Joint FAO/WHO/OIE Expert Consultation on Antimicrobial Use in Aquaculture and Antimicrobial Resistance

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Towards a risk analysis of antimicrobial use in aquaculture

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Preface

Thirty-five years ago Watanabe and his colleagues (Watanabe et al., 1971) gave a paper to the New York Academy of Science that first drew international attention to the potential risks associated with the use of antimicrobials in fish farming. They established that;

i) The use of antimicrobial in fish farms was associated with the emergence of resistance variants of bacteria associated with fish diseases.

ii) The resistances in these bacteria were encoded by genes that were located on plasmids.

iii) Theses plasmid-located genes could be transferred to terrestrial enteric bacteria.

Since that time there has been a huge volume of research performed and our understanding of the molecular basis of resistance and of resistance transfer mechanisms has increased phenomenally. We have also acquired significantly more information on the use, fate and local impact of antimicrobials in aquaculture.

It is, however, unclear that, with respect to the extent of the risk to human health, we are now in a position to say anything more than was said in 1971. Numerous agencies and expert groups have addressed the issue of the risk to human therapies of antimicrobial use in food animals but none have been able to formulate any substantial conclusions as to the role of aquacultural use of antimicrobials.

It is certain that the adverse effect, resistance to therapeutic antmicrobials in bacteria associated with human disease, has occurred. The question that has proved difficult to answer or to estimate is the part, if any, that aquacultural use of these antimicrobial agents played in the precipitation of that adverse effect? In a paper delivered to an OIE meeting on risk analysis in aquaculture in 2000 Smith (2001) argued that;

> *"The primary factors that have led to the emergence of antimicrobial resistance as a problem in human medicine are the enormous adaptive ability of bacteria coupled with the use and gross misuse of these agents by the medical profession. Any effect, in terms of human health, resulting from the use of these agents in aquaculture is, by comparison extremely minor."*

He also argued that our failure to make progress in establishing the contribution of aquacultural use of antimicrobials to the problem of resistance in human disease organisms could not be attributed to a lack of research interest. The reason for our repeated failure must either derive from the intractable nature of the problem (NRC, 1999) or because we have been approaching the problem by asking the wrong questions.

In preparing this briefing document we have taken the position that our failure to make meaningful progress in 35 years has been related to our failure to formulate the appropriate research questions.

For this reason we have attempted to use the risk analysis framework to develop systematically a set of questions which need to be addressed if we are to be in a position to make a science-based risk analysis of the issues raised by the use of antimicrobials in aquaculture.

"The scientific mind does not so much provide the right answers as ask the right questions."

Claude Levi-Strauss

"Science is built of facts just as a house is built of bricks, but a pile of facts is no more a science than a pile of bricks is a house."

Henri Poincaré

"An expert is someone who knows some of the worst mistakes that can be made in his subject, and how to avoid them."

Werner Heisenburg

"There is nothing more frightful than ignorance in action."

Johann Wolfgang von Goethe

1. Introduction

1. 1 Benefits of aquaculture

Aquaculture is a worldwide activity. In different countries it provides a major source of essential food or provides a major source in income.

The following comments on the importance of aquaculture are taken from the FAO report "Trends in Global Aquaculture Production: 1984-1996" by Krishen Rana and Anton Immink of the Fishery Information, Data and Statistics Service (FIDI).

"*As we move into the next millennium finfish and other aquatic products will be in greater short supply as domestic and international demand for both high and low valued species increases due to rising populations, living standards and disposable incomes. With globally dwindling yields from many traditional marine and inland capture fisheries, shortfalls in supply of aquatic products will largely need to be met from culture through increased utilisation and output of current and hitherto uncultured species.*"

"The potential of aquaculture to meet the challenges of food security and to generate employment and foreign exchange is clearly demonstrated by the rapid expansion of this sector, which has grown at an average annual rate of almost 10% since 1984 compared with 3% for livestock meat and 1.6% for capture fisheries production."

"Aquaculture represents one of the fastest growing food producing sectors, providing a product that is an acceptable supplement and substitute to wild fish and plants. By 1996, the total production of cultured finfish, shellfish and aquatic plants reached 34.12 million t which was valued at US$46.5 thousand million (billion). This represents an increase of around 11.0% and 6.2% over 1995 quantity and value, respectively…. Much of the

reported increase originated from the Low-Income Food-Deficit Countries (LIFDCs), in particular China."

"For food fish (fish destined for human consumption), over a quarter of total world supply (in 1996) was derived from aquaculture."

Aquacultural production has approximately doubled between 1996 and 2004 and during this period the yield from capture fisheries has remained almost static. It must, therefore be assumed that aquacultures contribution to the global total of aquatic products consumed by humans must have increased to well above 25%.

The importance of aquaculture has only been increased by the depletion of natural stocks of feral fish and the worldwide increase in the demand for seafood. It should also be noted that as the bulk of aquacultural production occurs in LIFDC's it frequently plays a major role in meeting subsistence nutritional requirements.

In addition to its importance as a source of food aquaculture also plays a major economic role in many countries. Again with particular relevance to LIFDC's, it is a vital source of employment, profit and foreign exchange earnings.

1.2 Diversity of Aquaculture.

The aquaculture industry is extraordinarily diverse. This diversity can be illustrated under a number of headings

1.2.1 Species farmed

In contrast with terrestrial farming systems, where the bulk of global production is based on a limited number of animal and plant species, the farming of more than 210 different aquatic animal and plant species was reported in 2000. However, the vast bulk of aquaculture production is composed of a small number of species. In 2000, 29 species accounted for 78% of the world production but it should be noted that these species belong to a number of different phyla.

Although the culture of high-priced species such as shrimp or salmon often receives more attention, it is important to note that low-value inland finfish (carp, tilapia…) produced in extensive or semi-intensive systems comprise the bulk of world aquaculture production.

The feed supplied to farmed fish will vary depending on both the species being farmed and the stage of the life cycle. The various feeds used in the various branches of the industry include commercially produced and processed diets at one end of the scale to untreated fishmeal mixes compounded on-farm at the other. In some, mainly subsistence, farms, fish nutrition is supplied indirectly by adding animal or in some cases human sewage to the rearing waters. In feeding juveniles, live algal or rotifer cultures, normally grown locally, are used as feed

1.2.2 Farming environment

The salinity of the waters used to rear fish varies from fresh water through to full oceanic salinity or higher. In 2000, more than half of global aquaculture production originated from marine, brackish or coastal water. Although brackish water production represented only 4.6% of total global aquaculture production by weight in 2000, it comprised 15.7% of total production by value. The main species groups reared in freshwater were finfish. High value crustaceans and finfish predominate in brackish water and molluscs, finfish and aquatic plants in marine waters.

Across the world the water temperatures at which fish are farmed vary over a 30º C range. Diurnal and annual temperature fluctuation can be considerable in some farming enterprises.

1.2.3 Farm structures/locations

There is a great diversity of structure within which fish farming is practiced. Some farms, using either fresh or marine waters, operate in enclosed recirculating systems. Others operate in raceways, lagoons, ponds, tanks or cages. Still others, mainly bivalve farms, grow their animals in structures placed in the open water.

The location of farms also varies. Fresh water farms may operate using spring or bore-hole water or they may operate in lakes of varying sizes or they may operate using water from rivers that may or may have been previously exposed to other anthropogenic influences.

Fresh water farms may also vary with respect to where or how they discharge water after they have used it. Some will discharge directly and untreated back into a river or lake whereas others will subject the water to filtration and/or other treatments before discharge.

The variations in the locations of seawater farms involve such parameters as proximity to the shore and land run-off, water depth and current flow. Marine farms can be located in relatively deepwater over a kilometre from shore and in strong tidal currents, but may also be located as on-shore walk-on facilities. They may also be located in areas with low currents or which experience little net water movement during a tidal cycle.

1.2.4 Size and economics of fish farms

Fish farms vary in size from small-scale farms involved in subsistence food production though small-scale commercial units through to large industrial scale productions units that may be operated by multinational companies. The same species are often cultured both at small scale and industrial scale. For instance, approximately 80% of the shrimp production in Asia is coming from small-scale aquaculture (1-2 hectares) while 100% of the shrimp production in Latin America comes from industrial aquaculture.

Two other factors tend to correlate with farm size. The larger farms are more often involved in international trade of their products whereas smaller farms often sell into very local markets. The frequency with which farm personnel have received any training in fish health will also vary with farm or company size. The employment, in-company, of vets or people with specific qualification in fish health tends to occur only in the very largest companies.

1.2.5 National economies

Fish farming is practiced in countries that have a variety of economic circumstances. Developing countries contribute to more than 90% of the total world aquaculture production with LIFDCs (Low Income Food Deficient Countries) accounting for more than 82% of the total. The LIFDC contribute more than 80% of the world finfish production of which 95% is derived from inland freshwater fish culture.

Developed countries contributed to less than 10% of the world aquaculture production.

1.2.6 National availability of scientific and technical support

Aquaculture is practiced in countries with very widely differing scientific and technical infrastructures. In general, the availability and the degree of sophistication of the scientific and technical support available to an aquaculture industry parallels the economic status of the country in which it operates.

It should also be noted that there has been a very great variation in which the international scientific community has concentrated on the issue raise by the farming of different species. Those species farmed in countries with highly developed economies and scientific infrastructures, such as salmon, have been the focus of significant research. The research effort given to species normally farmed in developing countries have received very much less attention.

1.2.7 General regulatory environment

Regulations governing aquaculture production vary greatly between countries. The extent to which any regulations that do exist are actually enforced also varies. Although it can be suggested that enforcement of any national regulations is often insufficient in developing countries it is not safe to assume that the degree of enforcement in developed countries is uniformly rigorous.

Aquaculture products directed towards the international trade are subjected to regulations that need to be respected by the producing countries in order to reach the market. Export oriented farmers need to comply with target market regulations, often more restrictive, in addition to their own regulations. Small farmers have more difficulty to access this information often due to a low technical level. However, the recent appearance of very dynamic producers associations is allowing the flow of information and the support to small farmers. Aquaculture products directed towards the domestic market are unlikely to be subjected to health-based regulations and therefore compliance with the regulations is not generally required and cannot be assumed.

1.2.8 National regulation of availability of antimicrobials

The extent to which general access to antimicrobials is regulated varies from country to country. In some countries antimicrobials for human and veterinary use are strictly regulated as prescription only medicines. In these countries the movement towards prudent use is actively being pursued by health professionals. At the other extreme are those countries where these drugs are freely available as over the counter products and health professionals have little opportunity to promote prudent use.

There is a general trend for the control of antimicrobial use in aquaculture to follow the national pattern of the control of the use of these agents in humans and animals.

1.3 Disease in aquaculture

Diseases are a primary constraint to the growth of many aquaculture species. Although the capability to manage aquaculture health issues has increased tremendously in the last 30 years, the rapid and on-going development of all aquaculture sectors continuous to "raise the bar" with new challenges. This is particularly apparent with increased interest in species diversification and new grow-out techniques.

Precise per annum figures of consequences of disease losses are difficult to pin-down, but some estimates are available, e.g. losses due to EUS (Epizootic Ulcerative Syndrome) in several Asian countries before 1990 exceeded US$10 million. In Thailand, losses between 1983-1993 were estimated at US$100 million. Report of shrimp disease losses range from US$400 million in China in 1993, US$17 million in India in 1994 or US$280 millions in Ecuador in 1999. In Western Europe, annual losses due to VHS (viral haemorrhagic septicaemia virus) were estimated at US$60 million. These estimates have been obtained during periods when the industry was affected by acute epidemics, mainly due to viral diseases. Often, losses due to chronic diseases, poor survival and low performance are not considered as a disease problem but a production cost. The cost of these types of diseases is likely to surpass the cost of the acute ones. A global estimate of disease losses to aquaculture by the World Bank in 1997 was in the range of US$3 billion per annum.

In addition to the obvious effect of large-scale aquaculture losses on rural communities, diseases also cause considerable financial impact on investor confidence. These losses are even more alarming where the success or failure of a harvest will determine the raising of families above or below the poverty threshold.

Again the general pattern of disease experienced in aquaculture depends on the species being farmed and the environmental situation of the farm.

With respect to shrimp culture, for example bacterial diseases are more relevant during early stages of development than in grow out. The higher environmental and feed quality demand and the larval increased susceptibility to bacterial infection/colonization leads to poor survival. Under "known optimal" rearing conditions survival rate of aquaculture animals range from 1%-40% (from egg to post larvae). However, bacterial diseases during grow out continue to be a common problem. Roughly, two types of bacterial diseases can be encountered. The ones caused by highly specific pathogens and the ones caused by opportunistic pathogens such as *Vibrio* spp. or motile Aeromonads.

1.4 Antimicrobial agents in aquaculture.

1.4.1 The uses of antimicrobial agents.

The rationale for antimicrobial agent use again shows the diversity that was demonstrated (section 1.2) for the production systems. However, the use of antimicrobial agents as growth promoters is not practiced in any branch of this industry.

In European and North American finfish farming (responsible for less than 10% of the world aquaculture production) antimicrobial agents are more or less exclusively used in metaphylaxis. That is, treatments are only administered to populations containing infected and moribund members. The vast majority of administrations of antimicrobials involve the incorporation of these agents into feed either in food-compounding plants or by farm workers on-farm. The administration of agents via injection or by bath occurs in only a very small minority of cases and normally only under very specific conditions. In these industries the majority of treatments are carried out under the direction of a veterinarian and are associated with laboratory diagnosis and susceptibility testing of the presumed aetiological agent. In these industries deliberate prophylactic use of antimicrobial is very rare or non-existent.

With respect to shrimp culture, bacterial diseases are more relevant during early stages of development than in grow out. During these stages, antimicrobials are often used in a prophylactic manner, and this is true particularly in small-scale farms. In shrimp farming it is common for antimicrobial to be administered by bath treatment or by additions to the rearing waters. Prophylactic treatments are, however, rarely used to control the infectious disease problems that are encountered in the grow-out phase. In the farming of this species the frequency of laboratory diagnosis and susceptibility testing varies and is more common in those industries where the farm sizes or companies involved in farming, are large. In small-scale commercial production any technical back up is rare and laboratory diagnosis and susceptibility testing are very uncommon.

It should be noted that possibly the majority of world aquaculture production occurs at the very small scale of subsistence farms. In common with much peasant agriculture these farms work on a low-input, low-output basis. Antimicrobial agent use in these farms, for any reason, is rare.

Ornamental fish culture represents another aspect of aquaculture. The use of antimicrobials in this industry and by private fish owners supplied by this industry is largely unregulated and undocumented. Thee are, however, grounds for believing that the use in this area may be significant.

1.4.2 Non-governmental regulation of antimicrobial agent use in aquaculture

There are a number of factors, other than government regulation, that have operated to limit or regulate the extent of antimicrobial agent use in aquaculture.

At an individual farm level antimicrobial agents can represent a significant component of production costs. There are, therefore, internal economic reasons driving the search for and implementation of alternative disease control strategies. In general the larger and more sophisticated fish farming companies become, the more they are able to address these alternatives.

Farmers or industry related associations have been the key factor in enforcing existing regulations or self-controlling their own production by developing best-practice guidelines. The introduction of 'quality marks' and other techniques have been effective tools in this regard. In less developed countries these organisations have been vital in education. They represent the most effective way of addressing the lack of knowledge on antimicrobial use in both, users and suppliers.

Market place factors have also had a serious impact on antimicrobial agent use. Increasingly, the quality standards of large-scale retailers are a major factor that must be considered in designing production and husbandry protocols. Often these retailers operate stricter standards, particularly with respect to drug residues, than many national or international governmental agencies. Possibly as important as the nature of their standards, are the penalties that retailers enforce in situations of non-compliance. Failing to meet the quality standards with respect to residues set by retail outlets may spell total economic ruin for a producer.

1.4.3 The regulation of antimicrobial agent availability in aquaculture

As discussed in 1.1.8 and 3.2.1 the extent of governmental regulation of the availability of antimicrobial agents for use in aquaculture varies widely from country to country and the extent of national regulation tends to follow that governing antimicrobial agent availability for human and animal therapies. Even in countries where regulations are well developed and policed there is a considerable variation in the number of agents that have been licensed and the conditions that have been attached to their use (see section 3.1).

There is, however, a large number of countries where either regulations exist that are not enforced or where there has been a failure to license any products for aquaculture. In these countries antimicrobial agent use is either limited by the availability of agents in the open market or, in more developed countries, by the willingness of veterinarians to prescribe off-label use.

It would be a mistake to assume that a failure to licence products for aquaculture is purely a problem in undeveloped countries. There are developed countries, including some with significant aquaculture production, where licensing of products has not been treated with sufficient urgency.

Although licensing is an expensive process and will inevitably increase the cost of therapeutants there are a number of consequences that tend to follow from inadequate licensing. These would include a lack of pharmacokinetic data (and therefore withdrawal time) for the unlicensed agents, a lack of standardized protocols for their use and a failure to develop safety protocols for their handling, storage and application.

1.4.4 Paradoxes in the regulation of antimicrobials for aquaculture.

There are clear arguments that the aquaculture industries in some countries are negatively impacted by the presence of rigorous regulation whereas in other countries the negative impacts derive from the absence of regulation.

In some highly developed economies, the use of antimicrobial agents in aquaculture is allowed only under prescription of licensed products in locations where specific permission to discharge into the environment has been obtained. Here the cost of licensing presents a major issue. As any industry operating in such an environment reduces, via use of improved husbandry and, the incidence of disease associated with bacterial infection, the sales of antimicrobial agents declines. As sales decline the relative cost of licensing increases. These countries characteristically have only very few (2-3) licensed products and the reduction in sales is having the effect that this number is decreasing as companies withdraw products whose sales cannot justify the licensing costs involved. Thus industries that have behave responsibly and appropriately with respect to health of their fish are increasingly being presented with a situation where they have no antimicrobial cover that would allow them to respond to any new emerging disease. Equally, these highly successful and well-run industries cannot contemplate investigating the farming of new species where the lack of knowledge of appropriate husbandry and lack of appropriate vaccines would necessitate, at least during the developmental phase, the use of antimicrobials.

Another situation exists in countries, and these are not just those in the developing world, where licensing of antimicrobials for aquaculture has either not been pursued by the national authorities or where regulations that exist are not enforced. This under-regulation or the insufficient enforcement has a number of consequences. It tends to lead to excessive and inappropriate use and this leads to the more rapid emergence of resistance. It facilitates the marketing and use of agents of poor quality and it tends to reduce the development of scientific knowledge of the appropriate and efficient use of antimicrobials.

In Asia, where small farmers with low technical knowledge, dominate the industry, antimicrobials are often available in the open market in a wide range of commercial products with dubious quality guaranty. Health support to the farmers is insufficient and diagnosis services for disease outbreaks, susceptibility testing antimicrobial prescription and technical back up is rare. The under-regulation or the insufficient enforcement of antimicrobial use leads to excessive and inappropriate use.

1.5 Benefits of antimicrobial agents to aquaculture

As with land-based farm animals, farmed fish are subject to diseases associated with bacterial infections. The impact of these diseases on animal health and the economics of farming disease can be limited by good husbandry and by the use of appropriate prophylactic measures. However, these measures rarely, if ever, have totally prevented the incidence of infectious disease in farmed animals. Thus, with the possible exception of ranching, some degree of antimicrobial cover has been found to be necessary in all animal production systems.

In aquaculture, the need for antimicrobial agent use arises in a number of specific situations.

1.5.1 Failure of disease prevention measures

Even when preventive measures such as good husbandry and vaccines are incorporated into the health management schemes of an aquaculture enterprise, this does not guarantee success. Aquatic animals subjected to stresses above and beyond what the organism is capable of enduring, such as unusually high water temperatures, nutritional toxicity or insufficiencies may develop depressed immune systems and compromised non-specific barriers (e.g. skin) enhancing susceptibility to bacterial or viral infection.

1.5.2 Emerging infectious disease

Emerging infectious disease would be defined as the appearance of a new disease/syndrome that has previously not been identified, but which increases in importance with time, causing changes in health management control measures, and impacting production practices, development, and possibly marketing of aquaculture products.

As our world becomes figuratively smaller and smaller, with better and faster transport, the risk of exotic disease transmission becomes greater. The relatively uncontrolled trade in the pet fish industry represents a, largely un-regulated, risk factor here.

1.5.3 Re-emerging infectious disease

Re-emerging infectious disease would be defined as a disease of importance that has been identified in the past, and after a period of quiescence or relative control of the disease, it becomes problematic once again. A number of factors can contribute to the re-emergence of a disease.

1. The entry of non-regulated fish or other organisms which are carriers of pathogens (ex. fish and or vectors in ship ballast, tropical fish importation), with subsequent transmission to wild or cultured stocks
2. The importation of fish for culture purposes, which are latent carriers of a pathogen, with subsequent transmission to cultured stocks
3. Changes of pathogenicity of disease causing organisms (ex. mutations or gene exchange events leading to changes in viral serotype or bacterial virulence factors)

1.5.4 New Species development

As mentioned previously, there is a large variety of species already cultured within the aquaculture industry (1.2.1) and new species are continually being evaluated for their aquaculture potential. Often in developing the farming technology needed for a new species, there is a lag phase between identification and characterization of disease agents and the development of disease control procedures such as vaccination or domestication breeding programs. Thus, the availability of antimicrobial agents for previously undiagnosed disease conditions or for known bacteria in novel contexts is essential for new species development. In the case of properly diagnosed bacterial diseases, selected antimicrobials are necessary to ensure the viability of the new species until alternative control measures can be incorporated into the production and health management program.

1.5.5 Developing culture technology

The development and utilisation of new culture technologies may change the manner in which pathogenic agents interact with the species concerned. As a consequence, they may, particularly during their development phase, require the availability of antimicrobial agents to provide disease cover for unforeseen epizootics.

Examples might include recirculation technologies, using elevated growing temperatures, using higher in-tank densities (ex. with liquid oxygen) and high concentrations of farms in limited geographic areas.

1.5.6 Animal welfare

The concerns for animal welfare are based principally on the premise that animals feel pain. Until recently, fish have escaped this sometimes anthropomorphically influenced judgement because of relatively unfamiliar behaviour and reactions to painful stimuli. Compared to warm-blooded animal welfare recognition, fish welfare is in its infancy. Aquatic animal health welfare is important for several reasons, and concern about these issues is likely to increase both within the veterinary profession and within consumers.

Although there is still a differing of opinions, more and more research is supporting the idea that fish are capable of feeling pain or at the very least discomfort. Ethically speaking, when using fish for food or research purposes, this “discomfort” needs to be minimised.

Considerations of animal welfare therefore place an obligation on those in charge of them to prevent, wherever possible, the occurrence of disease. Thus, the failure to provide good water quality, adequate nutrition, ideal stocking densities, growing temperatures, adequate transport conditions, and humane euthanasia, must be considered as unethical. Clearly, those in charge of fish have an obligation to provide adequate conditions for the animals under their care. However, as argued above, even when all reasonable care is taken, it is impossible to totally avoid the occurrence of overt disease.

Thus, ethical considerations lead to the conclusion that, for the well being of the farmed fish, therapies must be made available to treat the diseases that may occur in aquaculture enterprises.

The goal of any aquaculture operation is to sell a safe, wholesome product to consumers. In an environment where more and more consumers consider how their food is processed, treated and raised, it is imperative that fish growers be capable of demonstrating not only the safety and nutritional benefits of their product, but their concern for its welfare as well. This could become a product acceptance and marketing issue in the future.

1.6 Adverse effects of antimicrobial agents use in aquaculture

The adverse effects of antimicrobial use in aquaculture can be considered as those associated with direct toxicity of the biologically active chemicals or with increased frequencies of resistance to these agents that may occur in various bacterial groups.

These issues are the subject mater of the remainder of this document.

Bacterial resistance to antimicrobial agents is a complex issue and a few general comments will be made here.

Many discussions of bacterial resistance to antimicrobial agents appear to presume that everybody has a clear understanding of what the term resistance means. This is, however, an unwarranted assumption.

There has been much confusion generated by failures to define, either theoretically or empirically, the meaning being given to the term resistant.

A reading of the literature relating to the impact of antimicrobial agent use in aquaculture of the frequencies of resistance reveal that confusion as to what resistance is and how it can be measured are frequent. In many papers the data presented is largely meaningless due in no small part to the failure by the authors to understand these issues.

1.6.1 Theoretical definitions of resistance.

The validity of any definition of resistance depends on the context within which it is to be used. Two theoretical definitions can be considered.

In a general microbiological context a bacterial clone can be termed 'resistant' if it has the ability to function, survive or persist in the presence of higher concentrations of an antimicrobial agent than the members of the parental population from which it emerged. More loosely, a species can be termed resistant if its members have the ability to function, survive or persist in the presence of higher concentrations of an antimicrobial agent than the members of other species. Resistance, therefore, unlike properties such as cell shape or the ability to produce acid from glucose, is not a property that can be determined by studying a single strain. Resistance is always a relative term. It can be determined only by a comparison, under identical conditions, of the properties of two, or more, strains or species.

Resistance can also be defined in a clinical context. Here it refers to a bacterium whose contribution to a disease process is not inhibited by the therapeutic administration of the antimicrobial agent to a host or, in metaphylactic treatments, to a population. When used in this sense, resistance is a function not only of the phenotype of the bacterium but also of the properties of both the therapy and the host. Importantly, resistance in this sense cannot be determined simply from microbiological data alone. Equally importantly, the term resistance is host specific. A bacterium can be correctly termed resistant with respect to the therapy of a species of fish but may, because of host specific pharmacokinetic factors, be correctly classified as sensitive in the context of the therapy of a human infection.

1.6.2 Empirical determinations of resistance.

The practical determination of resistance or sensitivity in a bacterium is always a two-step process. The first is to obtain a measure of the susceptibility of the bacterium in laboratory media and the second is to interpret the meaning of that measure. Interpretation of laboratory susceptibility data involves the application of a breakpoint such that strain one side of the breakpoint are classified as sensitive and those on the other resistant. In many cases, tow breakpoints are employed and the strains falling between them are classified as belonging to an intermediate category.

It is important to note that breakpoints are neither absolute nor universal.

Any breakpoint is fundamentally linked to the laboratory susceptibility test protocols used to generate the data. Breakpoints developed for one protocol cannot be legitimately used for data generated by a different test protocol.

Breakpoints are species specific. A breakpoint developed for one species or bacterial group cannot automatically be assumed to be valid for a bacterium belonging to another group.

Clinically relevant breakpoints are host specific. The concentrations of an agent that can be achieved in a host during therapy influence the setting of clinically relevant breakpoints. As antimicrobial agents manifest different pharmacokinetics in different hosts, the breakpoints used for one host may not be relevant to another.

Clinically relevant breakpoints may vary depending on the mode of administration of the antimicrobial agent or the location of the bacterium in the host.

In fish, clinically relevant breakpoints may also have to be varied depending on the salinity or temperature of the water.

2. General statement of purpose.

The first step in a risk analysis is to develop a statement of purpose. This should specify the action being investigated and the potential adverse effects that are under consideration in the analysis.

Definition of the action

The use of antimicrobial agents in aquaculture

Definitions of the adverse effects

A Biologically mediated adverse effects

The increase of morbidity or mortality resulting from a reduction in the efficacy of a specific antimicrobial therapy of a disease as a consequence of the lack of susceptibility, to that agent, of the bacterium involved in the disease process.

B Chemically mediated adverse effects

The increase in morbidity or mortality resulting from the consumption of biologically active chemicals.

3 Hazards

The following definition of hazard adapted from the Codex Alimentarius document "PRINCIPLES AND GUIDELINES FOR THE CONDUCT OF MICROBIOLOGICAL RISK ASSESSMENT" CAC/GL-30 (1999) is used in this document.

A hazard is biological, chemical or physical agent with the potential to cause an adverse health effect. In terms of the action and the adverse effects identified above three classes of hazard can be identified.

3.1 Major classes of hazard

3.1.1. Antimicrobial agents

These can exert their effect either as a result of residues in food or via contamination of farm personnel during on-farm handling.

3.1.2. Resistant bacteria.

Bacteria that are associated with human, land-based animal or fish diseases and that are resistant to antimicrobial agents of significance in the control of these diseases.

3.1.3. Resistance genes

This hazard includes genes encoding resistance to antimicrobial agents of significance in human, land-based animal or fish therapies.

Under this hazard, the focus is on resistance genes that are capable of horizontal transfer. Thus, genes located on plasmids or associated with transposons or integrons are of most significance. As chromosomally located genes are normally inherited vertically, the hazards represented by bacteria possessing these genes are better treated under Hazard group 2.

3.2 Hazard group 1; Antimicrobials.

*3.2.1. **Range of antimicrobial agents used in world aquaculture.***

3.2.1.1 Europe

Few countries (with the exception of Norway) have compiled an authoritative list of antimicrobial agents used in their aquaculture industries.

With respect to the use of antimicrobial agents used in Europe, two sources have been used.

The first was a survey sent at the beginning of 2006, to more than thirty European agencies for Veterinary Medicinal Products (VMP), on antibiotics registered for fish in their countries.

Twenty-six countries answered the survey, among which six countries did not have any chemical VMP registered for fish.

Among the 20 countries that have (or will soon have) such products registered, nine only registered one active substance, four registered two actives substances, four registered three active substances, two registered four active substances, and only one country registered five different active substances.

At the European level, 8 different active substances and 50 distinct VMP are registered, mostly medicated premixes.

Instead of what would have been expected, the countries that registered the highest number of active substances and/or products are not the biggest farmed fish producers, but those that have the most ancient and diversified fish production.

Oxytetracycline is the most common antibiotic, with a wide range of national products: registered in 12 countries and it is commercialised as 19 different products.

In contrast, only five different products with trimethoprim-sulfadiazine are registered in seven countries, with one product being commercialized in four countries.

Oxolinic acid is registered in six countries (six products) and flumequine and florfenicol in five countries (and five products) each.

Three antibiotics are uncommon. Amoxicillin was registered in 3 countries and chlortetracyclin and sarafloxacin in one country each.

This survey shows that the therapeutic arsenal to treat bacterial fish diseases is very narrow in Europe, a threatening situation for the durable efficacy of available antibiotics.

A second source of information was less direct and was derived from a survey of laboratories performing susceptibility test of bacteria isolated from farmed finfish (Smith 2006). In this survey labs reported the agent to which the routinely reported susceptibility.

The survey of licensed agents is likely to produce an underestimate of the range of agents use as it will not include agents used off-label or agents used that are not licensed. The susceptibility testing survey may on the other hand represent an over estimate of the range of agents used. Table 1 below presents a comparison of the data obtained from the two sources and it is clear that there is a similarity in the picture that is obtained from both.

Table 1 Data on the range of antimicrobial agents used in European finfish culture

Agent	**Frequency of susceptibility testing in 29 laboratories Smith, (2006)**	**Frequency of registration in 20 European countries**
Oxytetracycline	28 (97%)	11 (55%)
Trimethoprim-sulfa	26 (90%)	7 (35%)
Oxolinic acid	20 (69%)	6 (30%)
Florfenicol	17 (59%)	5 (25%)
Flumequine	17 (59%)	5 (25%)
Enrofloxacin	12 (41%)	
Amoxycillin	11 (38%)	3 (15%)
Erythromycin	8 (28%)	
Ampicillin	5 (17%)	

Chloramphenicol, Ormetoprim-sulfa	4 (14%)	
Gentamycin	3 (10%)	
Naladixic acid, Furazolidone, Nitrofurantoin	2 (7%)	
Amikacin, Doxycycline, Marbofloxacin, Neomycin, Norfloxacin, Novobiocin, Penicillin and Streptomycin.	1 (3%)	

3.2.1.2 The Americas

In North America (the United States and Canada) only three classes of antimicrobials are registered for use in finfish . These include potentiated sulphonamides (ormetoprim-sulphadimethoxine and trimethoprim-sulphadiazine (Canada only), tetracyclines (oxytetracycline), and chloramphenicols (florfenicol). In South America oxolinic acid flumequine, florfenicol and oxytetracycline are registered for use in Chile.

3.2.1.2 Asia

In general, the range of antimicrobial agents available or in use in Asian countries is larger than in Europe or America.

Japan has a very large aquaculture industry. While the extent of use of antimicrobial agents is difficult to determine, it is thought to be considerable. There is published evidence of twenty-nine different antibiotics/antibiotic presentations authorised for use in Japan.

Estimates of antimicrobial agent use in other Asian countries are difficult to obtain but what data there is would suggest that a wide variety of agents are used. Some data can be obtained from the papers presented at the conference on the "Use of Chemicals in Aquaculture in Asia" held in Tigbauan, Iloilo, Philippines 20-22 May 1996. It should be noted that none of the papers differentiated between use on small-scale farms and large production units. There is anecdotal evidence that fewer antimicrobials are used on the larger farms.

A report from Vietnam recorded 122 antibiotic products being used in shrimp culture in that country. Many of these preparations contained similar agents, for example 77 contained quinolones, and so the total range of agents is probably much smaller.

Surveys from Sri Lanka indicated that erythromycin and tetracycline were the most commonly used antibiotics in the shrimp industry. Further, this report observed that these chemotherapeutic agents were used in an ad hock manner where farmers selected arbitrary dosages which were applied the agents as a bath or mixed with feed.

Reports from the Philippines also listed a significant number of antimicrobial agents used in shrimp culture (tetracycline, rifampicin, chloramphenicol, nitrofuran and erythromycin) and in the rearing of other food or ornamental aquatic animals (oxolinic acid, ormetoprim, sulfadimethoxine, sulfamerazine, sulfisoxazole, sulfisoxazole, trimethoprim/sulfadiazine, streptomycin, erythromycin, furazolidone, gentamycin, kanamycin, neomycin and nyfurpyrinol).

In India, a survey on the use of chemotherapeutants in carp farms in 1995 showed that in extensive farms 4 % used OTC and 2 % chloramphenicol, and in semi-intensive farms 12 % used OTC, 1 % chloramphenicol and 3 % other antibiotics. In extensive shrimp farms, 31 % used OTC, and 13 % other antibiotics; in semi-intensive shrimp farms 71 % used OTC, 3 % chloramphenicol and 2 % other antibiotics; in intensive shrimp farms 67 % used OTC.

3.2.2 Estimates (or lack of them) of the amounts of antimicrobial agents used in aquaculture

The data on the amount of antibiotics used in aquaculture are scarce but increasing, even if this question is still considered as highly sensitive because of commercial consequences on international trade.

However, more and more countries are now following the early example of Norway, that started publishing the amounts of antimicrobials used in salmonids farming at the beginning of the 1990s, and has continued to the present day (www.fiskerifir.no).

There are often difficulties comparing such data, because of the lack of homogeneity in the way they are presented. Some are given in annual weights of commercial products, others in weights of active principles, more often in amounts of active principles per tonne of animal produced. The biomass of treated animals would be an interesting parameter to obtain in order to evaluate the real therapeutic importance of each antibiotic, but is rarely available. In some cases, usage of therapeutic agents is only expressed as annual expenditures, or as a percentage of the farms using them, which can hardly be compared with any other usage data.

In Norway, the amounts of active substances used in salmon and trout farming increased from 3660 kg in 1980 (81 % OTC, 11 % sulfamerazine, 8 % TMPS) to a peak of 48,570 kg in 1987 (56 % OTC, 32 % nitrofurazolidone, 8 % OA, 4 % TMPS). The fish production also increased steadily in the same period, but the antibiotic use increased at a higher rate: 5 g/t of fish in 1980, 30 g/t in 1984 and more than 100 g/t in 1987 (Grave *et al.*, 1990).

From 1988 on, the use of antibiotics decreased regularly, most of all during the 1990s with the massive development of vaccination (Markestad & Grave, 1997). It should be noted that Hiney & Smith, (2000) have argued that improvements in husbandry also played a major role in this decline.

During the 1996-1999 period, the amounts of antibacterials used in Norwegian fish farming were approximately 1 tonne of active substances per year, and slightly less during the 2000-2004 period (Lunestad & Grave, 2005). In spite of a substantial increase in the farmed fish production, the amounts of antibacterial agents used in Norwegian aquaculture have decreased by 98 % since 1987.

In 2004, the antibacterials used to treat bacterial fish diseases in Norway were oxolinic acid (89 %), florfenicol (10 %), and flumequine and oxytetracycline (1 %), for a total amount of 1159 kg.

In Swedish fish farming, oxytetracycline was the dominant antibacterial drug between 1988 and 1993 (Björnerot *et al.* 1996). The heaviest usage of oxytetracycline was in 1990, and there was a marked decrease in the following years that resulted in a decrease of the total usage of antibacterial drugs in fish farming. The usage of antibacterials increased from 5 g/t in 1988 to 14 g/t in 1990, and decreased to 4 g/t in 1993. The amount of antibiotics used in Swedish fish farming decreased at the beginning of the 1990s to stabilize around 50 kg of active substance per year between 1998 and 2003 (SVARM, 2003). The use of antibiotics in 2003 is estimated to less than 2 g per ton of fish produced. The use of antibiotics for Arctic char (*Salvelinus alpinus*) is however 15 times higher than for rainbow trout.

During an environmental review of the British Columbia salmon farming industry, it was shown that in 1997 BC required 156 g of antibiotics to produce one metric tonne of Atlantic salmon (Fraser *et al.*, 2004). Oxytetracycline accounted for 90 % of drug use in BC, prescribed at the dose of 75 to 100 mg/kg BW, thus partially explaining this amount of drugs per tonne of fish.

In France, The National Agency for Veterinary Medicines (AFSSA-ANMV) publishes since 1999 a yearly survey on sales of antibiotics for veterinary use, as declared by pharmaceutical companies (Moulin & Roux, 2005). For farmed fish, the estimated weights of actives principles used were of 7,20 t in 1999, 5,96 t in 2001 and 4,22 t in 2005. A recent survey on prescriptions in French fish farms showed that antibiotics mostly used in 2001 in terms of tonnage of treated fish were FLU (31 %), TMPS (31 %), OTC (14 %), OA (13 %) and FFC (11 %). These therapeutic patterns markedly changed since then, and in 2005 antibiotics mostly used were TMPS (36 %), OA (22 %), FLU

(19 %), OTC (15 %) and FFC (8 %). The amount of antimicrobials per tonne of fish produced were 93 g/t in 2001, and 82 g/t in 2005, a decrease of 11.4 %.

In Vietnam, imports of drugs for aquatic use increased from approximately 640 t in 2001 to 85,500 t in 2004, from Thailand, USA, India, Taiwan, Germany, Hong-Kong, Indonesia, France and China (Van, 2005). Marine fish farmers use at least 8 antibiotics for disease treatment. Shrimp hatcheries use 5 to 19 different antibiotics during each production cycle.

No mention of amounts of antibiotics used for aquaculture in other Asian countries could be found (Arthur *et al*., 2000). There are comparisons (Table 2) however of estimated expenditures (in $ US/ha/yr) on chemicals, excluding fertilizers, but including antiparasitics and disinfectants, in several countries:

Table 2 Cost of chemotherapy ($ US/ha/yr) in various Asia countries

		Bangladesh	Cambodia	Nepal	Pakistan	Sri Lanka	Viet Nam
Carp	Extensive	0.7		12.6	0.7		0.1
	Semi-extensive	7.3	0.95	10.9	7.8		2.9
Shrimps	Extensive	4.5	0			15.3	2.3
	Semi-extensive	477.7				235.7	44.6
	Intensive		791.9			276.6	

3.2.3 Direct toxicity to or adverse effects in humans

Many of the issues raised by consideration of the consumption by humans of antimicrobial agents in fish are similar to those raised by these residues in other food stuffs The antimicrobial agents that may be present in farmed fish that reach the market place are not unique to aquaculture. The regulatory systems, the general principles as well as the basic parameters such as ADI and maximum residue levels values that have been established during consideration of residues in other animals still have relevance to fish.

The key factor that is unique to animals farmed in aquaculture is the pharmacokinetics and residue kinetics that are appropriate for each species. Here not only the variety of fish presents problem but also their poikilothermic nature.

Reimschuessel et al. *(*2005) have compiled a database of studies that presented data on the pharmacokinetics of antimicrobials in aquatic animals. These data reveal that there are considerable differences in the residue half-lives that have been estimated even for the same agent in the same species and therefore that withdrawal times cannot be established with a high degree of confidence. This has normally been dealt with by regulatory agencies by including a significant safety margin in the required withdrawal times.

The other observation that can be made from the database is that although there are possibly an adequate number of studies of the pharmacokinetics of some antimicrobials in some fish species, there are some species, notably shrimp, where there is a serious lack of adequate data.

3.2.4. Relationships between agents used in aquaculture and agents of importance in human and veterinary medicine

The most important relationship that has to be investigated between agents used in aquaculture and those of importance in human and veterinary medicine is that mediated by resistance.

Table 3 details the antimicrobial agents used most frequently in aquaculture and the agents to which they are most commonly linked by cross-resistance.

Table 3 Agents linked by cross-resistance patterns

Agents most commonly used in aquaculture	**Cross resistances**
Oxytetracycline	Other tetracyclines including doxycycline

Trimethoprim-sulfa / Ormethoprim/sulfa	Sulfonamides and potentiated sulfonamides
Oxolinic acid, Flumequine	Other quinolones increased susceptibility to mutations for resistance to newer fluroquinolones
Florfenicol	Chloramphenicol
Amoxycillin	Beta-lactam sensitive penicillins 1st generation Cephalosporins
Erythromycin	Some cross resistance with other macrolides

The World Health Organization has produced a document that provides a classification of critically important antibacterial agents for human that was specifically designed for risk management strategies of non-human use of antimicrobials (WHO, 2005)

They classified antibacterials using two criteria. Criterion 1 was that the agent was the sole therapy or one of few alternatives to treat serious human disease. Criterion 2 was that the agent was used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases causes by organisms that may acquire resistance genes from non-human sources

Those agents that meet both criteria were classified as critically important antimicrobials and those that meet one of the two were classified as highly important.

Tables 4 & 5 below have been abstracted from the WHO (2005) document. They include agents rated as critically or highly important for human therapy that can be considered relevant to the use of antimicrobial in aquaculture outlined in section 3.2.1 above or to those identified as demonstrating cross-resistance to those agents.

Table 4

Antibacterial agents considered as sole therapeutic option or one of few alternatives to treat serious human disease.

Class	Agents	Limited therapy for
Tetracyclines	chlortetracycline doxycycline minocycline oxytetracycline tetracycline	Infections due to *Chlamydia* spp. and *Rickettsia* spp.
Penicillins, aminopenicillins	ampicillin ampicillin/sulbactam amoxicillin amoxicillin/clavulanate piperacillin piperacillin/tazobactam*	*Listeria*
Quinolones	cinoxacin nalidixic acid pipemidic acid ciprofloxacin enoxacin gatifloxacin gemifloxacin levofloxacin lomefloxacin moxifloxacin norfloxacin ofloxacin sparfloxacin	*Campylobacter* spp., invasive disease due to *Salmonella* spp., and MDR *Shigella* spp. infections

Table 5

Antibacterial agents used to treat diseases caused by organisms that may be transmitted via non-human sources or diseases causes by organisms that may acquire resistance genes from non-human sources.

Class	Agents	Potential transmission
Sulfonamides, DHFR inhibitors and combinations	para-aminobenzoic acid pyrimethamine sulfadiazine sulfamethoxazole sulfapyridine sulfisoxazole	*Enterobacteriaceae* including *E. coli* from non-human sources

	trimethoprim	
Cephalosporins, 1st generation	**c**efazolin cephalexin cephalothin cephradine	*Enterobacteriaceae* including *E. coli* from non-human sources
Cephalosporins, 2nd generatio	cefaclor cefamandole cefuroxime loracarbef	*Enterobacteriaceae* including *E. coli* from non-human sources
Penicillins, aminopenicillins	ampicillin ampicillin/sulbactam amoxicillin amoxicillin/clavulanate piperacillin piperacillin/tazobactam*	*Enterococcus* spp. from non-human sources
Quinolones	cinoxacin nalidixic acid pipemidic acid ciprofloxacin enoxacin gatifloxacin gemifloxacin levofloxacin lomefloxacin moxifloxacin norfloxacin ofloxacin sparfloxacin	*Campylobacter* spp. and *Enterobacteriaceae* including *E. coli* and *Salmonella* from non-human sources

There is a preliminary list of Antimicrobials of Veterinary Importance compiled by OIE which needs to be further refined. The above exercise cannot. therefore, be performed in the veterinary context at this stage.

3.2.5 Fate of antimicrobial agents used in fish farming.

There was a period, during the 1990s, of concentrated study of the fate of antimicrobial agents used in marine salmon farming. These studies largely followed the intense use of these agents in Norway and Scotland in the late 1980s. Initially concerns were raised by the report of high and persisting concentrations of oxytetracycline in under-cage sediments Samuelsen et al (1992b). However, this study was performed at an experimental fish farm where there was massive over-presentation of medicated feed and subsequent studies have revealed that the data it presented was highly unrepresentative of the situation in commercial farms in Norway, Scotland, Finland, the USA and Ireland.

The following is a summary of the conclusions that can be drawn from the published data obtained from salmonid farms in Northern Europe and Northern America.

The majority of antimicrobial agents leave marine salmon farms in the water column. The concentrations detected in this component are, however, unlikely to reach the minimum concentration required to exert any selection for resistance in this environmental matrix.

The highest environmental concentrations occur in the under-cage sediments and are probably confined to a depth of 10 cm and an area of approximately twice the cage area.

The persistence of agents in sediments depends on current flow and oxygen concentration but half-lives of less than one month should be achieved at well run farms.

3.2.5.1 Fate of antimicrobial agents used in marine salmonid farms

The majority of studies have been performed in marine or brackish water salmonid farms. Essentially antimicrobial agents used in fish farms can have a number of fates.

i) Entry into farm workers

ii) Retention in the farmed fish

iii) Degradation in the farm environment.

iv) Entry into farm associated with feral fish

v) Entry into the water column

vi) Deposition on the under farm sediments

i) Entry into farm workers

Although there are no studies on this issue it is reasonable to assume that some of the antimicrobial agents used in fish farms will enter the farm workers. Inhalation of dust from commercially prepared feeds represents one pathway that requires investigation. Levy et al. (1976) have presented evidence that such contamination may occur in land-based farms.

The exposure of farm workers to antimicrobial agents will be increased in situations where the agents are mixed with the feed on-farm.

ii) Retention in the farmed fish

The fraction of the antimicrobial agents administered to farmed fish that reaches that target animals is a function of the feeding efficacy and the various bioavailabilities of the agents. However, as few of the agents are metabolised in fish it can be assumed that the target fish only represent a transitory location for the agents.

There have been a very large number of studies of the pharmacokinetics of therapeutic in fish and most have focused on residue kinetics. Reimschuessel et al. (2005) have provided a searchable database that contains the material from nearly 3000 investigations. It should be noted that this database reveals a significant difference between the amounts of pharmacokinetic data that has been collected for different species. For example, it only contains data obtained in 5 studies of pharmacokinetics in shrimp.

iii) Degradation in the farm environment.

The environmental stability of the agents most frequently used varies significantly. Ampicillin and amoxycillin are unstable in aquatic environments. Oxytetracycline is subject to photo-degradation and even at 1 m depth has a relatively short half-life in water. The quinolones oxolinic acid and flumequine are slowly degraded but the components of the potentiated sulfonamides appear to be stable in the aquatic environment (Lunestad et al., 1995). Samuelsen et al., (1994) demonstrated that in general antimicrobial agents were very significantly more stable in artificial marine sediment mesocosms, with half-lives in terms of months rather than weeks.

iv) Entry into farm associated feral fish

There are data indicating that concentrations of antimicrobial agent administered to caged fish can be found in feral fish, crabs and filter-feeding bi-valves in the immediate vicinity of the cages (Samuelsen et al. 1992a; Ervik et al., 1994; Capone et al. 1996). With respect to the filter feeders, however, the fraction of the input found in these animals is very small and the half-life of the agents is very short (Coyne et al., 1997).

v) Entry into the water column

Data has rarely been acquired as to the concentrations of antimicrobial agents that can be found in the water surrounding fish farms. However, a simple calculation can illustrate the concentrations that can be expected in the water leaving a fish farm.

This example uses data relevant to the administration of oxytetracycline at a marine salmon farm. Oxytetracycline has been chosen because it was an agent frequently used in this industry in the 1990s and because, as a function of its low bioavailability, larger amounts of this agent are used per treatment than is the case with the other agents such as quinolones, potentiated sulphonamides, amoxycillin and florfenicol.

A reasonably typical situation would involve a cage containing 80,000 fish of average weight 300 g and stocked at 10 kg/ $m^{3.}$ If these fish are fed OTC at 100 mg/kg/day the daily input of the agent will be 2.4 Kg or 2.4 x 10^6 mg. If the fish are stocked at 10 kg/m^3 the volume of the cage will be 2,400 m^3= 2.4 x 10^6 l. Thus if the total daily input remains in the cage its maximum concentration would be 1 mg/l or 1μg/ml. However, the absolute minimum water exchange rate consistent with fish health would be 1 exchange per hour. Thus the peak concentration of oxytetracycline that could be predicted to occur in the water would be 0.04 μg/ml.

In attributing any significance to this estimate it should be noted that in a marine environment the divalent cation concentrations result in an approximately 90% reduction in the biological activity of oxytetracycline (as well as for quinolones). Thus, the maximum concentration of oxytetracycline that could exert a selective pressure for the enrichment of resistance would be equivalent to 0.004 μg/ml.

vi) Deposition on the under farm sediments

Smith (1996) reviewed 16 studies of the distribution of oxytetracycline in the sediments under commercial salmonid farms in marine and brackish waters. He concluded that under normal commercial conditions approximately 1% of the total input ended up in the under-cage sediments. The median concentration of oxytetracycline in the top 2 cm of these sediments was 1.7 μg/g (range 10-0.1μg/g).

Fewer studies have investigated the horizontal distribution of oxytetracycline but the limited data available is consistent with the model of Coyne & Smith (1996) that indicated the likely footprint to be no more than twice the area of the treated cages.

Although the available data indicates that only a small fraction of the antimicrobial agents used in marine farms enters the under-cage sediments it should be noticed that the highest concentrations occur in this component of the environment. The half-lives of the persistence of antimicrobial agents in field situations (Coyne et al., 1994) can be very much shorter than those recorded in mesocosm studies (Samuelsen et al., 1994). Smith & Samuelsen, (1996) have discussed the data indicating that, in nature, re-suspension can result in a relatively rapid depletion of sediment concentrations. The rates of re-suspension are, however, increased by bioturbation and this process is dependent on sufficient oxygen being present in the sediments. In deeply anaerobic sediments the persistence of antimicrobial agent probably resembles that determined in laboratory studies. It should be noted that the data produced by Hektoen et al. (1995) on the persistence of antimicrobials in sediments were produced under experimental conditions that severely limited any bioturbation and these data, therefore, present an overestimate of probable persistence half-lives.

The biological activity of the quinolones and tetracyclines is dramatically reduced in the presence of divalent cations found in seawater and by interaction with freshwater and marine sediments. Although there are extensive data on the concentrations of antimicrobial agents that can be found in under-cage sediments there are significantly less on the concentrations that are required to exert a selection for resistance in this environment. In one of the few studies investigating this question O'Reilly and Smith (2001), determined that the concentrations of oxytetracycline and oxolinic acid needed to exert a selective pressure in fresh water sediments were 20 mg/kg and 0.63 mg/kg respectively. It is reasonable to expect that the equivalent figure for marine sediments would be significantly greater.

3.2.5.2 Fate of antimicrobials in fresh-water farms.

This topic has been less studied than the fate in marine farms largely as a result of the very much smaller quantities of agents used in these environments. In one study (Smith, et al., 1994a) it was demonstrated that the concentrations of oxytetracycline leaving a fresh water pond farm were below the levels of detection (0.02 mg/l). The quasi-totality of the input could be recovered by processing of the outflow through a rotating drum filter of nominal porosity 50 μm, indicating that most of the agent in the water leaving the farm was associated with suspended solids.

3.2.6. Impact of antimicrobial agents used in fish farms.

Smith et al. (1994b) have argued that the dominant impact of antimicrobial agents used in fish farms is on the frequencies of resistant variants in the prokaryotic microflora of the farm environment.

In this context the microflora can be divided into three groups; those associated with disease in fish, those associated with disease in other animals and those resident in the environment.

Although it is reasonable to expect that antimicrobial agent use in aquaculture has the potential to impact on the resistance frequencies in all three groups, major technical problems have been encountered in attempting to quantify these effects particularly with respect to the environmental group.

3.2.6.1 Studies on intestinal commensal microflora

In land-based animals and humans studies of the frequencies of resistance in intestinal microflora have provided a valuable insight into the selection for resistant variants. Studies of this type have much less relevance in fish. It is probable that fish do not have a commensal intestinal microflora of the type encountered in mammals and, therefore studies of this type have less relevance in fish.

A number of studies examining resistance frequency in intestinal microflora of fish have been performed but the data they have generated does not provide a simple picture. Large and significant changes in the frequency of resistance were detected by DePaola (1995) and DePaola et al. (1995) following the administration of oxytetracycline to catfish. Sugita et al., (1988) also reported an increase in the frequency of oxytetracycline resistance following the use of this agent in goldfish. However, Kerry & Smith (1997) failed to detect significant increases in oxytetracycline resistance in the intestines of marine salmon being fed feed medicated with this agent. Sugita et al. (1989) detected little change in resistance patterns following the administration of oxolinic acid to goldfish but Giraud et al. (2006) have presented evidence for the selection of resistance to low levels of oxolinic acid in sea bass after oral therapy with this agent.

3.2.6.2 Impacts on resistance in bacteria associated with fish diseases.

Smith et al 1994b reviewed the literature on the relationship between antimicrobial agent use and resistance in bacteria associated with disease in fish. Despite the problems resulting from the variation in susceptibility test methods employed and bias in the strain sets examined, they identified a general trend. Overall, in areas or industries where a particular agent was widely used, the probability of elevated frequencies of resistant variants was increased. They concluded that increased frequencies of resistance in bacteria associated with fish disease were the one consequence of the use of these agents for which there was unambiguous evidence. However, as has been the case in human and animal medicine, this relationship was less easy to observe on a local scale and the rate at which resistance emerged also appeared to vary between species.

Recent studies by Michel et al. (2003) investigated the relationship between florfenicol use and resistance to this agent in bacteria isolated from fish. They reported that "despite wide use, resistance to florfenicol does not seem to occur frequently in French fish farms."

3.*2.6.3 Impacts on resistance in environmental bacteria*

Measuring the impact of antimicrobial agent use on resistance frequencies

Resistance frequencies can be determined either by examining bacterial phenotype or by using molecular methods to determine their genotypes.

3.2.6.3.1 *Phenotypic methods*

The vast majority of studies of resistance frequencies in the environment of aquaculture facilities have involved measuring bacterial phenotypes.

Most of these studies of phenotypes involved culturing bacteria on aerobically incubated media. Best estimates suggest that less than 1% of the viable cells present in the aquatic environment can form colonies on laboratory media. The fraction of the environmental microflora that can be grown will vary from media to media but it is important to note that even media designed to be non-selective there is a very significant degree of selectivity.

Significant portions, (particularly under-cage sediments and fish intestines) of the aquaculture environment have very low oxygen concentrations. It is clear that many bacteria in these environments may have limited or negligible ability to form colonies in oxygen rich atmospheres.

As a consequence of these factors, all data published on the frequency of resistance in the environmental microflora of aquaculture facilities are necessarily partial and incomplete and this must be borne in mind in any interpretation given to them.

Two general approaches have been taken in attempts to measure the impact of antimicrobial agent use on the frequencies of resistance phenotypes in the microflora of aquaculture facilities.

In one approach individual bacteria have been isolated using laboratory media and their susceptibility then investigated. The problems with this approach are associated with deciding which susceptibility test protocols should be adopted. If the bacteria are associated with human or land-based animal diseases then standard protocols and interpretative criteria for defining resistance exist. If they are associated with fish disease, standard protocols exist for some of them but no breakpoints exist for defining resistance. If they are general environmental bacteria then neither standard protocols nor breakpoints have been developed.

In the other, population-based approach, differential plating or replica plating have been employed to enumerate the frequencies of resistant bacteria in the vicinities of aquaculture facilities without isolating or characterising them.

Independent of the approach taken studies aimed at detecting the impact of antimicrobial agent use must include data with respect to the microflora from appropriate control sites and must include details of the antimicrobial agent use in the potentially impacted site. Unfortunately these data have been included in only a minority of the studies that have been published. These flaws in experimental design severely limit the conclusions that can be drawn from much of the published literature.

Interpretation of data generated resistance studies.

Consideration of basic microbial methods

There have been a wide variety of protocols, particularly with respect to media and antimicrobial agent selection concentrations (and ways of dealing with the ionic concentrations in seawater) used in population-based studies of resistance frequencies. Thus, the empirical definitions of resistance employed in different studies have varied widely and few if any have been validated. At present there appear to be no major efforts being made to develop standard protocols appropriate for these types of investigations.

Considerations of background resistance frequencies.

A large number of studies have reported significant to high levels of resistance in the microflora of relatively pristine, unpolluted waters or water bodies free of known anthropogenic influence. These would include the studies of Kelch and Lee (1978), Jones et al. (1986), Alvero (1987), Bello et al. (1987), Amundsen et al. (1988), EI-Zanfaly et al. (1988), Magee and Quin, (1991), Boon (1992), McKeon, et al. (1995), Boon and Cattanach (1999).

These data demonstrate unambiguously that relatively high frequencies of resistant microflora in a water body can be observed in water that has had no recent contact with antimicrobial agents. Thus high frequencies of resistance in the microflora of a water body cannot be used as evidence of any recent introduction of antimicrobial agents into that water body.

These data also underline the absolute necessity for adequate controls in studies of resistance frequencies. It must be noted that the interpretation of the data presented in many published studies is complicated by their failure to include adequate controls.

Consideration of nutrient effects on resistance frequencies

There are ample data demonstrating that the frequency of bacteria capable of colony formation in the presence of antimicrobial agents will increase dramatically in environments where there is significant nutrient enrichment (Kapetanaki et al. 1995; Vaughan et al. 1996)). In laboratory mesocosm studies frequencies of resistance to oxytetracycline in samples taken from the aquatic environment can be increased from 2% to 100% purely by their incubation in the presence of drug-free fish feed.

This phenomenon may underlie the observations (McPhearson et al., 1991; Kerry et al. 1996) of elevated frequencies of resistance in environments impacted by an aquaculture facility but not by any antimicrobial agent use in that facility. Other studies have demonstrated rises in the frequencies of resistance but that the agents to which resistance increased were not those used on the farm (Austin, 1985. Similarly Schmidt et al. (2000) demonstrated a rise in resistance in bacteria in the ponds and outflows of Danish trout farms but reported no correlation between antibiotic usage and emergence of antibiotic resistance.

The Kapetanaki effect not only seriously confuses the interpretation of data from field studies of resistance frequencies in components of the aquacultural environment that have experienced nutrient enrichment but it also has an importance for laboratory methods for investigating resistance frequencies. Exposure of a sample of the aquatic environment to drug-free laboratory media has been shown to increase the frequency of bacteria with a resistant phenotype. Incubation of natural seawater in Tryptone Soya broth, for example, can result in the frequencies of the bacteria showing a phenotype of resistance to oxytetracycline rising to approximately 100%. This phenomenon probably underlies the observation that the use of replica plate methods result in significantly higher frequencies of resistance than are obtained from the same sample using differential plating methods.

It should be noted that in most aquaculture applications antimicrobial agents would enter the environment associated with nutrient rich material such as faeces or feed. The fact that both the nutrients and the agents have the potential to increase the frequencies of resistant bacteria seriously complicates any attempts to infer a causal link between the presence of the antimicrobial agent and any rise in resistance frequencies that is detected in the vicinity of a fish farm.

Intrinsic resistances

The bacteria in the aquatic environment have intrinsic resistance to various agents and each agent has only a limited spectrum of activity.

Most species of mesophilic Aeromonads have, for example, an intrinsic resistance to ß-lactam agents. Thus changes in the concentration of bacteria belonging to this group can have a major impact on the apparent frequency of resistance to ampicillin in a population.

Changes from a dominantly Gram-positive to a dominantly Gram-negative microflora can be expected to result in changes in the apparent frequencies of resistance to a wide variety of agents. However, very great care must be taken in attributing these changes in resistance frequencies to the impact of antimicrobial agents.

This issue raises problems that are relevant to the interpretation of data from population-based studies where the bacteria involved are not identified to the species level.

Cross-resistances

Some antimicrobial agents are, with respect to resistance, highly linked. Thus the mechanisms by which bacteria become resistant to oxytetracycline are also highly likely to confer resistance to tetracycline. Similar linkages can be postulated for other agents used in aquaculture. Common mechanisms should, for example, be assumed to underlie resistance to amoxycillin and ampicillin, or oxolinic acid and flumequine or trimethoprim/sulfmethoxazole and ormetoprim/sulfadimethoxine.

Many studies of bacteria present in the aquaculture environment have overestimated the frequencies of multiple resistance (MDR) by failing to take account of the extent of cross-resistance to highly related agents.

Limited value of phenotypic studies

The arguments presented above provide the basic reasons why, despite the performance of many studies, fundamental questions about the quantitative relationship between antimicrobial agent use in fish farms and the frequencies of resistance in the local microflora remain unanswered. These arguments would further suggest that these problems with phenotypic methods are fundamental and cannot easily be resolved. They strongly suggest that, where relevant, molecular methods should be considered.

Summary

It must be noted that the published literature in this area is highly contaminated with data collected in poorly designed studies and obtained with inadequate or invalid methods. These problems with the design and performance of various studies have allowed bias, unintentional or otherwise, to significantly influence the conclusions workers have drawn from their data.

There are also many studies where the data produced was appropriate for aims of the investigation but which cannot be easily applied to the problems raised by risk assessment.

Even examination of those studies where well-designed experimental protocols were used, appropriate controls were included and where the methods were valid, does not provide a coherent picture of the impacts. Guardabassi, et al. (2000) observed an increase in the frequency of oxolinic aid resistance in *Acinetobacter* spp in the outflow of a trout farm following the therapeutic use of this agent. However, Giraud et al. (2006) who also studied an oxolinic acid therapy found the increase in resistance frequency to be confined to the intestinal flora and found no effects in the outflow.

3.2.6.3.2 *Molecular studies of resistance gene frequencies*

One of the hazards being addressed in this document is that resulting from the enrichment of genes encoding resistance to antimicrobial agents as a result of antimicrobial use in aquaculture. The adverse effect that this hazard might have is that these genes might compromise antimicrobial therapies in humans or other land-based animal. Therefore it is the genes and not the bacteria, which can, in this context, be seen simply as transport mechanisms for genes that are of fundamental importance. The sequence of the genes encoding the majority of resistances are known and therefore it is technically possible to determine the presence, frequencies of occurrence and the concentrations of specific genes directly.

The previous section identified the problems involved in measuring resistance by examining bacterial phenotypes. These problems suggest that there are clear grounds for rejecting the use of bacterial phenotypes as proxies for their genotypes and would argue strongly in favour of the use of molecular methods that are capable of direct measurement of gene frequencies.

There is clear evidence that genes encoding resistance to antimicrobial agents can be found in the vicinity of fish farms. A number of studies (DePaola, et al., 1988; Sandaa, et al., 1992; Spangaard et al., 1993; DePaola, & Roberts, 1995; Rhodes et al., 2000; Miranda et al., 2003) have demonstrated the presence of specific resistance genes in bacteria isolated from the vicinity of aquaculture operations. The genes identified have been shown to be similar to those occurring in clinical isolates from humans (Rhodes et al., 2000; Furushita, et al., 2003). Further many of these studies have demonstrated that the genes are located on genetics structures that allow their transfer to other bacteria.

The evidence that there has been enrichment for these genes is much less persuasive. The majority (DePaola, et al., 1988; Spangaard et al., 1993; DePaola, & Roberts, 1995; Rhodes et al., 2000; Miranda et al., 2003) of the studies mentioned above did not relate the frequencies of the genes they detected to any event involving the use of antimicrobial agents and some even specified that such agents had not been used recently at the farms they studied. The conclusions that can be drawn from some of these papers are also limited by the absence of comparative data from control sites.

3.3 Hazard group 2; Resistant bacteria.

This hazard includes all bacterial groups that are associated with diseases, either in fish, humans or land-based animals, and that are resistant to the antimicrobial agents used in the therapy of those diseases. In this context the mechanisms and the genetics of the resistances are not factors that need to be considered.

Aspects of the ecology of a bacterial group and the epidemiology of the diseases associated with it are critically important in any risk analysis. Each bacterial group has a unique ecology and epidemiology and, therefore, will ultimately require an independent risk analysis.

A bacterial group can be considered as potentially relevant if it is present in the aquaculture environment where antimicrobial agents are used and it is associated with disease in one of the three host groups, humans, land-based animals and fish. This section provides a provisional list of the major bacterial groups that must be considered as potentially relevant in the context of these three host groups.

The aim of this treatment of the bacterial groups in which resistance may represent a hazard is not to provide a full description of each relevant bacterial group. Rather the aim is to provide sufficient detail to allow a movement towards a preliminary classification of the relative risks associated with each group.

Such preliminary classification is necessary in order to facilitate rational development of priorities and to inform decisions as to which bacterial groups should be given precedence in any full risk analysis.

Cautionary note

The information supplied in this section of the briefing draft must be considered as tentative and preliminary. It should not, any way, be treated as final or definitive. Its use should be confined to establishing the most significant issue to be investigated in a subsequent full and detailed risk analysis.

3.3.1 Risks of adverse effects in aquatic organisms.

Relevant bacterial groups

A wide variety of bacterial groups have been associated with diseases of fish. It can be argued from first principles that the size of the risks associated with increased frequencies of resistance in any bacterial group will be proportional to the importance of the disease associated with that group. Thus, the importance of any bacterial group will vary depending on the species of fish and the environment of the farms.

It should be noted that there is a potentially significant positive feedback loop in the use of antimicrobial agents in any industry. The bacterial groups that will most frequently be the target of antimicrobial therapy will be those that have importance in disease. In general, the more frequently a bacterial group is the target of therapy the greater will be the pressure for the selection and enrichment of resistant variants. Thus the bacterial groups within which resistance is most likely to present a risk of adverse effects in fish are those associated the most significant disease problems in fish farming.

Definitions of resistance

A start has been made in developing standard susceptibility test protocols for bacteria associated with diseases of aquatic animals. The present CLSI protocols (CLSI, M42-P and M49-P) represent the current state of an international cooperative process designed to produce a harmonisation of the methods used in this field. These protocols are, however, incomplete. They do not, as yet, present detailed test conditions or the relevant control data, for testing a number of important and more fastidious organisms. Most importantly no interpretative criteria (breakpoints) have been developed for any bacterial group associated with disease in aquatic animals. Thus, for two reasons, there are major difficulties in defining resistance in these bacteria.

The first reason is that the application of the CLSI standard methods is not yet universal. Recent surveys (Smith 2006a) have indicated that although there is an increasing movement towards the adoption of the standard CLSI test protocols many laboratories are still using older and local test protocols, Importantly nearly all the data available from periods before the development of these standard protocols was generated using a variety of methods and protocols. The use of different test protocols presents major difficulties in comparing, combining or interpreting the data that is available in the scientific literature.

The second reason is associated with the absence, for any of the test protocols including those of the CLSI, of any breakpoints that can be used to define resistance. The present situation is that laboratories interpret their data using a variety of different breakpoints, none of which have been demonstrated to have clinical validity. The current chaos in this field is illustrated by the data collected in a survey by the Permanent Advisory Network for Aquatic Disease.

Table 6. (taken from Smith, 2006b)

Frequency distribution of breakpoints (mm) currently in use for disc diffusion assays of susceptibility of non-fastidious, Gram-negative bacteria associated with fish diseases.

Numbers in the table indicate the number of laboratories employing a breakpoint within the specified range.

Zone (mm)	AMX 25 µg		ENR 5µg		ERY 15 µg		FLO 30µg		FLU 30µg		OXA 2µg		OTC 30 µg		SFO 25 µg		SFT 25 µg	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
6-7																**1**		
8-9																		**3**
10-11				**1**				**1**		**2**	**1**	**6**		**4**		**3**		**7**
12-13		**3**		**2**		**3**		**5**		**3**	**2**		**1**	**3**			**1**	**4**
14-15								**1**		**1**	**2**	**1**		**10**				**3**
16-17	**1**		**1**	**5**		**2**	**1**	**3**	**1**	**2**	**1**		**2**	**1**	**3**		**9**	**1**
18-19	**1**		**2**	**1**	**3**		**5**	**1**	**3**		**1**	**1**	**12**	**2**			**6**	
20-21			**1**				**4**	**2**	**2**	**3**			**2**	**1**			**1**	
22-23	**1**		**5**		**1**		**1**						**1**		**1**			**2**
24-25							**2**	**1**			**1**	**1**					**1**	**3**
26-27									**2**		**1**							

28-29					**1**								**1**				**3**	**1**
30-31			**1**				**1**		**2**				**1**					
32-33							**1**		**1**				**1**				**1**	
34-35														**1**			**2**	
36-37																		
38-39													**1**					
40-41																		
42-43																		
44-45									**1**	**1**								

Abbreviations: AMX, amoxycillin; ENR Enrofloxacin; ERY, erythromycin, FLO florfenicol; FLU, flumequine; OTC, oxytetracycline; OXA oxolinic acid; SFO, ormetoprim/sulfadimethoxine; SFT, trimethoprim/sulfmethoxazole;

S indicates breakpoints used to determine sensitivity.

R indicates breakpoints used to determine resistance.

At present there are no standard methods for determining resistance in bacteria associated with fish disease. A major factor in determining whether a bacterium is classified as resistant or not still remains the laboratory in which the testing was performed.

3.3.2 Risks of adverse effects in land-based animals

A treatment of the relative significance and of the properties of relevant bacterial groups in the context of the risks associated with land-based animals has not been attempted. However some general comments can be made.

Standard susceptibility test protocols with associated interpretative criteria (breakpoints) have been developed for bacteria of importance in animal disease. Thus the empirical definition of resistance in these bacteria presents no major practical problems.

There is little overlap between bacteria that are associated with diseases of fish and those that are associated with diseases of land-based animals. On the other hand, there may be significant overlap between the bacterial groups of significance to humans and those of significance to other land-based animals. In this context, however, some care in how bacterial groups are defined is necessary. If a wide definition is employed for any group, it may contain sub-groups with differential pathogenicity for different hosts. For example, different serotypes within the general group *Salmonella* spp. are known to occur more frequently in different land-based animals. There are reported associations between *S. enteritidis* and poultry, *S. dublin* and cattle and *S. enterica diarizonae* subspecies and sheep.

Some bacterial groups may have potential health impacts for both humans and other land-based animals but they may be of very much more significance with respect to one set of hosts that with respect to the other. *Erysipelothrix rhusiopathiae*, for example, have been associated with disease in both humans and pigs but their significance is much greater for pigs.

3.3.3 Risks of adverse effects in humans.

3.3.3.1.Bacterial groups entering humans via the oral route.

The issue of human infections associated with farmed fish was considered by a joint FAO/NACA/WHO Study Group in 1999 (WHO, 1999). This report suggested that bacteria associated with human infections that have been reported to be present in aquaculture products should be considered as either indigenous to the aquatic environment or introduced to it by anthropogenic contamination.

As these bacterial groups are human pathogens, both protocols and breakpoints for determining resistance are generally available and have been used. Unfortunately there are a number of different protocols (national and international) that can legitimately be used. Historically the issue of the variety of standard protocols and/or breakpoints has presented problems in both human and veterinary medicine but there are strenuous attempts being made to resolve these issues

With respect to analysing published data on frequencies of resistance in this group of bacteria, the data can, with some caution, be treated as reasonably comparable.

Indigenous bacteria

This group includes bacteria that are natural inhabitants of the environments where aquaculture is practiced. It can be assumed that the bacteria in this group are capable of multiplication in the aquaculture environment.

Bacteria in this group can be divided into those that are associated with disease in fish and those that are natural inhabitants of the aquaculture environment.

In discussing bacteria associated with fish disease, the FAO/NACA/WHO Study Group commented;

> "Few bacterial pathogens of farmed fish in temperate climates are capable of infecting humans. The risk of fish pathogens producing human disease is therefore low. In warmer climates, where organisms such as *A. hydrophila* and *Edwardsiella* spp. are important fish pathogens, this may be less true."

Pre-harvest contaminating bacteria

This group includes those not naturally present in the aquatic environment but present as a result of deliberate or accidental contamination with human or animal faeces, or otherwise introduced to the aquatic environment. The ability of bacteria in this group to multiply in the aquatic environment will vary but may be low.

Post-harvest contaminating bacteria

This group includes those present as a result of contamination with human or animal faeces, or otherwise introduced to the fish after their removal from the aquatic environment.

Bacteria in this group cannot, by definition, have been present during any application of antimicrobial agents during the production process. They, therefore, have no relevance to the issues discussed in this risk analysis.

However, it is important to note that in analysing any data on the epidemiology of human disease associated with the consumption or handling of fish, care must be taken to eliminate those cases arising from post-harvest contamination.

3.3.3.2. Bacteria using 'Fish as food' as the vector

Table 7 presents a list of the bacteria that have been associated with infections resulting from the consumption of aquaculture products by various authors. This table also includes the observations made by the FAO/NACA/WHO Study Group (WHO, 1999) with respect to the risks they represent.

Table 7 Bacteria associated with human disease transmitted by consumption of aquaculture products.

Bacterial Group	**FAO/NACA/WHO Study Group Comments***
Indigenous	
Vibrio parahaemolyticus	V. parahaemolyticus has been recognized as a major cause of gastroenteritis and is particularly associated with the consumption of raw marine crustaceans and fish.
Vibrio vulnificus	Has been associated with primary septicaemia following ingestion of raw bivalves and crabs. Confirmed cases of septicaemia and gastrointestinal disease caused by *V. vulnificus* following consumption of products from aquaculture have not been reported.
Vibrio cholerae	The risk associated with eating fish is likely to be low
Aeromonas hydrophila	Epidemiological evidence suggests public health risks from *Aeromonas* and *Plesiomonas* spp. in farmed fish are low.
Plesiomonas shigelloides	
Listeria monocytogenes	Fish produced in temperate inland aquaculture systems may be contaminated with L. monocytogenes and thus present a potential health risk when consumed raw or without heat treatment.
Edwardsiella tarda	Not mentioned
Pre-harvest contamination	
Salmonella spp	Aquacultural products constitute a very low risk to public health with respect to salmonellosis.

Other *Enterobacteriaceae*	Little risk of infection associated with the consumption of farmed fish products.
Campylobacter spp.	The risk associated with the consumption of farmed fish products is low.

Note 1

The above Table treats bacterial groups that have been associated with human disease and that have been also reported to be present in aquaculture. It must, however be noted that in some cases the members of the bacterial group that have been reported as present in aquaculture facilities belong to a different sub-group than those associated with human disease.

Vibrio parahaemolyticus

Although members of this group are frequently encountered in aquaculture environments, the isolation of human pathogenic strains is very much less frequent.

Vibrio cholerae

The strains of *V. cholerae* isolated from aquaculture normally belong to the non O1/O139 sub-group. Strains of the serotypes O1 and O139 of *V. cholerae* that are associated with cholera in humans are extremely rare in the aquaculture environment.

Listeria monocytogenes

L. monocytogenes is widely distributed in the terrestrial environment. It has been reported as present on fish in fish farms in temperate waters and has been implicated in human disease via the consumption of smoked fish. There are, however, data indicating differences in the strains of this organisms isolated from fish and from fish processing plants. This indicates that the *L. monocytogenes* involved in infections resulting from the consumption of smoked salmon may not have been present in the farms where antimicrobial agents were used.

Note 2

Human disease has also been associated with *Clostridium botulinum* contamination of fish and fish products. Botulism is, however, an intoxication rather than an infection and this bacterial group has, therefore, not been considered here.

3.3.3.3. Bacteria using water as the vector

In discussing risks associated with waterborne bacteria, the FAO/NACA/WHO Study Group (WHO, 1999) commented;

> "In developed countries, the possibility that bacteria resistant to antimicrobials used in aquaculture might reach the public through the drinking-water chain is remote."
>
> "Although there is a theoretical risk of the transfer of resistant pathogens to humans through drinking-water in the tropics, the risk is judged to be small…."

Given these comments no further consideration were made of bacterial groups of possible relevance to this infection pathway

3.3.3.4. Infections acquired via contact/wound route

Lehane & Rawlin (2001) have documented the more significant bacterial groups that have been associated, or putatively associated, with infections transmitted from fish or water to humans via direct contact. Their list included; *Erysipelothrix rhusiopathiae, Leptospira interrogans, Mycobacterium marinum, Photobacterium (Vibrio) damsela, Streptococcus iniae* and *Vibrio vulnificus*.

Erysipelothrix rhusiopathiae

Erysipelothrix rhusiopathiae can be found widely distributed in soil and water and has been frequently associated with fish (Di Fidalgo et al., 2000). Although primarily associated with disease in swine erysipeloid is known as an occupational disease for those handling fish. Person to person transmission is not thought to occur. Antimicrobial therapy of erysipeloid is of value and the agent of choice is penicillin.

The incidence of erysipeloid in developed countries is very low. In England in 2002-03, for example, 14 erysipeloid incidents resulted in a total of 0.0001% (34) of hospital bed days. Data for the situation in tropical and developing countries has not been accessed.

Leptospira interrogans

Outbreaks of leptospirosis are usually caused by exposure to water contaminated with the urine of infected animals. The disease is not known to be spread from person to person.

Doxycycline or penicillin are reported to be antimicrobial agents of choice.

The evidence would suggest that aquaculture activities do not result in the enrichment of this bacterial group.

The incidence of leptospirosis is generally very low in developed countries. In Western European countries, the incidence of leptospirosis has been decreasing and it has almost disappeared in the Netherlands, Belgium, Germany and Switzerland. In the US, the annual number of recorded cases ranges from 142 cases in 1964 to 40 cases in 1984 and approximately 50% of these occurred in Hawaii. However, in developing and tropical countries, leptospirosis is either emerging or is still highly prevalent. Urbanization in poor social conditions, such as in Brazil, has led to severe outbreaks similar to epidemics. (Baranton & Postic, 2006).

Mycobacterium marinum

Infections with *M. marinum* occur worldwide, most commonly in individuals with occupational and recreational exposure to freshwater and saltwater. With respect to aquaculture associated infections the most common risk factor is working with tropical fish tanks.

In developed countries *M. marinum* is an uncommon infection. In the United States, studies report an approximate annual incidence of 0.27 confirmed cases per 100,000 patients.

The disease typically remains localized without significant morbidity in patients who are immunocompetent. Invasive infections are very rare with only 24 cases being reported in the literature over the last 30 years (Lahey, 2003)

Although many therapeutic choices exist, rifampin and ethambutol were used most often in invasive infections,

Photobacterium (Vibrio) damsela

This bacterial group is associated with disease in fish. Infections in human appear to be rare, associated with wound infections more common in tropical environments and relatively severe (Yamane et al. 2004).

Antimicrobial therapy is recommended and the regime of choice is a maximum dosage of ceftazidime (or cefotaxime) and fluoroquinolone or tetracycline (or doxycycline).

Streptococcus iniae

Streptococcus iniae is associated with relatively rare but serious infections of farmed fish. Aquaculture can, therefore, increase the concentration of the organism.

Reports of human infection via puncture wounds from infected fish are rare. Information to date suggests that the risk of healthy humans getting infections from diseased fish is apparently very low and the risk to this group is minimal (Shoemaker and Klesius 1997). The median age of people who have been infected is 69, suggesting that age and possibly poor general health may be risk factors. Erythromycin would be the agent of choice in therapy.

Vibrio vulnificus

The CDC has reported that *V. vulnificus* is a rare cause of disease but can cause an infection of the skin when open wounds are exposed to warm seawater. Males are particularly susceptible to *V. vulnificus* septicaemia and those who are immunocompromised are at higher risk for invasion of the organism into the bloodstream and potentially fatal complications.

The recommended antimicrobial therapy is doxycycline with a third-generation cephalosporin.

3.3.3 5. Therapy in the treatment of infections identified as potential risks to humans

As mentioned above, the extent to which resistance in any bacterial group represents a hazard to humans is a function of the frequency with which its infections in humans requires antimicrobial therapy by the agent or agents to which the bacterium is resistant. In the context of the hazard represented by resistant bacteria, any specific resistance is relevant only if it compromises the therapies that are used to control infections by that particular bacterial group.

The Control of Communicable Diseases Manual of the American Public Health Association recommend that, as most gastro-intestinal infections are self-limiting, antimicrobial therapy is not appropriate for these conditions. With orally acquired infections antimicrobial therapy is only indicated in those rare cases, frequently limited to immunocompromised hosts, where systemic infection occurs.

Antimicrobial therapy is, therefore, relevant mainly to contact and wound infection and to those rare, orally-acquired infections that lead to systemic dissemination.

The Table below presents some information on the treatments that have been recommended by some authorities for the control of diseases resulting from infections by the various bacterial groups discussed above.

Table 8. Recommended treatment for human infection by various bacteria

Bacterial Group	**FAO/NACA/WHO Study Group Comments**
Food associated	
Vibrio parahaemolyticus	Generally treatment is not required Treatment with tetracyclines or quinolones in cases of extended diarrhoea.
Vibrio vulnificus	1st choice tetracyclines 2nd choice ceftoxamine or ciproloxacin
Vibrio cholerae	Little value
Aeromonas hydrophila	

Plesiomonas shigelloides	Little value
Listeria monocytogenes	1st choice ampicillin 2nd choice potentiated sulphonamide
Edwardsiella tarda	Ampicillin
Salmonella spp	Ampicillin, amoxycillin, potentiated sulphonamides,
Other *Enterobacteriaceae*	
Campylobacter spp.	Erythromycin
Contact or wound infections	
Vibrio vulnificus	Doxycycline with a third-generation cephalosporin.
Mycobacterium marinum	Rifampin and ethambutol
Erysipelothrix rhusiopathiae	Penicillin
Leptospira interrogans	penicillin
Streptococcus iniae	Erythromycin

3.4 Hazard group 3 Resistant genes

3.4.1 Genetic basis of resistance.

The genetic basis of bacterial resistance to antimicrobial agents has been extensively studied and, on the basis of how bacteria acquire these resistances, two main categories can be identified. The first are resistances developed following mutation of the DNA in such a way that the bacterium is resistant to a given antimicrobial's activity. Mutations of this type frequently involve modification of the target molecule. The second are those resistances that are acquired following the exchange of DNA coding for antimicrobial resistance between resistant and susceptible bacteria. These are most frequently associated with specific or non -specific (multi-efflux pumps), positive function resistance mechanisms.

It should be noted that although it is probably legitimate, at least for the purposes of risk assessment, to treat resistances acquired by mutation as vertically inherited and non-transferable, the converse is not true. It would not be legitimate to treat all resistances resulting from the possession of genes encoding positive function resistance mechanisms as transferable. The chromosomally located β-lactamases that are very widely disseminated within the mesophilic Aeromonads are a case in point. These genes may originally have been acquired by gene exchange but by now can be treated as essentially non-transferable, intrinsic resistances. Thus, in this risk analysis exercise it is appropriate to discuss the risks represented by these positive function genes in mesophilic Aeromonads under hazard 2 (resistant bacteria) and not under hazard 3 (resistant genes).

In treating bacterial resistance to antimicrobial agents it is also necessary to mention the possible involvement of phenotypic persistence mechanisms recently discussed by Balaban, et al. (2004). Cells manifesting persistence mechanisms are phenotypically resistant variants present in genetically sensitive populations. Persistence mechanisms allow bacterial clones to survive an antimicrobial agent challenge without acquiring any genetically encoded functions or chromosomal mutations. Smith et al. (1994) have presented evidence that these mechanisms may occur in bacterial associated with fish disease, however, as they are phenotypic in nature and not fully stable they are of no consequence in the present discussion.

3.4.1.1 Resistance to quinolones

In the context of aquaculture, resistance via mutation is encountered most frequently with respect to members of the quinolone group of agents (Sørum, 2006). Resistance to quinolones in Enterobacteriaceae and in most bacteria associated with fish disease most commonly arises stepwise as a result of mutation usually accumulating in the genes encoding primarily DNA gyrase and also topoisomerase IV (*gyrA, parC*). Decreased permeability by changes in the nature and amount of porins (in particular OmpF) or increased efflux by mutations in regulatory genes of chromosomally encoded multidrug resistance pumps (Acr) or their regulatory systems (MarA, SoxS) may cause increments in quinolone resistance. The genes responsible for these resistances are chromosomally located and the extent to which they are capable of horizontal transfer in nature is generally accepted to be low**.**

Not all genes encoding quinolone resistance can, however, be treated as non-transferable. Nordman and Poirel (2005) have reviewed the emergence of resistance to quinolones encoded by Qnr type mechanisms. The genes responsible, which have been found in aquatic bacteria (Poirel et al. 2006), can transfer horizontally and can confer low-level resistance to fluoroquinolones in recipient bacteria. So far these Qnr type mechanisms have been shown to have a very widespread distribution geographically but to be relatively rare in any specific environment (Nordman and Poirel, 2005).

From a risk analysis perspective a major significance has to be given to the degree of horizontal transfer. It is appropriate to discuss the risks represented bacteria with non-transferable quinolone resistances under hazard 2 (resistant bacteria) and not under hazard 3 (resistant genes). However, those with Qnr type resistances are more appropriately treated under hazard 3.

The existence of both transferable and non-transferable genetic mechanisms encoding resistance to quinolones underlines the advantage of molecular methods over phenotypic methods in the surveillance of resistance frequencies.

3.4.2 Movement of resistance genes

Plasmid transfer or conjugation is the most studied mechanism of DNA transfer and acquisition by bacteria. Although it is not the only mechanism by which bacteria exchange genes it is thought to be the mechanism that plays the most significant role in the horizontal transfer of genes between bacteria in the natural environment.

Plasmids are circular extrachromosomal DNA within the bacterium. Larger plasmids carry the information necessary to allow conjugation, which permits movement of DNA between bacteria. Smaller plasmids, and these have been demonstrated in some strains of *Aeromonas salmonicida* containing the TetC resistance determinant, do not have this capacity and are dependent upon other plasmids for their passive mobilisation (Sørum, 2006).

Plasmids may also harbour transposons or integrons, which are DNA sequences necessary for insertion and excision in plasmid or host DNA. Resistance genes in plasmids are frequently associated with these genetic elements and this association facilitates their movement between different genetic structures within a cell as well increasing the efficiency with which they can be horizontally transferred between bacteria of the same species, and those of other genera. These elements also play an important role in the development and transfer of new gene combinations in the bacterial population.

It has been known for a long time that single plasmids may carry genes involved in resistance to more than one class of antimicrobial agents. Thus these plasmids can transfer multiple resistances and the selective pressure exerted by the presence of one agent may also exert a pressure for the selection of genes encoding resistance to other agents that are located on the same plasmid. It is now becoming apparent that resistance genes are frequently more closely linked. Evidence now suggests that resistance genes are often grouped in integron-associated cassettes. Thus not only can movement between hosts involve multiple resistance genes but similar considerations also govern the movement of these genes between genetic structures within a host.

Resistance determinant transmission between bacteria may be examined as a two-step process.

The first step is that the genes must move or be moved to a new host, and this process is greatly facilitated by a plasmid location. If the resistance genes are to be stably inherited in the new host they must be located on a plasmid that is itself stable in the new host or it must be relocated to a genetic structure that is stably inherited. Not all plasmids are capable of being stably inherited in all the bacteria to which they are transferred. The presence of other plasmids in the recipient host, whether of the same or different incompatibility groups, may restrict plasmid stability in that new host cell. It should be noted that plasmid may have a variety of effects, other than conferring a resistance phenotype, on their new hosts and some of these may influence the host growth rate or survival in various environments. Thus, the demonstration, in laboratory tests, of the stable inheritance of a plasmid within a pure culture cannot be used to infer that the plasmid containing strain will persist in the environment (Smith et al, 1994). The continued presence of selective agents such as antimicrobials may also have an effect on plasmid stability. Brazil et al. (1986), for example, observed the acquisition of a plasmid encoding chloramphenicol resistance by a strain of *Aeromonas salmonicida* during the use of this agent to control an outbreak of furunculosis in a salmonid farm. Although this plasmid was stably inherited in the laboratory, it disappeared from the strains isolated from the farm soon after the end of the therapy.

The second step, which may be involved in effective plasmid-mediated transfer of resistance genes in nature is related to possible gene relocation within the new recipient host. In situations where a plasmid can transfer to, but is not stably inherited in, a new host, it is still possible that the resistance genes initially present on that plasmid can achieve stability. Transposon and integron associated genes or gene cassettes can be moved between various plasmid or chromosomal DNA molecules within a cell.

The ecological consequences of this two-step process can be illustrated by data from the Aeromonads. Sørum et al (2003) and Rhodes et al. (2000) have demonstrated that, in this group of bacteria, TetA genes are strongly associated with Inc U plasmids and it is known that Inc U plasmids are capable of transfer to a wide variety of Gram-negative bacteria. TetA genes are widely disseminated in other bacterial genera but the Inc U plasmids are found mainly, but not exclusively, in Aeromonads. These data therefore indicate that although TetA has spread widely through bacterial genera, the plasmids with which it associated in different host are not the same.

3.4.3 Antimicrobial resistance associated with fish pathogens

Most published studies of antimicrobial resistance associated with aquaculture concern genera of bacteria that are associated with diseases of aquatic organisms. Logically, pathogens for which antimicrobial therapy is common would be a likely starting place for the identification and characterisation of resistance genes. The *Aeromonas* (*A. hydrophila* and *A. salmonicida*) and *Vibrio* genera are by far the most represented in the literature. These are well known pathogens of salmonids and cyprinids (among other species) and are easily cultivated and manipulated in a laboratory environment. Resistance to antimicrobial therapy has been identified in these species since the 1950's.

The earliest studies (Watanabe, et al., 1971; Aoki *et al*. 1984; Waltman *et al*. 1989) concentrated on demonstrating the plasmid associated transfer of resistance phenotypes and the incompatibility groups and size of the plasmids involved (Hedges et al. 1985; Brazil et al. (1986)). The recent advances in molecular biology have facilitated studies of the molecular nature of resistant determinants. These studies, recently reviewed by Sørum (2006), have allowed the determination of the phylogenetic relationships between resistant genes and their host and have helped elucidate the involvement of transposons, integrons and gene cassettes. They have also allowed the development of molecular probes that can allow the detection of specific genes or gene sequences. The application of these techniques has led to a rapid expansion of out knowledge of the molecular basis of antimicrobial resistance. As yet, however, these technical advances and the data they have provided have not produced a similar expansion in our understanding of the critical issue of the ecology of resistance genes in the environment or the factors influencing the dynamics of gene flow in the environment.

3.4.4 The source of resistance genes and selection in the aquaculture environment

For antimicrobial agent use in aquaculture to select for an increase in the frequency of genes encoding resistance these genes must be present in the aquaculture environment. Consideration of this issue raises the question of the source of any resistance genes present in the environment prior to the use of antimicrobial agents in aquaculture.

3.4.4.1. Sources of resistance genes

It is generally accepted that human activites, including the disposal of municipal sewage and hospital effluent, and agricultural activity, including veterinary interventions, are major sources of antimicrobial resistance genes in the general environment. Recent research indicates, however, the possibility that one of the principle sources or reservoirs may in fact be indigenous environmental bacteria.

As discussed in a previous section there is ample evidence to support the hypothesis that bacteria possessing genes encoding positive functions resistance to antimicrobial agents are present in the environment of all aquaculture facilities. Indirect evidence would suggest that this is true even of those fed by spring water. Further the available data would support the position that all aquaculture environments should be considered as containing bacteria with the potential ability to transfer these genes encoding resistance to other bacterial hosts.

The studies that have been performed on the presence of genes within indigenous bacteria isolated from the aquaculture environment, whether indigenous or introduced by anthropogenic contamination, have nearly all been performed at locations where no antimicrobial agent use was occurring or even in many cases had occurred in the recent past ((DePaola, et al., 1988; Spangaard et al., 1993; DePaola, & Roberts, 1995; Rhodes et al., 2000; Miranda et al., 2003). One exception to this, was the work of Sandaa et al. (1992). In this work resistance genes were detected immediately under cages in which the fish had been fed oxytetracycline for 10 days. Unfortunately this study provided no data from samples taken from any control site.

In interpreting the data presented on the presence of resistance genes in the aquaculture environment it is important to note that there are equally extensive data demonstrating that resistant bacteria can be detected in water sources that are not only free of aquacultural influence but also of any recent anthropogenic influence at all.

3.4.4.2 Selection for resistance genes

Although there are data demonstrating that resistance genes are present in areas where detectable concentrations of antimicrobial agents may occur, there are none that clearly demonstrate that a selection for an increased frequency of these genes does in fact occur in these environments. Whereas the available data provide useful information on the existence of genes that could be the subject of selection, they do not provide any information on the extent of any selection that does occur in these environments.

3.4.4.3 Summary

There is now ample data demonstrating that bacteria harbouring resistance genes can easily transfer these elements to other genera including those associated with human and land-based animal disease (cf. Kruse, and Sorum, 1994). This evidence, first produced over 30 years ago, is now so overwhelming that there would appear to be little value in collecting any more information that simply reinforces this observation

There is evidence that the frequency of resistance genes in bacteria associated with fish disease has increased in areas where these agents have been used by fish farms. There are data suggesting that emergence of resistance in these bacteria is more likely to arises in situations were chronic and continued antimicrobial agent use is practiced. However, current evidence would suggest that, in situation where antimicrobial agent use follows prudent, best-practice guidelines, this linkage can only been detected when isolates from a wide geographic area, collected over a reasonably long time period are examined.

What is currently lacking is any detailed information on the extent to which antimicrobial agent use in fish farms increases the frequency of the bacteria possessing such genes in the bacteria present in the environment of aquaculture facilities.

Stating that there is no evidence that an event has occurred is, of course, not the same as stating that the event has not occurred.

3.4.5 Modelling gene flow in the environment

The advent of molecular techniques that can be applied to bacterial genetics has presented increasing amounts of data that suggests that gene flow between bacteria is a more common event than had previously been supposed. These data have led to various researchers considering whether the concept of a common bacterial gene pool would have merit in modelling patterns of gene flow within prokaryotes. Such a concept would be consistent with the observations by Rhodes et al. (2001) and others that genes involved in resistance that are detected in bacteria associated with fish disease are the same as those detected in bacteria associated with human disease.

It is clear that the concept of a general prokaryotic gene pool has advantages in simplifying the task of building provisional models of the dynamics of gene frequencies and gene movements. Essentially, the concept would suggest that, as there is a common gene pool, selection in any environment would have an effect on the total gene frequencies within the pool. This position could be developed by further suggesting that the relative contribution of each area of antimicrobial usage (human, land-based animal, horticultural and aquacultural) to the changes in the frequencies of genes within the pool would be a function of the selective pressures exerted by the use of these agents.

If the gene pool concept is to be of value it is also necessary to postulate some linkage between the frequencies of any resistance gene in the pool and chances of it entering any particular bacterium. Here, however, there are some difficulties. Currently there are data suggesting that the frequencies of resistance in *Salmonella* serotypes associated with different animal hosts show significant differences. Thus, over the last few years, the frequencies of resistance genes in *Salmonella* spp. have been more related to the hosts they infect than to their access to a common gene pool. .

It is possible to argue that the movement of genes between bacteria has a time component. The relationship between the frequencies of gene in the common pool and those in individual bacterial groups is dynamic and has a relatively slow response time.

It is, however, equally clear that an over simplification of the common gene pool concept will lead to misleading and erroneous conclusions.

The common gene pool concept allows us to consider the movement of genes from aquaculture to human environments but also allows consideration of the reverse flow from human to aquaculture. Arguments have been presented about the effective direction of gene flow between aquaculture and the human. Angulo (1999???) has argued that as the *flo R* gene was first reported in 1993 (Kim & Aoki, 1993) in *Pasteurella piscicida,* a bacterium associated with fish disease, and was then subsequently observed in Salmonella (Bolton et al., 1999: Briggs & Fratamico, 1999). This can be taken as evidence as to the direction, in this case, of gene flow from aquaculture to humans. However, arguments based on the first report of an event are inherently weak, as they cannot account for differential research efforts in different areas. In this case more recent publications have revealed the presence of the *floR* gene in terrestrial bacteria first isolated before 1993 and well before florfenicol was first introduced into aquaculture. Meunier et al (2003) reported the gene as present in a *Salmonella enterica* strain first isolated from a turkey in 1990 and Cloeckaert et al. (2001) also reported the gene to have been present in a *Klebsiella pneumonia* strain isolated in the 1970s.

3.4.6 Resistance gene ecology; a possible self-regulating complex system.

Recent developments in systems biology and in our understanding of the dynamics of self-regulating complex systems (Kauffman, 1995) has led workers to examine the relevance of these concepts to medical and ecological problems (cf. Ahn et al., 2006).

The ecology of resistance genes is clearly a very complicated field and there are grounds to justify an investigation into whether it is best treated as a complex system. From the perspective of risk analysis the demonstration that resistance gene ecology represented a complex system would have major theoretical and practical importance. At a theoretical level it would call into question the value of simple cause and effect analysis (Bateson, 1987). In a non-complex system the variation in responses to a stimulus can be described by a normal distribution curve and therefore, a dose response curve is a meaningful concept. In complex systems, however, the response to a stimulus is contingent upon the state of the system at that time and the relationship between them is described by a power law distribution. Thus, if resistance gene ecology represents a complex system, we would expect that the system response to any selection event would normally be small, either in space or time, but that, increasingly rarely, the system could show major responses. The log of the frequency of any system response would show an inverse linear relationship to the log of the intensity of that response.

At a practical level the demonstration that resistance gene ecology was best treated as a complex system would indicate the need for those with experience of systems biology to be involved in the risk assessment process

4. Exposure assessment

4.1 Exposure Pathways

4.1.1 Introduction

The four main purposes of developing an exposure pathway are;

i. It allows us to clarify out thinking about the process.

ii. It allows us to develop a list of questions that we will have to answer if we are to provide a meaningful assessment of risk.

iii. It facilitates the identification of critical gaps in the available data and therefore, to identify key research areas.

iv. It allows the identification of critical control points and therefore represents an essential step if we are to design effective ways of managing the risk.

4.1.2 Definitions

Exposure pathways attempt to itemise the linkages between an action and an adverse effect or series of adverse effects. In order to facilitate clear thinking it is necessary to define both the action and the adverse effects that are the subject of any analysis. With respect to the work of this group the following definitions of the action and adverse effects have been used.

Definition of the action

The use of antimicrobial agents in aquaculture

Definitions of the adverse effects

A Biologically mediated

The increase of morbidity or mortality resulting from a reduction in the efficacy of a specific antimicrobial therapy of a disease as a consequence of the lack of susceptibility, to that agent, of the bacterium involved in the disease process.

B Chemically mediated

The increase in morbidity or mortality resulting from the consumption of biologically active chemicals.

4.1.3 General consideration of exposure pathways

How many exposure pathways will be required?

Clearly different exposure pathways will be required for the consideration of biologically mediated and chemically mediated adverse effects. In addition host issues and hazard issues will influence the number of pathways that will be required.

Host issues

In considering the exposure pathways it is valuable to separate adverse effect that occur in various hosts;

a) humans

b) other land-based mammals.

c) fish or farmed aquatic animals

Hazard issues

In considering the exposure pathways it is valuable to separate adverse effect that occur via various specified hazards arising from the use of antimicrobial agents in aquaculture;

a) Antimicrobial agents

b) Bacteria resistant to antibacterial agents

c) Genes encoding resistance to antimicrobial agents.

Methods used to develop exposure pathways.

The key characteristic of exposure pathways is that they are question-based and not data-based. This means that the construction of a pathway is not and must not be determined by what we know but must be informed by a systematic consideration of what we **need** to know.

As one of the major outputs of an exposure pathway is a list of topics about which data is required, it would be pointless and misleading to use the available data to determine the exposure pathway.

Although exposure pathways are not data-based they must be informed by a general understanding of the process that has been, directly or indirectly, informed by our general experience.

Thus, in this work, the first step has been to develop a general model of the linkage between the action to the adverse effects. This model building stage is often omitted, or not made explicit, when a risk analysis is addressing a relatively simple set of linkages. It, however, becomes more relevant and essential as the linkages under consideration increase in complexity.

4.2 Development of hazard and host specific exposure models

Section A

Hazard: Antimicrobial agents

Adverse effects: The increase in morbidity or mortality resulting from the consumption biologically active chemicals.

A 1 Fish or farmed aquatic animals

Agent use in primary aquaculture farm.

Pathway A.1.1

A. 1.1.1 Exit of agent from farm associated with feed, faeces.

A. 1.1.2 Entry of agent into secondary aquatic organism via consumption of feed or faeces

Pathway A.1.2

A. 1.2.1 Exit of agent from farm associated with water

A. 1.2.2 Absorption of agent from water by secondary aquatic organism.

Pathway A. 1.3

A. 1.3.1 Exit of agent from processing plant into the aquatic environment

A. 1.3.2 Entry of agent into secondary aquatic organism

Adverse effects in secondary hosts

A. 2 Land-based animals

Agent use in primary aquaculture farm

Pathway A.2.1

A. 2.1.1 Exit of agent from farm associated with water

A. 2.1.2 Entry of agent into land-based animals via consumption of water.

Pathway A.2.2

A. 2.2.1 Exit of agent from farm associated with mortalities or processing waste

A. 2.2.2 Use of farmed organism mortalities or processing water as soil fertilizer

A. 2.2.3 Entry into animals via contact with fertilizer.

Pathway A. 2.3

A. 2.3.1 Exit of agent from farm associated with mortalities or processing waste.

A. 2.3.2 Use of wastes or mortalities as animal feed

A. 2.3.3 Entry into animals via consumption of feed.

Pathway A. 2.4

A. 2.4.1 Exit of agent from processing plant.

A. 2.4.2 Entry of agent into aquatic environment

A. 2.4.3 Entry of agent into land-based animals via consumption of water.

Adverse effects in land-based animals

A. 3 Humans

Agent used in primary aquaculture farm.

Pathway A.3.1

A. 3.1.1 Exit of agent from farm associated with aquatic organism

A. 3.1.2 Entry into humans via consumption of aquatic organism.

Pathway A.3.2

A. 3.2.1 Exit of agent from farm associated with feed, faeces.

A. 3.2.2 Entry of agent into secondary aquatic organisms via consumption of feed or faeces

A. 3.2.3 Entry into humans via consumption of secondary aquatic organism.

Pathway A.3.3

A. 3.3.1 Exit of agent from farm associated with water.

A. 3.3.2 Entry of agent into humans via consumption of water.

Pathway A.3.4

A. 3.4.1 Exit of agent from farm associated with water.

A. 3.4.2 Entry of agent into humans via skin lesions.

Pathway A. 3.5

A. 3.5.1 Direct entry into farm worker via handling of medicated feed or pre-mix

Adverse effects in humans

Section B

Hazard: Resistant bacteria

Adverse effects: The increase of morbidity or mortality resulting from a reduction in the efficacy of a specific antimicrobial therapy of a disease as a consequence of the lack of susceptibility, to that agent, of the bacterium involved in the disease process.

B. 1 Farmed aquatic organism

Agent use in primary aquaculture farm

Enrichment of resistant variants of bacteria associated with aquatic animal disease.

Pathway B.1.1

B. 1.1.1 Exit of resistant variants associated with farmed organism.

B. 1.1.2 Introduction into a second farm of farmed aquatic organism with covert (or overt) infection with resistant variants.

B. 1.1.3 Development of epizootic requiring therapy.

Pathway B.1.2

B. 1.2.1 Exit of resistant variants associated with feral aquatic organism.

B. 1.2.2 Introduction of resistant bacteria to aquatic organism in second farm via contact with infected feral aquatic organism.

B. 1.2.3 Development of epizootic requiring therapy.

Pathway B.1.3

B. 1.3.1 Exit of resistant variants associated with farm wastes (faeces, suspended solids).

B. 1.3.2 Infection of farm organism in second farm by consumption of wastes from primary farm.

B. 1.3.3 Development of epizootic requiring therapy.

Pathway B.1.4

B.1.4.1 Exit of resistant variants associated with processing plant wastes (water, suspended solids).

B.1.4.1 Infection of aquatic organism in farm by exposure to water and/or ingestion of suspended solids.

B.1.4.3 Development of epizootic requiring therapy.

Adverse effects in aquatic organism. (NB as adverse effect involve the failure of therapy they can only occur in farmed organisms).

B.2 Land-based animals,

Agent use in primary aquaculture farm

Presence of bacteria associated with disease of land-based animals.

Enrichment of resistant variants of bacteria associated with land-based animals.

Pathway B. 2.1

B 2.1.1 Exit of resistant bacteria from farm associated with water

B 2.1.2 Entry of resistant bacteria into land-based animals via consumption of water.

B. 2.1.3 Development of disease requiring therapy.

Pathway B. 2.2

B. 2.2.1 Exit of resistant bacteria from farm associated with mortalities or processing waste

B. 2.2.2 Use of aquatic organism mortalities or processing water as soil fertilizer

B. 2.2.3 Entry into animals via contact with fertilizer or soil.

B. 2.2.4 Development of disease requiring therapy.

Pathway B. 2.3

B. 2.3.1 Exit of resistant bacteria from farm associated with mortalities or processing waste.

B. 2.3.2 Use of wastes or mortalities as animal feed

B. 2.3.3 Entry into animals via consumption of feed.

B. 2.3.4 Development of disease requiring therapy.

Pathway B. 2.4

B 2.4.1 Exit of resistant bacteria from processing plant associated with water

B 2.4.2 Entry of resistant bacteria into land-based animals via consumption of water.

B. 2.4.3 Development of disease requiring therapy.

Adverse effects in land-based animals.

B. 3 Humans

Agent use in primary aquaculture farm

Presence of bacteria associated with disease of humans.

Enrichment of resistant variants of bacteria associated with humans.

Pathway B. 3.1

B 3.1.1 Exit of resistant bacteria from farm associated with farmed aquatic organism.

B 3.1.2 Entry of resistant bacteria into humans via consumption of aquatic organism.

B.3.1.3 Development of disease requiring therapy.

Pathway B. 3.2

B 3.2.1 Exit of resistant bacteria from farm associated with water

B 3.2.2 Entry of resistant bacteria into humans via consumption of water.

B 3.2.3 Development of disease requiring therapy.

Pathway B. 3.3

B 3.3.1 Exit of resistant bacteria from farm associated with water

B 2.3.2 Entry of resistant bacteria into humans via contact with water.

B 2.3.3 Development of disease requiring therapy.

Pathway B. 3.4

B 3.4.1 Exit of resistant bacteria from processing plant associated with water

B 3.4.2 Entry of resistant bacteria into humans via consumption of water.

B 3.4.3 Development of disease requiring therapy.

Pathway B. 3.5

B 3.5.1 Exit of resistant bacteria from processing plant associated with water

B 2.5.2 Entry of resistant bacteria into humans via contact with water.

B 2.5.3 Development of disease requiring therapy.

Adverse effects in humans.

Section C

Hazard: Resistant genes (in these models chromosomally located genes not known to be associated with transposons or integrons will not be considered.)

Adverse effects: The increase of morbidity or mortality resulting from a reduction in the efficacy of a specific antimicrobial therapy of a disease as a consequence of the lack of susceptibility, to that agent, of the bacterium involved in the disease process.

C. 1 Farmed aquatic organism

Agent use in primary aquaculture farm
Presence of bacteria possessing resistance genes in aquaculture farm environment.
Enrichment of any bacteria possessing resistance genes.

Pathway C1.1

C.1.1.1 Co-habitation, within the farm environment, of any bacteria possessing resistance gene with any bacteria associated with aquatic organism disease
C.1.1.2 Transfer of resistance gene to bacteria associated with aquatic organism disease
C.1.1.3 Movement of resistance gene to a location conferring stability in new host.
C.1.1.4 Development within the farm of disease requiring therapy.

Pathway C1.2

Movement of enriched resistant bacteria to new farm via
C.1.2.1.1 Water
C.1.2.1.2 Feral aquatic animals
C.1.2.1.3 Farmed aquatic animal
C.1.2.1.4 Processing plant waste

then as C1.1.1 to C.1.1.4

Adverse effects on farmed aquactic organisms

C.2 Land-based animals,

Agent use in aquaculture farm
Presence of bacteria possessing resistance genes in aquaculture farm environment.
Enrichment of any bacteria possessing resistance genes.

Pathway C2.1

C.2.1.1 Movement of enriched resistant bacteria to land-based animals via water

C.2.1.2 Transfer of resistance gene to bacteria associated with land-based animals in water

C.2.1.3 Movement of resistance gene to a location conferring stability in new host.

C.2.1.4 Infection of land-based animal

C.2.1.5 Development of disease requiring therapy.

Pathway C.2.2

C.2.1.1 Movement of enriched resistant bacteria to land-based animals via water

C.2.1.2 Entry into land-based animal via consumption of water

C.2.1.3 Transfer of resistance gene to bacteria associated with land-based animals in animal intestine

C.2.1.4 Development of disease requiring therapy.

Pathway C 2.3

As C2.1 but with aquatic organism wastes applied as fertilizer as vector

Pathway C 2.4

As C2.2 but with aquatic organism wastes applied as fertilizer as vector

Pathway C 2.5

As C2.1 but with aquatic organism wastes applied as animal feed as vector

Pathway C 2.46

As C2.2 but with aquatic organism wastes applied as animal feed as vector

Adverse effects in land based animals

C.3 Humans,

Agent use in aquaculture farm

Presence of bacteria possessing resistance genes in aquaculture or processing plant environment.

Enrichment of any bacteria possessing resistance genes.

Pathway C3.1

As C2.1 but with humans as hosts.

Pathway C3.2

As C2.2 but with humans as hosts.

Pathway C3.3

As C3.1 but with 'aquatic organism as physical product' as vector.

Pathway C3.4

As C3.2 but with 'aquatic organism as physical product' as vector.

Pathway C3.5

As C3.1 but with 'aquatic organism as food' as vector.

Pathway C3.6

As C3.2 but with 'aquatic organism as food' as vector.

Adverse effects in humans.

4.3 Exposure pathways

In presenting these exposure pathways we are conscious of their provisional nature. Clearly the pathways presented here could and should be further developed and refined by a peer review process. There are, however, a number of reasons why it was felt necessary to develop and present explicit exposure pathways.

They are required to clarify thinking about the various risks.

They allow the identification of the types of information on the properties of the various hazards that will be required for any risk assessment.

They allow the development and promotion of rational research strategies.

However, in terms of the meeting in Seoul one of the advantages of presenting these exposure pathways is that they provide a framework for the preliminary rating of the risks presented by the various hazards in the various aquaculture contexts. This preliminary rating will be essential if we are to develop appropriate priorities in selecting risks that should be the subject of a full risk analysis.

A further comment can be made about the process of developing exposure pathways. In performing this task it was clear the difficulty in establishing clear and unambiguous pathways increased as we moved from hazard group 1 through to hazard group 3.

Antimicrobial agents (hazard group 1) are chemical entities and relatively simple, linear models of their movement can be produced.

Resistant bacteria are, however, biological entities that have to move through and survive in, a variety of environments. The impact of environmental conditions and intra-microbial competition significantly complicate the modelling of these movements.

Modelling the movement of resistance genes, however, present further levels of complexity. As genes must move in hosts, all the factors that complicate the modelling of resistant bacteria are still relevant. However, these are further complicated by issues of the stability of the association of the genes with the hosts and the movement of the genes between genetic structures in the hosts. Resistance genes are always physically linked to other DNA coding sequences and the impact of these sequences on the survival of the hosts in various environments will also need to be accounted for.

4.3.1 Exposure pathways for hazard 1 – antimicrobial agents.

In this document exposure pathways have not been specifically developed for this hazard. This decision was taken for two main reasons.

i) Exposure pathways for chemical residues in food, present little theoretical difficulty, are well understood and have been developed in other studies.

ii) The only aspects of this issue that is specific to aquatic organisms are those relating to pharmacokinetics and residue kinetics in fish.

4.3.2 Exposure pathways for hazard 2 - resistant bacteria

4.3.2.1

FRAMEWORK FOR DEVELOPING EXPOSURE PATHWAYS FOR THE ADVERSE EFFECTS IN HUMANS MEDIATED BY THE BIOLOGICAL HAZARD REPRESENTED BY BACTERIA RESISTANT TO ANTIMICROBIAL AGENTS.

Preliminary steps.

A. Identification of a specific bacterium or bacterial group.

Priority should be given to bacterial groups identified by two criteria.

Those that have been associated with food borne infections where an aquatic organism has been identified as a significant vector.

Those which are known to be involved in human disease and which have been reported to be present in the environment of aquaculture enterprises.

B. Identification of the antimicrobial agent of relevance to that bacterial group

The antimicrobial agents to which resistance is of significance are those that are used in the therapy of the diseases associated with infections of humans by the selected bacterial group. Attention should also be paid to agents linked to those used in human therapies by the degrees of cross-resistance conferred by known resistance mechanisms.

Key events in the linkage

1. Presence of bacterium in the aquaculture environment.

2. Selective pressure, in the aquaculture environment, for the emergence of resistance in that bacterium.

3. Impact of selective pressure on resistance.

4. Routes by which resistant bacteria leave the aquaculture environment.

5. Survival in vector system

6. Routes by which the resistant bacteria enter humans

7. Mortality and morbidity associated with infections by members of the bacterial group.

8. Value and frequency of antimicrobial therapy provided to diseased individuals.

Questions relevant to a detailed consideration of the key events

1. Presence of bacterium in the aquaculture environment.

What is the frequency of the occurrence of this bacterial group in aquaculture environments and at what concentrations do they occur?

> The answers here will clearly vary depending on the environment of the aquaculture enterprise. Separate data will be required for fresh and marine environments and probably for temperate and tropical waters. In-shore offshore farms may also have to be considered separately?

Is the bacterial group part of the natural flora of the aquaculture environment or a transient? Are they capable of growth in the aquacultural environment?

> The answers here will clearly vary depending on the environment of the aquaculture enterprise.

Are all the members of the bacterial group associated with disease in humans?

> The quality and value of data on the enumeration and/or identification of members of bacterial groups in the aquaculture environment will be method dependent. Do the methods identify human pathogens or groups that may contain some human pathogens?

What is the distribution (frequency and concentration) of the bacterial group within various components of the aquaculture environment (water, sediment, farmed or feral fish, other organisms)?

Does the bacterial group enter the aquatic environment from a controllable, or potentially controllable terrestrial source?

2. Selective pressure, in the aquaculture environment, for the emergence of resistance in that bacterium.

Are any relevant antimicrobial agents used in the aquaculture enterprise and how frequently are they applied?

> The answers here will vary from country to country and also may vary with respect to the species of aquatic organism farmed.

What is the fate of any antimicrobial agents used?

> Here we will need to establish the concentration and persistence of the relevant agents in various compartments (water, sediment, farmed or feral fish, other organisms) of the aquatic enterprise?
>
> Data will also be required on the antimicrobial agent 'footprint' over which any impact can be expected.

To what extent do the distributions of antimicrobial agents in the aquacultural environment allow it to exert a selective pressure on the bacterial group?

> Within the aquacultural environment how does the distribution of the antibacterial agent map onto the distribution of the bacterial group?
>
> Within the aquacultural environment how does the distribution of the antibacterial agent map onto the areas where the members of the bacterial group under consideration are capable of multiplication? (Enrichment for resistance variants is very much more efficient in growing populations)

What is the minimum concentration of any relevant antimicrobial agent in any environment that can exert a selective pressure?

> The biological activity of all antimicrobial agents is context-dependent. In order to interpret data on the presence (concentration and persistence) of antimicrobial agents it is necessary to know their minimum effect concentrations with respect to selection of resistance variants.

3. Impact of selective pressure on resistance in the relevant bacterial group.

What empirical definitions of resistance are of value in this context?

> Any investigations of resistance will require standardised methods for measuring susceptibility.

What are the frequencies of resistance in the bacterial group in the microflora entering the aquaculture environment or present in that environment in the absence of antimicrobial agent use?

> Measurement of the frequencies of specific resistances in a particular bacterial group can be made by establishing either the frequency of variants manifesting these resistances within the group or by measuring the concentration of resistant variants in the aquaculture environment. These are independent variable that may not co-vary.

What are the frequencies of resistance in the bacterial group within the aquaculture environment during or after antimicrobial agent use?

> Measurement of the frequencies of specific resistances in a particular bacterial group can be made by establishing either the frequency of variants manifesting these resistances within the group or by measuring the concentration of resistant variants in the aquaculture environment. These are independent variables that may not co-vary.

4. Routes by which resistant bacteria leave the aquaculture environment.

Within which vector or vehicle do the bacterial group (and its resistant members) leave the aquaculture environment?

The main categories here will be the aquatic organism produced by the farm and the water leaving the farm. Additional consideration may also have to be given to feral fish (including shellfish) as secondary vectors.

With what frequency are these vectors (or vehicles) contaminated with resistant bacteria as they leave the farm or farm environment?

What concentrations of the bacterial group occur in these vectors?

5. Survival in vector system

With respect to bacterial groups using 'aquatic organism as food' as the vector, what are the effects of handling, storage and cooking (in any) on the survival of the bacterial group?

With respect to bacterial groups using 'aquatic organism as physical product' as the vector, what are the effects of handling and storage on the survival of the bacterial group?

With respect to bacterial groups using water as a vector, what governs the survival of the bacterial group in the aquatic environment?

6. Routes by which the resistant bacteria enter the human environment.

To what extent are aquatic organisms the vector involved in infection of humans?

To what extent are aquatic organism-associated infections a result of handling aquatic organism, or food preparation or food consumption?

The relative importance of these routes of infection can be established by examination of record of epidemiological studies of human infections.

To what extent is water the transport medium?

To what extent are water-associated infections a result of drinking the water or contact during recreational use or during contact necessitated by work?

With respect to contact transmission, what is the role of pre-existing lesions?

To what extent are aquatic organisms, other than those produced at the farm where the antimicrobial agents are used, involved in the transmission to humans?

7. Mortality and morbidity associated with infections by members of the bacterial group.

What is the variety of disease syndromes associated with infections by the bacterial group?

Are the different disease syndromes associated with different routes of infection?

What are the predisposing factors involved in infection associated morbidity or mortality?

With what frequency are infections by the bacterial group asymptomatic?

What is the incidence of human disease (of each type) associated with the bacterial group?

What sources and vectors, other than those associated with aquaculture, are associated with infections by the bacterial group?

What are the mortality rates associated with these infections?

8. Value and frequency of antimicrobial therapy provided to diseased individuals.

In what percentage of cases is antimicrobial treatment of therapeutic value?

What other factors influence the decision to treat with antimicrobial therapy?

What are the 1st line antimicrobial agents of choice?

What are the 2nd line antimicrobial agents of choice?

What is the frequency of resistance to any of these therapeutic agents?

4.3.2.2.

FRAMEWORK FOR DEVELOPING EXPOSURE PATHWAYS FOR THE ADVERSE EFFECTS IN LAND-BASED ANIMALS MEDIATED BY THE BIOLOGICAL HAZARD REPRESENTED BY BACTERIA RESISTANT TO ANTIMICROBIAL AGENTS.

Preliminary steps.

A. Identification of a specific bacterium or bacterial group.

Priority should be given to bacterial groups which are known to be involved in land-based animal disease and which have been reported to be present in the environment of aquaculture enterprises.

B. Identification of the antimicrobial agent of relevance to that bacterial group

The antimicrobial agents to which resistance is of significance are those that are used in the therapy of the diseases associated with infections of land-based animals by the selected bacterial group. Attention should also be paid to agents linked to those used in land-based animal therapies by the degrees of cross-resistance conferred by known resistance mechanisms.

Key events in the linkage

1. Presence of bacterium in the aquaculture environment.

2. Selective pressure, in the aquaculture environment, for the emergence of resistance in that bacterium.

3. Impact of selective pressure on resistance.

4. Routes by which resistant bacteria leave the aquaculture environment.

5. Survival in vector system.

6. Routes by which the resistant bacteria enter land based animals.

7. Mortality and morbidity associated with infections by members of the bacterial group.

8. Value and frequency of antimicrobial therapy provided to diseased individuals.

Questions relevant to a detailed consideration of the key events

1. Presence of the bacterium in the aquaculture environment.

What is the frequency of the occurrence of this bacterial group in aquaculture environments and what at what concentrations do they occur?

> The answers here will clearly vary depending on the environment of the aquaculture enterprise. Separate data will be required for fresh and marine environments and probably for temperate and tropical waters. In-shore and offshore farms may also have to be considered separately?

Is the bacterial group part of the natural flora of the aquaculture environment or a transient? Are they capable of growth in the aquacultural environment?

> The answers here will clearly vary depending on the environment of the aquaculture enterprise.

Are all the members of the bacterial group associated with disease in land-based animals?

> The quality and value of data on the enumeration and/or identification of members of bacterial groups in the aquaculture environment will be method dependent. Do the methods identify land-based animal pathogens or group that may contain some land-based animal pathogens?

What is the distribution (frequency and concentration) of the bacterial group within various components of the aquaculture environment (water, sediment, farmed or feral fish, other organisms)?

Does the bacterial group enter the aquatic environment from a controllable, or potentially controllable terrestrial source?

2. Selective pressure, in the aquaculture environment, for the emergence of resistance in that bacterium.

Are any relevant antimicrobial agents used in the aquaculture enterprise and how frequently are they applied?

> The answers here will vary from country to country and also may vary with respect to the species of aquatic organisms farmed.

What is the fate of any antimicrobial agents used?

> Here we will need to establish the concentration and persistence of the relevant agents in various compartments (water, sediment, farmed or feral fish, other organisms) of the aquatic enterprise?
>
> Data will also be required on the antimicrobial agent 'footprint' over which any impact can be expected.

To what extent do the distributions of antimicrobial agents in the aquacultural environment allow it to exert a selective pressure on the bacterial group?

> Within the aquacultural environment how does the distribution of the antibacterial agent map onto the distribution of the bacterial group?
>
> Within the aquacultural environment how does the distribution of the antibacterial agent map onto the areas where the members of the bacterial group under consideration are capable of multiplication? (Enrichment for resistance variants is very much more efficient in growing populations)

What is the minimum concentration of any relevant antimicrobial agent in any environment that can exert a selective pressure?

> The biological activity of all antimicrobial agents is context-dependent. In order to interpret data on the presence (concentration and persistence) of antimicrobial agents it is necessary to know their minimum effect concentrations with respect to selection of resistance variants.

3. Impact of selective pressure on resistance in the relevant bacterial group.

What empirical definitions of resistance are of value in this context?

> Any investigations of resistance will require standardised methods for measuring susceptibility.

What are the frequencies of resistance in the bacterial group in the microflora entering the aquaculture environment or present in that environment in the absence of antimicrobial agent use?

> Measurement of the frequencies of specific resistances in a particular bacterial group can be made by establishing either the frequency of variants manifesting these resistances within the group or by measuring the concentration of resistant variants in the aquaculture environment. These are independent variable that may not co-vary.

What are the frequencies of resistance in the bacterial group within the aquaculture environment during or after antimicrobial agent use?

> Measurement of the frequencies of specific resistances in a particular bacterial group can be made by establishing either the frequency of variants manifesting these resistances within the group or by measuring the concentration of resistant variants in the aquaculture environment. These are independent variables that may not co-vary.

4. Routes by which resistant bacteria leave the aquaculture environment.

Within which vector or vehicle do the bacterial group (and its resistant members) leave the aquaculture environment?

> The main categories here will be the water leaving the farm, mortalities and waste products from processing.

With what frequency are these vectors (or vehicles) contaminated with resistant bacteria as they leave the farm or farm environment?

What concentrations of the bacterial group occur in these vectors?

5. Survival in vector system

With respect to bacterial groups using water as a vector, what governs the survival of the bacterial group in the aquatic environment?

With respect to bacterial groups using 'aquatic organisms waste as fertilizer' as the vector, what are the effects of handling, storage, processing (if any) and exposure in soil on the survival of the bacterial group?

With respect to bacterial groups using 'aquatic organisms waste as animal feed' as the vector, what are the effects of processing and storage on the survival of the bacterial group?

6. Routes by which the resistant bacteria enter the land-based animal environment.

To what extent is water the vector involved in infections of land-based animals?

To what extent is 'aquatic organisms waste as fertilizer' the vector involved in infections of land-based animals?

To what extent is 'aquatic organisms waste as animal feed' the vector involved in infections of land-based animals?

> The relative importance of these routes of infection can be established by examination of record of epidemiological studies of land-based animal infections.

7. Mortality and morbidity associated with infections by members of the bacterial group.

What is the variety of disease syndromes associated with infections by the bacterial group?

With what frequency are infections by the bacterial group asymptomatic?

What is the incidence of land-based animal disease (of each type) associated with the bacterial group?

What sources and vectors, other than those associated with aquaculture, are associated with infections by the bacterial group?

What are the mortality rates associated with these infections?

8. Value and frequency of antimicrobial therapy provided to diseased individuals.

In what percentage of cases is antimicrobial treatment of therapeutic value?

What other factors influence the decision to treat with antimicrobial therapy?

What are the 1st line antimicrobial agents of choice?

What are the 2nd line antimicrobial agents of choice?

What is the frequency of resistance to any of these therapeutic agents?

4.3.2.3.

FRAMEWORK FOR DEVELOPING AN EXPOSURE PATHWAY FOR THE ADVERSE EFFECTS IN AQUATIC ORGANISMS MEDIATED BY THE BIOLOGICAL HAZARD REPRESENTED BY BACTERIA RESISTANT TO ANTIMICROBIAL AGENTS.

Preliminary steps.

A. Identification of a specific bacterium or bacterial group.

Priority should be given to bacterial groups known to be involved in aquatic organism disease.

B. Identification of the antimicrobial agent of relevance to that bacterial group

The antimicrobial agents to which resistance is of significance are those that are used in the therapy of the diseases associated with infections of fish.

Key events in the linkage

1. Presence of bacterium in the aquaculture environment.

2. Selective pressure, in the aquaculture environment, for the emergence of resistance in that bacterium.

3. Impact of selective pressure on resistance.

4. Routes by which resistant bacteria persist or move within the aquaculture environment.

5. Incidence of disease, associated with a resistant bacterial group that requires therapy.

6. Availability of alternative therapeutic or disease control options.

Questions relevant to a detailed consideration of the key events

When antimicrobial agent use in aquaculture is metaphylactic, it is reasonable to assume that some bacteria associated with aquatic organism disease are present, or are thought to be present every time antimicrobial agents are administered.

When, however, agents are used prophylactically this assumption may not be valid.

1. Presence of bacterium in the aquaculture environment.

With what frequency and at what intensity is the bacterial group present in the aquaculture environment?

What is the frequency of disease associated with infections by the bacterial group?

2. *What is the selective pressure, in the aquaculture environment, for the emergence of resistance in that bacterial group?*

How frequently are antimicrobial agents administered to treat infections associated with the bacterial group in question?

Is antimicrobial agent use appropriate in terms of choice of agent?

What is the frequency with which selection of a therapeutic regime is supported by of microbial diagnosis and susceptibility testing?

Is antimicrobial agent use appropriate in terms of administration protocol?

The use of inappropriate administration protocols may increase the rate at which resistant variants emerge.

3. Impact of selective pressure on resistance in the relevant bacterial group.

What empirical definitions of resistance are of value in this context?

> Any investigations of resistance will require standardised methods for measuring susceptibility.

What are the frequencies of resistance in the bacterial group within the aquaculture environment during or after antimicrobial agent use?

What are the sources of resistance genes?

> Mutational resistance (quinolones) can arise de-novo, but positive function resistances must be derived from another, donor bacterium. Even intense selective pressure cannot have any impact on the frequencies of positive function resistance in the target bacterial group if the relevant genes are not already present in the microflora present in the environment.

Where does selection for resistance occur?

4. Routes by which resistant bacteria leave the aquaculture environment.

Within which vector or vehicle does the bacterial group (and its resistant members) move between farms?

> The main categories here will be the target fish, feral fish, fomites used in farm operation and the water leaving the farm. Additional consideration may also have to be given to cross-farm contamination arising from harvesting and processing practices.

With what frequency are these vectors (or vehicles) contaminated with resistant bacteria as they leave the farm or farm environment?

What concentrations of the bacterial group occur in these vectors?

5. Incidence of the disease associated with the bacterial group under consideration.

(see 1 above)

6. Availability of alternative therapeutic or disease control options.

In the case of the bacterial group manifesting resistance to one agent, are there any other alternative and effective agents available?

Are there alternative prophylactic treatments or husbandry procedures that can effectively treat the diseases?

4.3.3. Exposure pathways for hazard 3 - resistance genes

The exposure pathways presented here for hazard group 3 are incompletely developed and will require further work.

4.3.3.1.

FRAMEWORK FOR DEVELOPING EXPOSURE PATHWAYS FOR THE ADVERSE EFFECTS IN HUMANS MEDIATED BY THE BIOLOGICAL HAZARD REPRESENTED BY GENES ENCODING RESISTANCE TO ANTIMICROBIAL AGENTS.

Preliminary steps.

A. Identification of specific genes of critical importance.

Priority should be given to genes that encode resistance to agents that have been identified as critically important in human medicine.

Key events in the linkage

1. Presence of the gene in the aquaculture environment.

2. Genetic structures associated with resistance gene.

3. Selective pressure, in the aquaculture environment, for the enrichment of the gene in the microflora.

4. Impact of selective pressure on resistance gene frequency.

5, Movement of resistant genes between the aquaculture environment and the human environment.

6. Entry of the resistant genes into bacteria associated with human disease.

7. Stabilisation of resistance gene in bacteria associated with human disease.

8. Mortality and morbidity associated with infections by bacterial carrying the resistance gene.

Note

The sequence in which issues 5 & 6 need to be treated will vary depending on where the transfer of genes to bacteria associated with human disease is postulated to occur.

Scenario 1. Transfer occurring in the aquaculture environment.
Scenario 2. Transfer occurring during passage in the vector (vehicle).
Scenario 3. Transfer occurring within the human compartment.

Questions relevant to a detailed consideration of the key events

1. Presence of the gene in the aquaculture environment.

What is the frequency with which the gene is present in the aquaculture environment prior to antimicrobial use?

What is the concentration of the gene in the aquaculture environment prior to antimicrobial use?

To what extent are the sources of the resistance genes in the aquacultural environment anthropogenic and controllable?

2. Genetic structures associated with resistance gene.

With what frequency are resistance genes chromosomally located?

With what frequency are the genes of interest associated with transposons and/or integrons?

With what frequency are the genes of interest located on self-transmissible plasmids?

With what frequency are the genes of interest physically associated with genes encoding resistance to other antimicrobial agents?

3. Selective pressure, in the aquaculture environment, for the enrichment of the gene in the microflora.

Are any antimicrobial agents, capable of exerting a selective pressure for the enrichment of gene of interest, used in the aquaculture enterprise and how frequently are they applied?

The answers here will vary from country to country and also may vary with respect to the species of aquatic organism farmed.

What is the minimum concentration of any relevant antimicrobial agent in any environment that can exert a selective pressure?

The biological activity of all antimicrobial agents is context-dependent. In order to interpret data on the presence (concentration and persistence) of antimicrobial agents it is necessary to know their minimum effect concentrations with respect to selection of bacteria possessing resistance genes.

To what extent do the distributions and concentrations of antimicrobial agents in the aquacultural environment allow it to exert a selective pressure on the bacteria possessing the resistance gene?

Within the aquacultural environment how does the distribution of the antibacterial agent map onto the distribution of the bacterial possessing the resistance gene?
Within the aquacultural environment how does the distribution of the antibacterial agent map onto the areas where the bacterial possessing the gene under consideration are capable of multiplication? (Enrichment for resistance variants is very much more efficient in growing populations)

4. Impact of selective pressure on resistance gene frequency.

(Selection for enrichment of a gene in the environment can only occur via the selection of bacteria possessing that gene.)

What empirical definitions of resistance are of value in this context?

Any investigations of resistance will require standardised methods for measuring susceptibility.

What percentage of bacteria manifesting phenotypic resistance can be treated as possessing resistance genes?

What are the frequencies and concentrations of bacteria possessing resistance genes within the aquaculture environment during or after antimicrobial agent use?

5 & 6, Movement of resistant genes between the aquaculture environment and the human environment and transfer to bacteria associated with human disease

Scenario 1. Transfer occurring in the aquaculture environment.

What is the incidence and concentration of bacteria associated with human disease in the aquaculture environment?

What is the frequency of transfer of resistance genes to bacteria associated with human disease in the aquaculture environment?

What are the survival rates of bacteria associated with human disease as they move from the aquaculture environment to humans?
Does the acquisition of resistance genes affect these survival rates?

Scenario 2. Transfer occurring during passage in the vector (vehicle).

Within the vector what are the incidences and concentrations of bacteria possessing resistance genes and whose frequency was increased in the aquaculture environment?

What is the incidence and concentration of bacteria associated with human disease in the vectors?

What is the frequency of transfer of resistance genes to bacteria associated with human disease in the vector?

What are the survival rates of bacteria associated with human disease as they move in the vector to humans?
Does the acquisition of resistance genes affect these survival rates?

Scenario 3. Transfer occurring within the human compartment.

What are the incidences and concentrations in humans of bacteria possessing resistance genes and whose frequency was increased in the aquaculture environment?

What is the frequency, within humans, of the transfer of resistance genes from bacteria derived from the aquaculture environment to bacteria associated with human disease humans?

7. Stabilisation of resistance gene in bacteria associated with human disease.

With what frequency are resistance genes, when they enter bacteria associated with human disease, physically located on structures that can be stably inherited in those new hosts?

With what frequencies can resistance genes move to structures that are stably inherited?

8. Mortality and morbidity associated with infections by bacterial carrying the resistance gene.

What increases in morbidity or mortality are associated in humans infected with bacteria possessing resistance to any specific agent.

4.3.3.2.

FRAMEWORK FOR DEVELOPING EXPOSURE PATHWAYS FOR THE ADVERSE EFFECTS IN LAND-BASED ANIMALS MEDIATED BY THE BIOLOGICAL HAZARD REPRESENTED BY GENES ENCODING RESISTANCE TO ANTIMICROBIAL AGENTS.

No explict exposure pathway has been developed for this risk. It would appear, however, that the factors to be considered are in the majority of respects similar to those identified in 4.3.3.1 above.

4.3.3.3.

FRAMEWORK FOR DEVELOPING EXPOSURE PATHWAYS FOR THE ADVERSE EFFECTS IN AQUATIC ORGANISMS MEDIATED BY THE BIOLOGICAL HAZARD REPRESENTED BY GENES ENCODING RESISTANCE TO ANTIMICROBIAL AGENTS.

Preliminary steps.

A. Identification of specific genes of critical importance.

Priority should be given to genes that encode resistance to agents that are used in aquaculture.

Key events in the linkage

1. Presence of the gene in the aquaculture environment.

2. Genetic structures associated with resistance gene.

3. Selective pressure, in the aquaculture environment, for the enrichment of the gene in the microflora.

4. Impact of selective pressure on resistance gene frequency.

5, Entry of the resistant genes into bacteria associated with aquatic organism disease.

6. Stabilisation of resistance gene in bacteria associated with aquatic organism disease.

7. Mortality and morbidity associated with infections by bacterial carrying the resistance gene.

Questions relevant to a detailed consideration of the key events

The issues raised by key events 1 – 4 are essentially similar to those discussed in 4.3.3.1.

5, Entry of the resistant genes into bacteria associated with aquatic organism disease.

What is the frequency with which two different bacteria, both associated with aquatic organism disease, co-habit the same environmental niche?

What is the frequency of transfer, within the aquatic environment, of resistance genes between bacteria associated with aquatic organism disease?

6. Stabilisation of resistance gene in bacteria associated with aquatic organism diseases.

With what frequency are resistance genes, when they enter new bacteria associated with aquatic animal disease, physically located on structures that can be stably inherited in those new hosts?

With what frequencies can resistance genes move to structures that are stably inherited?

8. Mortality and morbidity associated with infections by bacterial carrying the resistance gene.

What increases in morbidity or mortality are associated in aquatic organisms infected with bacteria possessing resistance to any specific agent.

5. Risk assessment

5.1 The nature of risk assessment

The nature of the risk analysis process and the time that has been available for the preparation of this document are the main reasons why no risk assessment has been attempted.

It is further, strongly argued that, at this stage of the process none could or should be made.

As the Codex Alimentarius Commission (2004) in the 14^{th} edition of their procedural manual, stressed effective communication and consultation with all interested parties should be ensured throughout the risk analysis process. Clearly, with respect to the risks addressed in this document, this essential and fundamentally important process of communication and consultation couldn’t be initiated

Clearly modesty in what we hope to achieve and claim to have achieved, is not only prudent but is also absolutely necessary.

It is also argued that no reasonable progress can be made without taking into account the diversity of aquaculture and the complexity of the multiple exposure pathways that it generates.

5.2 The diversity of aquaculture

The earlier sections of this document have extensively documented the diversity of the aquaculture enterprises that are encountered around the world. They have also identified the wide diversity of hazards and the complexity of the exposure pathways by which those hazards might exert their adverse effects.

These considerations clearly suggest that attempting a global analysis of the risks for human or animal health presented by the use of antimicrobial agents in aquaculture is not possible and does not make sense from a scientific point of view.

If progress is to be made it is clear that the whole problem will have to be broken down into smaller task that can be explicitly and clearly formulated. The discussions in the earlier sections suggest a multitude of different, specific risks could be analysed.

Given the cost and effort that would be required to perform an adequate analysis of any one specific risk, it is clear that some attempt at prioritising the various risks will be essential

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Comment: That should be the outcome of the meeting. we may keep it for the final draft.

5.3 Defining a reasonable target.

In a background paper prepared for a Joint FAO/WHO Consultation held in Kiel, Germany on March 18-22, 2002, Buchanan and Dennis (2002) addressed the lesson learnt in previous risk analyses. They identified the commissioning of a risk assessment as a critically important initial step.

> "*Commissioning a risk assessment is perhaps the most critical step in the conduct of a risk assessment because it establishes what the question(s) that the risk assessment will address, the resources that will be brought to bear, and the expectations that the risk managers have in relation to both the product that will be produced and the timeframe in which the activity can be completed.*"

Thus, the experience of these authors argues that the first step in commissioning a risk assessment is that of specifying the risk that is to be addressed.

It is argued here that this issue of identifying which risk assessment(s) should be commissioned represent a reasonable and achievable target for the Expert Consultation Group meeting in Seoul.

We, therefore, recommend that, at this stage, the prioritisation of the various risks that can be identified should be our primary objective.

It is important in defining a risk that it is specified in sufficient detail. As discussed above, a general risk that is too broad in its scope will lead to confusion not only in the risk assessment phase but also, and more importantly, it will preclude and hinder the development of effective risk management strategies.

The discussions in the previous sections would suggest that a risk definition should include a number of items and should as a minimum include;

i) Definition of a specific hazard and not a general hazard group (see section 3)

ii) Definition of the specific aspect of the worldwide aquaculture industry that is to be the territory of the investigation. Here it will be necessary to specify the species farmed and the environmental conditions of the enterprises.

iii) Definitions of the geographical area with respect to which the risk analysis is to be performed. In this sense the geographical area provides a proxy classification of the socio-economic and scientific infrastructural of the aquaculture operation.

6. Risk Management

Risk assessment and risk management are closely linked. Buchanan and Dennis (2002) emphasised the necessity, in any risk analysis, of a continuously iterated feedback between risk managers and risk assessors

The previous section addressed the reasons why a risk assessment cannot be developed rapidly and why, at this stage, none could be attempted.

Clearly if the development of a detailed risk assessment is premature then this also holds true for scientific based risk management.

Both logic and prudence argue that you cannot attempt to manage a risk until you have assessed it. Importantly the risk assessment process and its associated critical analysis of exposure pathways provide data that is critical for the development of effective management strategies. Regulations designed to reduce a risk that are not adequately informed by the data derived from assessment are liable to be ineffective, intrusive and non-functional.

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Comment:

At this stage a number of observations about risk management can, however, be made.

The extent to which any regulations aimed at managing a risk impact on any activity should be proportional to that risk associated with that activity.

Any regulations aimed at minimising the risks associated with an activity must, if they are to be effective, take account of the socio-cultural, economic context of that activity. They must also take account of the scientific, technical and administrative resources that are available to implement any proposed regulations.

Arguments that risk management strategies and risk management regulations must be developed in conjunction with risk assessment do not preclude the development of guidelines for the prudent use of antimicrobial agents in aquaculture. Not should they preclude the dissemination of these guidelines or their use in educational programmes.

APPENDIX I

Towards Prudent use of antimicrobials in aquaculture

In veterinary medicine prudent use has the overall goal to reduce antimicrobial usage, with particular emphasis on the relative use of broad-spectrum and critically important drugs. Rational use refers to rational administration of antimicrobials with the purpose of optimising clinical efficacy while minimising development of resistance.

General reasons for prudent and rational use

Ecological considerations

Antimicrobial agents are biologically active compounds and as such should be used with prudence.

General principles of appropriate stewardship of the planet and its biosphere would demand that biologically active compounds should not be needlessly distributed within the environment.

In the case of these particular compounds, we are aware that the major response of the biosphere to their presence is an increase in the relative frequency of bacteria that manifest an increased tolerance of their action. It is possible that antimicrobial agents may have a negative impact on important metabolic and degradative activities of prokaryotes in the environment. Evidence suggests, however, that such effects will be transitory and will be rapidly compensated for by the emergence groups capable of performing these functions in the presence of antimicrobial agents.

Increases in the frequency of tolerance (resistance) to antimicrobial agents probably have little negative impact on prokaryotic ecology or the biosphere generally. Bacteria have always and will always be, in a continuous state of adaptation and re-adaptation to novel environmental factors,

Even when we have no specific reasons to suspect that an adverse effect will result from their use, we never know enough to be sure. Humility requires caution and caution requires prudence.

Considerations of resistance in general

The major significance of increased frequencies of bacteria that can tolerate elevated concentrations of antimicrobial agents arises in situations where we, as humans, wish to use these agents to manipulate bacteria for our own purposes. To the extent that our use of these agents to control bacteria is the dominant factor leading to an increase in the frequency of tolerance (resistance) among those bacteria, our use of them is under negative control. As a consequence of this negative control loop, the value to us of antimicrobial agents, is roughly in inverse proportion to the frequency with which we use them. The current evidence suggests that this statement can be made more specific. The more we use them for a particular purpose the less likely they are to serve that purpose.

What is abundantly clear, for example, is that the major factor that has led to the emergence of the resistant bacteria that limit the value of antimicrobial agents in the control of human infections has been the use of these agents to control previous infections in humans.

The same argument can be made for the use of antimicrobial agents in aquaculture. The major consequence of the use of antimicrobial agents in aquaculture has been the development of resistances that limit the value of these agents in controlling bacterial infections in aquaculture (Smith et al., 1994).

As a consequence it is clear that prudent use of antimicrobial agents is in the self-interest of all users including those involved in aquaculture.

Considerations of resistance to aquaculture users

It has been argued earlier that aquaculture is an important and in some situations, vitally important industry. It has also been demonstrated that even given optimum husbandry and environmental conditions antimicrobial agents will be needed on occasions.

In aquaculture the imprudent and excessive use of antimicrobials is not only short sighted, it is also fundamentally bad economics. Imprudent or excessive use is expensive and there is ample evidence that it leads to a more rapid emergence of resistance. If aquaculture is to avoid entering the pre-antibiotic era the avoidance of selecting for resistance in bacteria associated with aquatic organism diseases should be an important aim of all therapies aimed at disease control. In the absence of resistance therapeutic interventions are more effective, and recurrence of disease is reduced. The costs associated with a disease outbreak including those associated with medication, manpower, and losses of sales due to prolonged or repeated treatments are but a few examples.

Importantly for the arguments being debated in this document, the case for prudent use of antimicrobial agents in aquaculture would be strong and compelling even if it were demonstrated that the risks for human health associated with this use were negligible.

Considerations of resistance to other users.

There does exist the possibility of transmission of antimicrobial resistance from aquaculture to bacteria that are pathogenic to humans, although validated scientific studies, which document this type transmission, are rare to non-existent. After due consideration of the available data, it may be demonstrated that the use of antimicrobial agents in some aspects of aquaculture represented a significant or unacceptable risk of adverse effects on human therapies. Were this situation to pertain it would increase the urgency with which prudent use should be pursued but it would not significantly alter the prudent use guidelines outlined below.

Guidelines for prudent and rational use of antimicrobials in aquaculture

Due to the incredibly vast differences in aquatic species, approved medications, and culture practices between countries, it is difficult to formulate specific recommendations that encompass all antimicrobial usage in aquaculture. It is apparent however, that intervention at both the governmental/regulatory level and at the industry level is necessary to provide adequate resources and a coordinated approach to resolve or avoid problems associated with antimicrobial use. There are a number of general recommendations concerning judicious use of antimicrobials which if applied would likely limit antimicrobial resistance development and promote residue avoidance. The following text outlines these principles of judicious use.

Recommendations at the governmental/regulatory level

1. Ensure diagnostic support for a developing industry

To ensure the proper application of antimicrobial therapy an accurate diagnosis is essential. In those areas which have no consultative services or where the size/type of industry is such that a diagnostic service cannot be self-supporting, the development of government sponsored diagnostic support should be encouraged.

2. Develop extension services in collaboration with producers associations where they exist

The presence of professionals which have been trained in principles of aquaculture health management are extremely important resources for producers. Responding to producers questions, conducting on farm interventions during disease outbreaks, making recommendations on therapeutic options, as well as providing important information to producers concerning regulations, their application and their importance are among the many practical services they can provide.

3. Establish regulations concerning the use of antimicrobials

These regulations should include the permitted products and allowable withdrawal times. Antimicrobials should be specifically designed for aquatic animals, and licensed by the competent authorities. A monitoring system should be developed to follow the amounts used in aquaculture, either monitoring sales or prescriptions, or both.

4. Develop infrastructure to enforce existing regulations

Once the regulations are established, it is of crucial importance to have the infrastructure to enforce them. This is often missing in developing countries. Control of importation, commercialization and use of antimicrobials which are not permitted for use in aquaculture should be established. The imposition of inspections for antimicrobial residues in aquaculture products for domestic or export markets may help to discourage the use of forbidden substances.

5. Improve the availability of antimicrobials specifically designed and licensed for aquatic animal species

In most countries there are very few products registered for aquatic animal species, if any. The use of the most active substance and the rotation of substances are paramount for the limitation of the development of antimicrobial resistances. This will only be achieved by the improvement of the availability of products specifically designed and licensed for aquatic animal species.

6. Support appropriate research

Aquaculture is a relatively young industry. In many areas there is a shortage of the basic scientific knowledge necessary for its development. In the context of prudent use of antimicrobials a number of research priorities can be identified, although their relative importance will vary from location to location. Research into improved husbandry techniques, into appropriate breakpoints for attributing species-specific meanings to susceptibility test data and into antimicrobial agent administration techniques and the resultant pharmacokinetics are all areas where there are significant gaps in our knowledge.

Recommendations at the industry level

1. Establish an accurate diagnosis

The efficacious use of an antimicrobial is necessarily dependant upon the presence of a susceptible causal infectious agent in the animal population. The accurate identification of the disease present is of paramount importance when planning health management strategies. Viral disease for example, would clearly be a poor candidate for therapeutic intervention. Likewise, not all bacterial diseases are conducive to antimicrobial treatments. Mitigated results are often obtained following treatment of certain chronic bacterial diseases such as Bacterial kidney disease *(Renibacterium salmoninarum)* which may argue for the utilisation of preventive measures such as vaccination or depopulation rather than antimicrobials.

2. Remediate underlying disease processes

Bacterial infections may be encountered secondary to underlying disease conditions. As mentioned in the preceding example, it would be of little use to apply an antimicrobial regime in a situation where a bacterial septicaemia is secondary to viral disease. However, this co-infection may lead a grower to orient control and treatment strategies in the wrong direction. Certain conditions which stress aquatic animals may provoke a disease outbreak. Excessive parasitism, which may be encountered with sea lice in Atlantic salmon (*Salmo salar*), or *Gyrodactylus* spp. in speckled trout (*Salvelinus fontinalis*) are two examples. Concentrating therapeutic efforts on a secondary bacterial septicaemia, and ignoring the instigating factor of the disease outbreak is a guarantee for recidivism of the disease.

3. Establish sensitivity patterns for the bacterial pathogen responsible for disease

The correct choice of antimicrobial for therapeutic intervention in aquaculture should be based on antimicrobial sensitivity patterns of the offending organism. The utilisation of standard methods of sensitivity testing by the collaborating laboratory is primordial for obtaining information applicable to the bacterial species in question. Proposed guidelines for standard susceptibility testing methods concerning non-fastidious aquatic bacteria have recently been published by the CLSI (Clinical and Laboratory

Standards Institute), and should be applied when possible. In the absence of susceptibility tests, or when confronted with bacteria for which standard susceptibility determination protocols are inappropriate, such as with *Vibrio* spp. the choice of antimicrobial can be based on previous antimicrobial sensitivity results, a history of success or failure of previous applications of medication, as well as drug availability and economic feasibility for the producer.

A careful follow-up of the affected aquaculture organisms should be made, noting efficacy of the selected antimicrobial (decrease in mortality, and delay of identifiable action of the antimicrobial, and mortality). Susceptibility testing should be repeated if the diseased aquatic organisms are non-responsive to treatment, or mortality persists following the prescribed duration of treatment. Re-submission of samples for necropsy and changes in husbandry (ex. decrease culture densities, increase oxygenation etc.) should also be considered in cases of persistent bacterial disease.

3. Ensure proper utilisation of antimicrobials; accurate dosage, recommended duration, and appropriate administration route

Careful calculation of the antimicrobial dosage is essential for therapy success. This implies necessarily that knowledge of fish or aquatic animal biomass is known or can be accurately estimated. Treatment of bacterial disease with a sub-therapeutic dosage of antibiotics is known to be an effective method for selection of resistant bacteria. Antimicrobial formulations which are licensed for use in aquaculture generally provide recommendations on administration dosages and techniques. They frequently contain less than 100% active ingredient, and the calculation of the quantities of medication required must take this into account. Antimicrobials are available in medicated feed in some locations, whereas in others, on-farm "topdressing" of a medicated premix is the privileged method of administration. If the latter is the case, adequate mixing of the premix and the feed must be ensured to permit a homogenous administration of the calculated medication to the aquatic animals.

The recommended duration of medication administration is extremely important and must be respected. Because of the expense of medication, there is a temptation to stop antimicrobial administration when the level of mortality is acceptable, but not completely arrested. However, if a treatment is interrupted too rapidly, there is a risk of selection of moderately susceptible strains in the bacterial population. Rapid recrudescence is a likely sequel, as well as repeated antimicrobial treatments.

4. Ensure ingestion of oral antimicrobial therapy

Antimicrobials in most cases are administered orally in aquaculture enterprises. Logically, for therapy to be effective, the medication must be ingested. Depending on the disease organism and the species of cultured animal, inappetence may become a problem with the progression of the disease, making oral administration of antimicrobials difficult. In this case, the feeding rate should be decreased to ensure that all medicated feed is absorbed, and restrict the spoiling of uneaten medicated feed.

Certain medications, such ormetoprim-sulfadimethoxine are refused by some fish species at recommended treatment levels, so therapeutic doses of this drug can at times be difficult to attain. When this problem is encountered, an alternative medication should be considered if available. Antimicrobial therapy in cases where the medication will not be ingested should be avoided.

5. Avoid repeated use of the same antibiotic

Repetitive treatments with a single antimicrobial class have been identified as an important risk factor associated with the development of antimicrobial resistance. Where feasible, a rotation of antimicrobial classes in treatment of bacterial disease should be considered. To allow such rotation, the availability of registered antimicrobials for aquatic animal species is of the higher importance, most of all in countries with rapidly increasing aquaculture production.

6. Use a narrow- spectrum antibiotic whenever possible and avoid indiscriminate use of drugs

An antibiotic that is specific for the infectious agent in question is the ideal choice when considering antimicrobial therapy. An antimicrobial with a larger spectrum of action than required will likely impact a greater number of bacteria in the surrounding environment. A higher number of species exposed to an antimicrobial increases the chances that a bacterium containing resistant genetic elements will be enriched or selected. The combination of antibiotics in treatment is not recommended unless synergism is likely to occur.

7. Observe recommended withdrawal periods

Antimicrobial withdrawal periods must be respected to ensure the innocuity of the aquaculture product for consumers. The recommended withdrawal periods for approved medications may vary with temperature or dosage, and in general have been developed for a limited number of species. Whenever possible products licensed for the species in question should be used. If none are available, the utilisation of a product licensed for another food-producing species should be considered. In cross-species prescription, an application of a minimal withdrawal period of at least 500ºCd is recommended.

8. Keep accurate records concerning antimicrobial use

Information concerning the diagnosis, the drug prescribed, the date and duration of treatment, the number of fish treated and withdrawal times to be respected should be kept for all groups of aquatic animals under treatment.

9. Store antimicrobial products in an appropriate manner

Antimicrobials are labile compounds which are subject to degradation if stored in an inappropriate manner. Storage in a dark, cool, dry area is generally recommended. The quality of the chemicals should equally be ensured by utilizing only licensed products obtained from reputable wholesalers, and expired products should not be used. Utilisation of expired products, those stored in an inappropriate, or those of inferior quality, may result in the administration of sub-therapeutic dosages to diseased aquatic animals.

10. Do not use antimicrobials in a preventative manner

Antimicrobials are not used as growth promoters in aquaculture, but are used occasionally in a preventive manner. The utilisation of antimicrobials in the treatment of an anticipated disease should be discouraged. Rather, preventive measures targeting husbandry of the species in question should be encouraged to avoid disease outbreaks.

11. Maximize waste management strategies

The objective of antimicrobial therapy is to attain a complete administration of a therapeutic dose of medication to the animal, e.g. decreasing the feeding rate during the medication period. . However, there will be a fraction that is lost to the water column following leaching of the medicated feed, and a portion of non-absorbed medication excreted by the aquatic organism in the urine and feces. Recuperation of the fecal portion by using waste recuperation techniques such as sedimentation or filtration will help reduce the quantity of antimicrobial released to receptor watersheds. The binding of certain antimicrobials with benthic sediments, as well as degradation by microbial processes and exposure to sunlight will decrease the quantity of available active medication in the environment.

12. Maximise the utilisation of disease prevention strategies which will minimise the necessity of antimicrobial utilisation.

The use of antimicrobials should at no time be considered in isolation of other good production practices. The implementation of biosecurity measures, the utilisation of vaccines, nutritional optimisation, husbandry changes, and others are among the elements of a successful disease prevention program. The implication of a veterinarian or fish health professional when available is extremely useful when considering the application of preventative disease measures to the health management of an aquaculture enterprise. Their expertise with the species in question, as well as pathology, pharmacology, immunology, physiology and other connected disciplines makes them a vital resource when planning disease control strategies.

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