for standardized validated methods of analysis that can help to fill the data gaps. One of the commonest phrases found in the literature whilst researching this article was the need for appropriate and prudent use of antimicrobial agents by physicians and veterinarians. The United Nations considers that heavy use of antibiotics in people and animals is encouraged by commercial pressures and has the potential to cause significant antibiotic contamination of microbial communities in the natural environment (e.g. sewers, soil and receiving waters), which could be transferred back into human and animal disease organisms (UN Earthwatch, 2003). On the other hand, it is also claimed that modern industrialized food production adds extra emphasis on lowering the use of antibiotics in all parts of agriculture, husbandry and fish farming because these food products are distributed to very large numbers of humans compared to more traditional smaller scale niche production (Sørum and L'Abee-Lund, 2002). However, maybe therein lies the problem because with increased globalization of trade and a rapidly expanding population the possibilities of rapid spread of antimicrobial resistance through the food chain and travel is infinitely greater. Smaller scale local production would almost certainly provide less impact and have less need to use antimicrobial agents. The primary factors that have led to the emergence of antimicrobial resistance as a problem in human medicine and food animals are the enormous adaptive ability of bacteria coupled with the use and misuse of these agents. Any effect, in terms of human health, resulting from the use of these agents in aquaculture is, by comparison, extremely minor (Smith, 2001).

The current debate about which environmental ecosystem is to blame for the rapid rise of antimicrobial resistance deflects attention away from the real priorities. We need to stop continually looking in on aquaculture to try and prove that there are unacceptable risks associated with the use of veterinary drugs and chemicals that somehow are having serious impacts through the food chain. Instead, we should recognise that there are in fact some minor potential risks and these may warrant improved control measures. From this premise, we could then redirect our energies towards addressing how we can use aquaculture to provide a partial answer to more important issues, such as increasing the contribution of aquaculture to food security and poverty alleviation. Aquaculture is regarded as an important domestic provider of high-quality animal protein and other essential nutrients, as well as providing employment opportunities, cash income and valuable foreign exchange, with developing countries alone producing over 90% of total aquaculture production by weight in 1998 (Tacon, 2001).

"...microbes are educated to resist..." (Alexander Fleming warning against the misuse of penicillin in 1945)[1]

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[1] The full quote attributed to Alexander Fleming is: "The greatest possibility of evil in self-medication is the use of too small doses so that instead of clearing up infection, the microbes are educated to resist penicillin and a host of penicillin-fast organisms is bred out which can be passed to other individuals and from them to others until they reach someone who gets a septicemia or a pneumonia which penicillin cannot save." (New York Times, 26 June, 1945).

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