

Antimicrobial resistance in aquaculture

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Summary

Appropriate antimicrobial therapy represents one of the most effective management responses to emergencies associated with infectious disease epizootics. The use of these agents, however, has the potential to increase the frequencies of bacterial resistance and this would have a negative impact on the subsequent use of these agents to control infectious disease in aquaculture. There is also a possibility that the enrichment of resistant bacteria or genes encoding resistance could have an adverse impact on the use of antimicrobial agents to control diseases in humans and other land-based animals. Attempts to apply formal risk analysis to this problem have been frustrated by the extreme diversity of aquaculture and by the general shortage of relevant data. A central argument made in this paper is, however, that not only do we lack the data this exercise would require; we also lack validated methods for collecting those data in the first place. At the most fundamental level we do not even possess validated methods for determining whether a bacterium isolated from an aquaculture site should or should not be classified as resistant. In the absence of any significant risk assessment, current attempts at risk management are centred on the development of lists of critically important antimicrobials for the various users of these agents. It is argued here that studies of gene ecology and models of gene flow in the environment are urgently required if we are to be able to evaluate this risk management approach, to predict its consequences or to generate more appropriate strategies.

Keywords

Antimicrobial agent – Aquaculture – Critically important antimicrobial – Public health – Resistance – Risk analysis – Susceptibility test methods.

Introduction

Antimicrobial agents have the potential to play an important role in promoting human health. This role may be mediated directly, by their use to control disease in humans, or indirectly, by their use to control disease in animals reared for consumption by humans. In addition to their role in human health, antimicrobials also have the potential to play a major role in promoting animal welfare.

The use of these agents involves an unavoidable and inexorable negative feedback loop. This feedback loop is a function of the awesome ability of bacterial populations to respond to adverse environmental conditions. When any bacterial population is exposed to inhibitory concentrations of antimicrobial agents, variants will

emerge within that population that are able to continue to function in the presence of those agents. The automatic consequence of this ability of bacterial populations to respond to antimicrobial agents is that the more we use these agents to control bacterial infections the more frequently will we encounter bacteria resistant to their action. Thus, the fundamental position is that the more we use antimicrobials the less value they will have.

The slow realisation of the inevitability of this negative feedback loop has led to an increasing awareness that we must use these agents only when they are necessary and, when we use them, our use must be, to the best of our ability, rational. Kruse and Guardabassi (60) have defined rational use as the adoption of treatment regimen that have improved clinical efficacy but that result in minimal selective pressure for emergence of resistant variants.

The use of antimicrobials in aquaculture

Any agricultural or aquacultural farming operation that relies on the routine and regular use of antimicrobials to control losses is, in the long run, unsustainable. The continued use of antimicrobials will lead to the emergence of resistance in the target bacteria. Thus, such a dependence on antimicrobials not only represents an unacceptable and imprudent use of these valuable agents, but it will almost certainly prove to be self-defeating.

In any population of farmed animals, maintaining appropriate living conditions, employing appropriate husbandry protocols and using, when they are available, vaccines against enzootic or frequently encountered infections are the primary and most effective methods by which losses to infectious disease can be limited. However, the aim of all of these prophylactic procedures is to limit the occurrence of infectious disease and it is unrealistic to expect them to entirely prevent any occurrence of these diseases. Even in well-run farming operations, infectious disease emergencies must be expected and must be planned for. The application of the prophylactic measures mentioned above will mitigate the impact of any emergency epizootic, but once that epizootic has started the only action that can be taken to promote animal welfare is the administration of a therapeutic treatment. In this context, the administration of antimicrobials has been demonstrated to be the most effective treatment option. Thus, the inevitability that disease emergencies will occur requires that we learn how to use antimicrobials in such a way as to maximise their efficacy whilst minimising the pressure for increased frequencies of resistant variants that is automatically a consequence of their use. If we are to achieve these aims we must first have some general understanding of how these agents are being used in aquaculture.

Which agents are employed in aquaculture?

As a general observation we can state that all antimicrobial agents in use in aquaculture are also used in human or veterinary medicine. There are no antimicrobial agents that have been specifically developed for aquacultural use and simple economic considerations suggest that this will always be the case.

In attempting to list the range of agents that are employed in aquaculture, Schnick *et al.* (87) commented that there were wide variations in the quality of the available data. Some lists appear to include all agents that might ever have been used or might have been considered for use, whilst others would appear to be seriously incomplete.

The regulation of antimicrobial agent availability to aquaculture is one factor that influences the range of agents used; however, the degree of regulation and enforcement of regulations varies widely between countries. In some countries, generally those of Northern Europe, North America and Japan, regulations governing access are reasonably strict, and the products that are licensed in each country may give some indication of the agents actually used. At the other extreme, there are many countries with significant aquaculture industries where there is little effective regulation of access to, or use of, antimicrobials.

With respect to Europe, Guichard and Licek (40) have identified the agents contained in the products that have received market authorisations in various countries. They reported that the most frequently licensed products are those containing oxytetracycline (16 countries), first-generation quinolones (12 countries), potentiated sulphonamides (9 countries), florfenicol (8 countries), and amoxicillin (3 countries). However, in each country it is common that no more than 2 or 3 antimicrobial agents are licensed for use in aquaculture. Listing licensed products will, however, nearly certainly lead to an underestimation of the range of agents being used in European aquaculture. There are countries in this region that have significant aquaculture industries but have no licensed antimicrobials. Anecdotal evidence suggests that, in these countries, the use of antimicrobial agents is widespread and may include the use of agents not included in the list prepared by Guichard and Licek (40). In addition, in many countries, off-label use of antimicrobials may be significant and may also result in the use of a wider range of agents.

The range of antimicrobials used in countries where there are no strict regulations governing access to these agents is even more difficult to assess. Arthur *et al.* (10) edited the proceedings of a meeting on the use of chemicals in aquaculture in Asia, which provide some data, much of it anecdotal, on the range of agents used in various Asian countries. The papers presented to this meeting suggest that the range of agents used in Asia includes those licensed in Europe, but that in addition, a wide variety of other compounds have or are being used. For example, it has been estimated (106) that up to 122 different preparations containing various antimicrobials have been applied in Vietnamese shrimp culture.

To what extent are antimicrobials used in aquaculture?

Assessing the amounts of antimicrobial agents used in land-based food animal production in Europe has proved difficult (21). Even greater problems are encountered in attempting to estimate the amounts used in world aquaculture. Table I presents an attempt to estimate the use of antimicrobials in aquaculture in some countries. The

most accurate statistics are those available from Scandinavian countries. The data presented for other countries are estimates and the original sources should be consulted to assess their probable accuracy.

Table I suggests that there is a very wide variation in the use of antimicrobials. Although we lack much of the required data, it is reasonable to suggest that the dominant species farmed, the degree of regulation and the extent of technical services available are all factors that may contribute to this variation. One important factor that can be identified as influencing antimicrobial use in an industry is the availability of vaccines that provide effective immunoprophylaxis for the dominant infectious diseases it experiences. The very low use of antimicrobials in Norway has been attributed to the availability of vaccines for furunculosis and cold-water vibriosis (69). In Chile, where the dominant species farmed are similar to those farmed in Norway, the high use of antimicrobials is likely to be related to the lack of an effective vaccine for the rickettsial infections that are experienced by this industry.

Table I
Estimates of antimicrobial use in the aquaculture industries of various countries

Country	Estimated antimicrobial use (g per tonne production)	Source
Norway	1	(65)
Sweden	2	(103)
Greece	100	(82)
Canada (British Columbia)	156	(33)
Chile	200	(19)
Vietnam	700	(106)

How are antimicrobial agents administered to aquatic animals?

There are three methods – medicated feed, bath and injection – by which antimicrobial agents can routinely be administered to aquatic animals. Again, there are little hard data that allow an authoritative statement to be made about the relative frequencies with which each of the three methods is used in global aquaculture. As a general indication, however, it can be suggested that administration by injection tends to be used only in the treatment of very large fish or of highly valuable brood stock. Administration via addition of the agent to the rearing water tends to be employed in the treatment of very small juveniles and larvae in hatcheries. For the majority of farmed species, the presentation of medicated feed would appear to be the dominant mode of administration in the grow-out phase of production.

Therapy, prophylaxis or metaphylaxis?

Classification of antimicrobial treatments

The antimicrobial treatments of large, normally land-based, animals can usefully be classified as either prophylactic or therapeutic. Prophylactic treatments are those administered to uninfected and often healthy animals with the aim of preventing the initiation of infections or promoting growth. Therapeutic treatments, in contrast, refer to those situations where an agent is administered to an animal that is already infected. The aim of therapeutic treatments is to kill, or sufficiently inhibit, the infecting bacterium and to facilitate the host in its attempt to eliminate the bacterium and to recover from the disease.

In aquaculture and in some land-based agriculture sectors, antimicrobial agents are normally administered to populations of animals rather than individuals and, in this situation, we need a slightly different classification of treatments.

In the context of treatments administered to populations the term prophylaxis is still valuable and can be used to describe those treatments administered to populations where there is no evidence of infection in any of the individual members of that population. However, as it is extremely rare for all members of a treated population to be infected, the term therapeutic, as defined above, is not strictly relevant. Treatments of populations that contain infected members are better classified as metaphylactic (99).

Antimicrobial treatments in aquaculture

There have been few publications that allow us to identify the relative frequencies of prophylactic and metaphylactic treatments in aquaculture. However, the approach taken by Cabello (22), who appears to treat all administrations in aquaculture as prophylactic, is almost certainly misleading. The only data that Cabello cites in support of his treatment of all aquacultural use as prophylactic are derived from the Norwegian finfish farming industry. Ironically, this is the only industry for which a detailed analysis of the rationale for antimicrobial use is available (64), and this demonstrated that only 1 of the 5,493 treatments analysed over a 10-year period could be classified as prophylactic.

Again, lack of data limits our ability to present anything other than generalisations. However, the following comments might give some guidance. Prophylactic treatments, when they are employed, are mostly confined to the hatchery, juvenile or larval stages of aquatic animal production. This is thought to be particularly relevant to crustacean and mollusc production systems. Prophylactic treatments are also thought to be more common in small-scale production units that cannot afford, or cannot gain

access to, the advice of health care professionals. In contrast, it is generally believed that metaphylactic treatments dominate in the out-growing phases of shrimp production, in the treatment of finfish generally and in large-scale farms involved in international trade.

In all forms of aquaculture, antimicrobials are rarely, if ever, used as growth promoters.

How do antimicrobial treatments achieve clinical efficacy?

The aim of developing rational antimicrobial treatment regimen is to improve clinical efficacy whilst at the same time reducing selection for resistant variants. Defining the required clinical effect is, in general terms, relatively simple. If, however, we are to develop rational therapies we need a detailed understanding of how any therapy actually achieves its clinical effect.

The arguments presented above suggest that metaphylactic treatments via medicated feed are the dominant way in which antimicrobials are administered in aquaculture. Thus, the question of how orally administered antimicrobials actually act in reducing mortalities when they are administered in a metaphylactic context is one of fundamental importance.

With respect to treatments of finfish and probably other aquatic animals, the central problem relates to the observation that infected individuals often show reduced appetite or stop feeding all together. This would suggest that in a population that contains infected members at least three sub-populations could be identified:

- those that are infected and are not feeding
- those in the early stages of infection that may be feeding at normal or a reduced rate
- those that are healthy, not infected and are feeding at a normal rate.

It is clear that orally administered antimicrobials cannot influence the fate of the sub-population that is not feeding. They will receive no drug and, therefore, an administration can have no impact on the course of their infections or disease. In considering the efficacy of oral treatments the key question is, therefore, whether their efficacy is primarily related to their function in:

- facilitating the elimination of the bacterium from those that are infected but still feeding
- preventing *de novo* infections in the uninfected and healthy sub-population.

Surprisingly, there are very few published studies that allow us to decide which of these is the more significant. The data produced by Coyne and her co-workers (26, 27, 28) would suggest that prevention of infection is probably the more significant. Having examined nearly 200 salmonid fish sampled at the end of five different therapies in commercial fish farms they found only one that contained both the infecting bacterium and significant concentrations of the agent. Studies in Norway (T.E. Horseberg, personal communication) and the United Kingdom (UK) (D.J. Alderman, personal communication) have also been interpreted as evidence that the primary function of oral treatments to populations is to prevent the initiation of infections in the healthy fish in that population. If these conclusions are correct, and they appear to be consistent with the intuitive but experience-based assumptions of many veterinarians, then oral administrations to populations of aquatic animals may have to be classified as metaphylactic on a population level but prophylactic on an individual level.

The mode of action of oral metaphylactic administrations has important implications for the development of rational, science-based therapies. The implication of our current understanding of the mode of action for both measuring and improving clinical efficacy will be considered below.

Monitoring the efficacy of treatments

Monitoring the efficacy of any antimicrobial treatment is essential for any farmer and also generates vital feedback for validating the interpretive criteria (such as clinical breakpoints or epidemiological cut-off values – see later section) that are being used by laboratory scientists to assess resistance in the target bacterium. Interpretive criteria are valid to the extent to which their application facilitates an accurate prediction of the clinical outcome of a therapy. Thus, the ability to assess clinical success or failure is critical to the development of valid methods of defining resistance.

In laboratory studies, efficacy can be assessed by comparing the patterns of mortality (or morbidity) that are observed in a treated population with those that are observed in a control population that did not receive treatment. However, in commercial conditions, it is very rare that such untreated control populations are available. In the absence of control populations, efficacy can only be assessed by estimating whether the observed pattern of mortality in the treated population more closely resembles that which would have been predicted for that population if it had received no treatment or that which would have been predicted if the treatment had been efficacious.

Predicting the patterns of mortality that would be expected in an untreated population is complicated by the fact that

all epizootics have a time-course and few result in the deaths of all members of a population. Even in an untreated population, mortalities will eventually decline, but we rarely possess the information that would allow us to predict precisely when that decline would occur and how fast the decline would be. However, we can say that, as a consequence of the natural kinetics of epizootics, an observation of a decline in mortalities in a treated population cannot, of itself, be taken as evidence of treatment efficacy.

Predicting the patterns of mortality that would be expected during and after an efficacious treatment is also complicated. Metaphylactic treatments are normally initiated only when some rise in mortality has been observed in the population and we can, therefore, assume that populations undergoing such treatments will contain a percentage of infected and non-feeding fish. As these fish will not receive therapeutically adequate concentrations of an orally administered agent they will continue to die at a rate uninfluenced by the initiation of the treatment. As a consequence, it is reasonable to predict that, even when a treatment is highly efficacious, mortalities may continue and may even increase in the days after the initiation of an oral treatment. The extent of mortalities that can be expected after the initiation of a successful treatment will be a function of the number of infected and non-feeding fish present at the start of the therapy and of the kinetics of the disease process.

Using clinical efficacy data to assess the validity of interpretative criteria is important if we are to develop meaningful empirical definitions of resistance. This approach will, however, encounter two major problems. Firstly, clinical failure may result from factors other than the lack of susceptibility in the target bacterium and secondly, the available clinical efficacy data may be ambiguous or difficult to analyse. Valuable information can, however, be gained from situations where the interpretation of laboratory susceptibility data predicted clinical success but field observation demonstrated clinical failure. As Smith and O'Grady (97) have argued, there is an urgent need for the systematic collection of data of this type.

Pharmacodynamics

In human and to a lesser extent veterinary medicine, the addition of pharmacodynamics (PD) to pharmacokinetics (PK) has allowed the design of therapeutic regimens that minimise the emergence of resistance (62) and has facilitated the rational estimation of clinically significant breakpoints for determining resistance in target bacteria (104).

The PK/PD approach involves establishing the clinically significant relationship between the minimum inhibitory

concentration (MIC) of the agent against the target bacterium and PK measures such as peak serum concentrations (C_{max}), the area under the curve during a 24 h period (AUC_{24}) or the percentage of a 24 h period during which serum concentrations are greater than the MIC ($t>MIC$). For different agents the relevant PK/PD parameter may be C_{max}/MIC , AUC_{24}/MIC or $t>MIC$ (104). In human medicine the critical value of the relevant parameter that will result in the required clinical outcome can be established from animal infection studies. If the PK/PD approach is to be applied to treatments of fish or aquatic animals in general, we need to be able to define the required clinical outcome. The arguments presented above indicate that we have no certainty as to which of two possible clinical outcomes will prove to be the most relevant. Are orally administered metaphylactic treatments effective because they prevent the initiation of *de novo* infection in healthy animals or because they facilitate the elimination of the infecting bacterium from infected fish?

Until we know the answer to this question we will not be able to apply the full power of the PK/PD approach either to the design of treatment regimens that minimise the development of resistance or to the setting of clinical breakpoints that provide an empirical definition of resistance. At an even more fundamental level, until we know in which of the sub-populations the critical events are occurring, we do not know in which we should make our PK measurements.

Measurement of resistance to antimicrobials

The joint Food and Agriculture Organization of the United Nations (FAO)/World Organisation for Animal Health (OIE)/World Health Organization (WHO) expert consultation on antimicrobial use in aquaculture and antimicrobial resistance (111) recommended that 'Measures should be developed and implemented at national and international levels to prevent development and spread of antimicrobial resistance in aquaculture'. If this is to be anything other than a pious aspiration, it is essential that we develop valid methods for detecting resistance and that some harmonisation in their application is achieved.

Modern biological science is dominated by a deeply ingrained molecular reductionism. This often unconscious bias has led to a serious imbalance in our knowledge concerning resistance. We possess a reasonably sophisticated understanding of the molecular and particularly the genetic basis of the resistance manifested by bacteria associated with aquaculture. This topic has been recently reviewed by Sørum (100) and will not be

treated in detail here. Our molecular biological knowledge of resistance mechanisms must be contrasted with the very limited ability we have to determine, with any efficiency or validity, whether, in the context of any particular therapy of aquatic animals, a particular bacterium should be classified as resistant (4). Not only are we limited in our ability to detect clinically significant resistance in bacteria associated with aquatic animal disease, but there are also significant and largely unresolved problems with the attempts that have been made to determine the frequencies of resistance in bacteria present in the environment of aquaculture operations.

Measuring susceptibility and detecting resistance in clinically relevant bacteria

Laboratory detection of resistance is a two-part process. The first part involves arriving at a quantitative measure of *in vitro* susceptibility. The second involves interpreting the clinical meaning of that measure.

Measuring susceptibility

There are essentially two groups of methods available to generate an *in vitro* measure of susceptibility. One group includes all those that measure the minimum concentration that is required to inhibit some aspect of bacteria activity, normally related to cell division (MIC). These methods produce measures of susceptibility in units of mg/l. The second group rely on measurements of the zones of inhibition produced by discs containing the antimicrobial agent and generate measures of susceptibility in units of mm. There is still a debate as to the relative merits of these two methods (77). When clinical breakpoints derived from PK or PK/PD determinations are available, MIC methods would have obvious advantages. Jones (46) has, however, argued that disc diffusion is the more accurate of the two methods. In practice, largely for logistical and cost reasons, the majority (90%) of laboratories involved in susceptibility testing of clinical isolates from aquaculture use disc diffusion methods (92).

An important aspect of all laboratory measures of susceptibility is that the numerical value recorded for any strain will depend not only on the method used (MIC or disc diffusion) but also on the specific details of the test protocol employed. Thus, in order to facilitate communication between laboratories, to allow meaningful comparisons of their data and to harmonise the interpretive criteria they apply, some standardisation of test protocols is essential (4).

In the last decade considerable progress has been made in developing standard susceptibility test protocols relevant to aquaculture. The first step forward was the publication of provisional protocols developed by 24 scientists from 17 countries who met in Weymouth (the UK) in 1989 (4).

These protocols have been further developed by the Clinical and Laboratory Standards Institute (CLSI) in their guidelines M42-A (23) and M49-A (24). There are, however, areas where the test protocols specified in the CLSI guidelines are, as yet, incomplete. CLSI divide the bacteria encountered in aquatic animal disease into 5 groups. Group 1 includes those species that grow on Mueller-Hinton agar at 22°C to 28°C in at least 48 h. Group 2 includes the obligate halophiles that require additional NaCl. The other groups are the gliding bacteria (Group 3), the streptococci (Group 4) and all other species that have fastidious growth requirements (Group 5). The current versions of the CLSI guidelines present the quality control requirement for Group 1 strains, but for the other groups these have yet to be developed. With respect to the appropriate media and incubation conditions, there are strong and probably final recommendations for Groups 1 and 2, but final consensus has not yet been reached on the most appropriate test conditions for many species in the other groups. Work to address some of these outstanding issues is ongoing and the next revision of the guidelines will include further details.

The harmonising of antimicrobial susceptibility testing in human and animal medicine has been seriously hindered by the independent development of a number of different standard protocols by national and international agencies. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is currently engaging in the long and complex task of attempting to harmonise these protocols and the various interpretive criteria associated with them (48). In the field of aquatic animal therapy there is a strong possibility that we could avoid the confusion that developed over time in human and veterinary medicine. As the CLSI protocols are clearly the most developed and the most validated protocols available, a very strong case can be made for their adoption as a worldwide industry standard. A recent survey of current practice in laboratories involved in testing clinical isolates from aquaculture revealed that 70% were using either CLSI protocols or those of Alderman and Smith (4).

At present it is difficult to see any justification for not using the test protocols specified in either M42-A (23) or M49-A (24) in all studies of the antimicrobial susceptibility of bacteria associated with aquatic animal disease.

Interpreting susceptibility data

Alderman and Smith (4) deliberately separated the issue of the test protocols from the more difficult and complex issue of the development of valid and clinically relevant criteria of interpretation (clinical breakpoints). This two-step approach has also been adopted by CLSI. Thus, the current situation is that we have standard protocols for generating data but we lack validated breakpoints that would allow the attribution of clinical meaning to the data

we produce. Following a recent survey, Smith (92) reported wide variations between laboratories with respect to the breakpoints they used to interpret the data they generate from disc diffusion studies (Figs 1 and 2). He also noted that some laboratories were using the same numerical breakpoints for all species and for all agents. He argued that we could not have confidence that, at least in some cases, the interpretations being supplied by laboratories to health care professionals were not both misleading and erroneous.

The issue of setting interpretive criteria is now an urgent one. This urgency derives not only from the need to supply health care professionals and aquaculture producers with appropriate advice, but also from the increasing demands from international agencies for the monitoring of resistance in bacteria associated with aquaculture. Until we can agree how to measure 'resistance' in the laboratory, any monitoring programmes will only be able to collect data that we do not understand and that is fundamentally meaningless.

Breakpoints or epidemiological cut-off values?

As defined by EUCAST (www.srga.org/Eucastwt/eucastdefinitions.htm), clinical breakpoints and epidemiological cut-off values (ECO) represent two distinctly different methods of providing interpretive criteria for susceptibility data. Clinical breakpoints attempt to provide interpretive criteria that are of direct clinical relevance, whereas ECO divide bacteria purely on the grounds of their *in vitro* susceptibility.

Turnidge and Paterson (104) have provided a first class review of the data required to set clinical breakpoints in human medicine. These attempt to take account of the distribution of susceptibility measures for a particular bacterial species, the nature of the infection being treated, properties of the administration (pharmacokinetics), the properties of the *in vivo* interaction between the agent and the target bacterium (pharmacodynamics) and historical data on clinical outcomes of previous therapies. Clinical breakpoints are, in the first instance, expressed in MIC values and regression analysis of MIC and disc diffusion data would be required before any clinical breakpoints could be expressed in units relevant to disc diffusion data. In contrast, the setting of ECO values requires only the distribution of susceptibility measures (MIC or disc diffusion) for a particular species.

Setting clinical breakpoints for bacteria associated with aquaculture

A consideration of the Turnidge and Paterson (104) review reveals the large amount of data that is required to set any clinical breakpoints. This alone would suggest that setting such breakpoints relevant to therapies in aquaculture would take a very significant and coordinated effort by many laboratories. Such an effort would take a lot of time

and money and, consequently, clinically validated breakpoints could not be achieved in the immediate future. However, there are other considerations, largely deriving from the diversity of aquaculture, that suggest that even when breakpoints were developed for this industry they would have a significantly lower precision than those that have been developed for the single, homoeothermic species *Homo sapiens*. Aquaculture encompasses the farming of a wide variety of species, genera and even phyla in environments that vary with respect to temperature and salinity (32, 94). In attempting to generate the PK and PD data required to set clinical breakpoints we would have to decide whether each particular combination of therapeutic regimen, species and environment would have to be examined separately or whether it would be acceptable to produce values that represent a group of conditions. Would it, for example, be legitimate to attempt to use a single set of PK/PD data for all salmonids in cool seawater? If we do not allow such grouping then the workload becomes horrendous, but if we do, there must surely be some loss of precision.

Although clinical breakpoints must be considered as the 'holy grail' we must also consider the current situation where we can have little confidence that errors in interpretation of susceptibility data are not being made on a regular basis (92). In this context we must examine the possibility that the development of ECO might represent a way in which we could reduce error.

Epidemiological cut-off values

The aim of setting ECO values is to provide criteria for classifying any clinical isolate as belonging to one of two groups (31, 48). One group consists of those that are fully susceptible, or 'wild-type' (WT). The other group consists of all those that have a reduced susceptibility, and these are termed non wild-type (NWT). ECO values categorise isolates as WT or NWT on the basis of their *in vitro* susceptibility phenotypes and take no account of the PK or PD data or the data on clinical efficacy of any therapy. The question, therefore, arises as to what clinical meaning should be given to these categories. That is, what recommendation should be given when a WT or a NWT isolate has been identified?

In general, when the target bacterium has been identified as WT it would be safe to report that the susceptibility testing provides no grounds for recommending that a therapy would be inappropriate. Prudence would, however, suggest that the identification of the target bacterium as NWT would be grounds for recommending that a therapy should not be initiated. This application of ECO values is essentially conservative in that there may be isolates that have a decrease in susceptibility that is sufficient for them to be classified as NWT, but is not sufficient for them to be clinically resistant.

Do we need ECO values?

There are laboratories that have been involved in susceptibility testing for many years and it might be thought that, over time, they had developed reasonable and effective criteria for assessing the probable clinical significance of their own data. An analysis of the breakpoints being used in aquaculture (92) suggests, however, that there are no grounds for complacency. Figure 1 shows the breakpoints (black bars) that would be applied by responding laboratories to zones generated by oxytetracycline (30 µg discs) plotted against the distribution of zone sizes obtained for *Aeromonas salmonicida* with these discs in three independent studies (grey bars). This figure indicates that the majority of the breakpoints in use fall into the relatively large gap between WT and NWT strains. This reasonably satisfactory situation can be contrasted with that illustrated in Figure 2. This figure presents a similar analysis of data for oxolinic acid (2 µg) discs. Here it is clear that not only is the gap between the WT and NWT smaller, but also that

the majority of the breakpoints in use do not fall into the gap between them. It is reasonable to fear that some errors are occurring in the interpretation of oxolinic acid susceptibility data and this underlines the need to address the rational setting of ECO values.

Is setting ECO values difficult?

The difficulty in setting ECO values is related to the extent of the changes in susceptibility that result from the type of resistance mechanisms that are encountered. As a generalisation it can be stated that when an NWT phenotype arises as the consequence of the acquisition of an additional gene encoding specific, positive function resistance, the reduction of susceptibility is large. With respect to aquaculture this situation was apparent in the studies of oxytetracycline susceptibility of *Aeromonas salmonicida* by Uhland and Higgins (105), Miller and Reimschuessel (70), and Smith *et al.* (98). In these situations the difference in the zone sizes recorded for WT and NWT was such that little difficulty could be expected

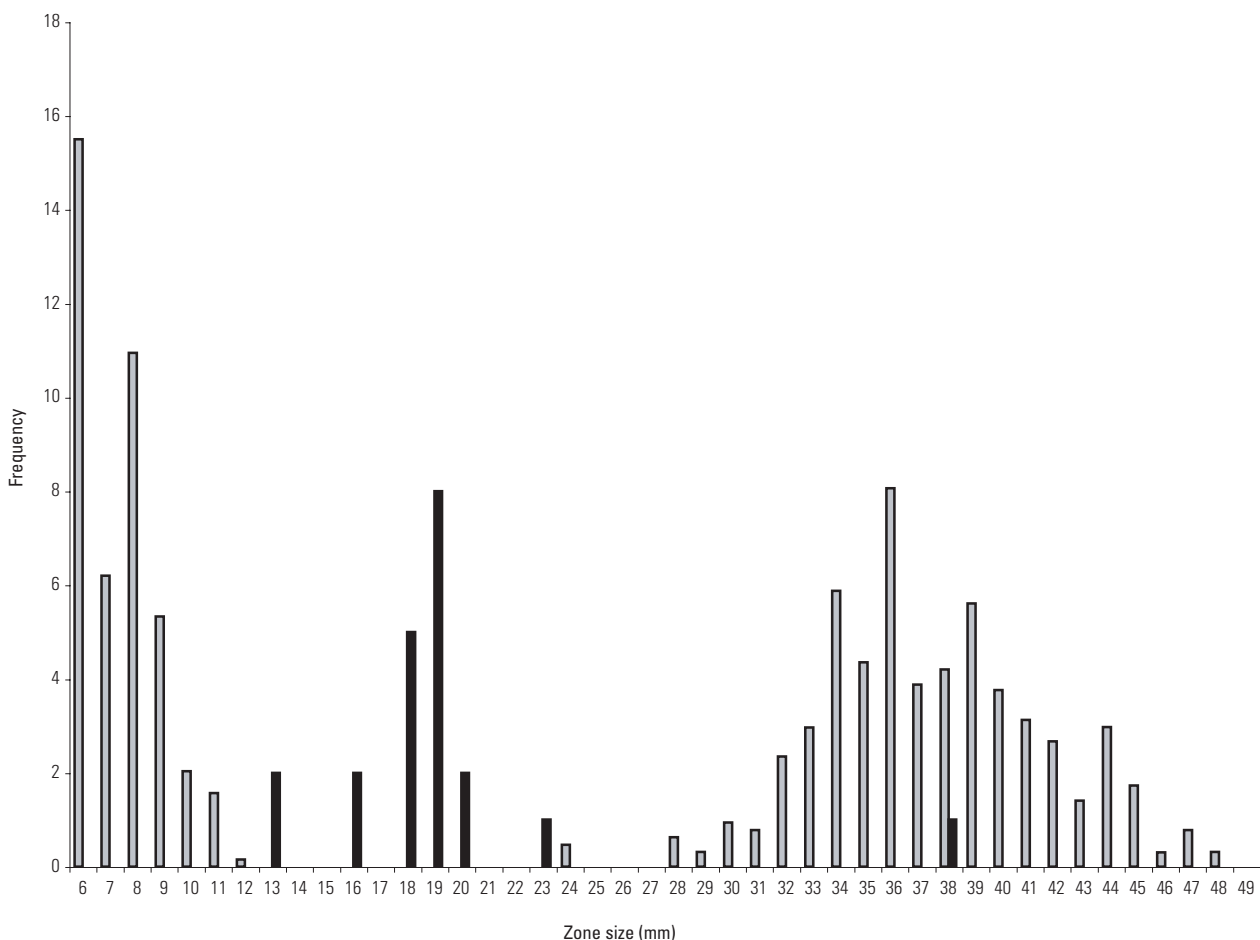


Fig. 1
Histogram showing the distribution of zone size obtained by 30 µg oxytetracycline discs against 373 strains of *Aeromonas salmonicida* (grey bars) and the breakpoints currently being used (black bars)

The *A. salmonicida* data were obtained from Uhland and Higgins (105), Miller and Reimschuessel (70) and Smith *et al.* (98). The breakpoints were from Smith (92)

in setting an ECO that could be applied to their characterisation (Fig. 1).

The difficulties in setting ECO values arise when there is not a large difference in the zone sizes recorded for WT and NWT strains. This situation is most likely to occur when reduction of susceptibility results from a modification of the target site as a result of a chromosomal mutation, as is frequently the case with the first-generation quinolones (36, 43, 54, 100). Multiple low-level resistance (MLLR) has been recorded in bacteria associated with aquaculture (13, 38, 110) and these also result in small differences between WT and NWT isolates. MLLR phenotypes have been associated with modifications of membrane permeability (14, 76) or alterations in the regulation of multi-drug efflux systems (34, 79). The recent work by Balaban *et al.* (12) provides evidence for the involvement of phenotypic persistence mechanisms in bacterial resistance to antimicrobials. Smith *et al.* (96) had previously suggested that persistence mechanisms might account for some of the

relatively unstable low-level resistances that have been reported.

Setting ECO values

The issues of how to set ECO have, of course, been addressed by those concerned in human and veterinary medicine and it would, therefore, appear that we should be able to learn from their experience. The extent to which we can do that is, however, limited by three considerations. Firstly, it is not clear that those involved in human medicine have, despite the strenuous efforts of EUCAST, actually reached a consensus position. Secondly, we need to bear in mind the very significant differences in size and throughput of the laboratories involved in hospitals and those involved in supporting aquaculture. Smith (92) reported that of the laboratories involved in susceptibility testing of clinical isolates of bacteria associated with aquatic animal disease, 70% reported handling fewer than 100 isolates a year. Thirdly, we must consider that, in all

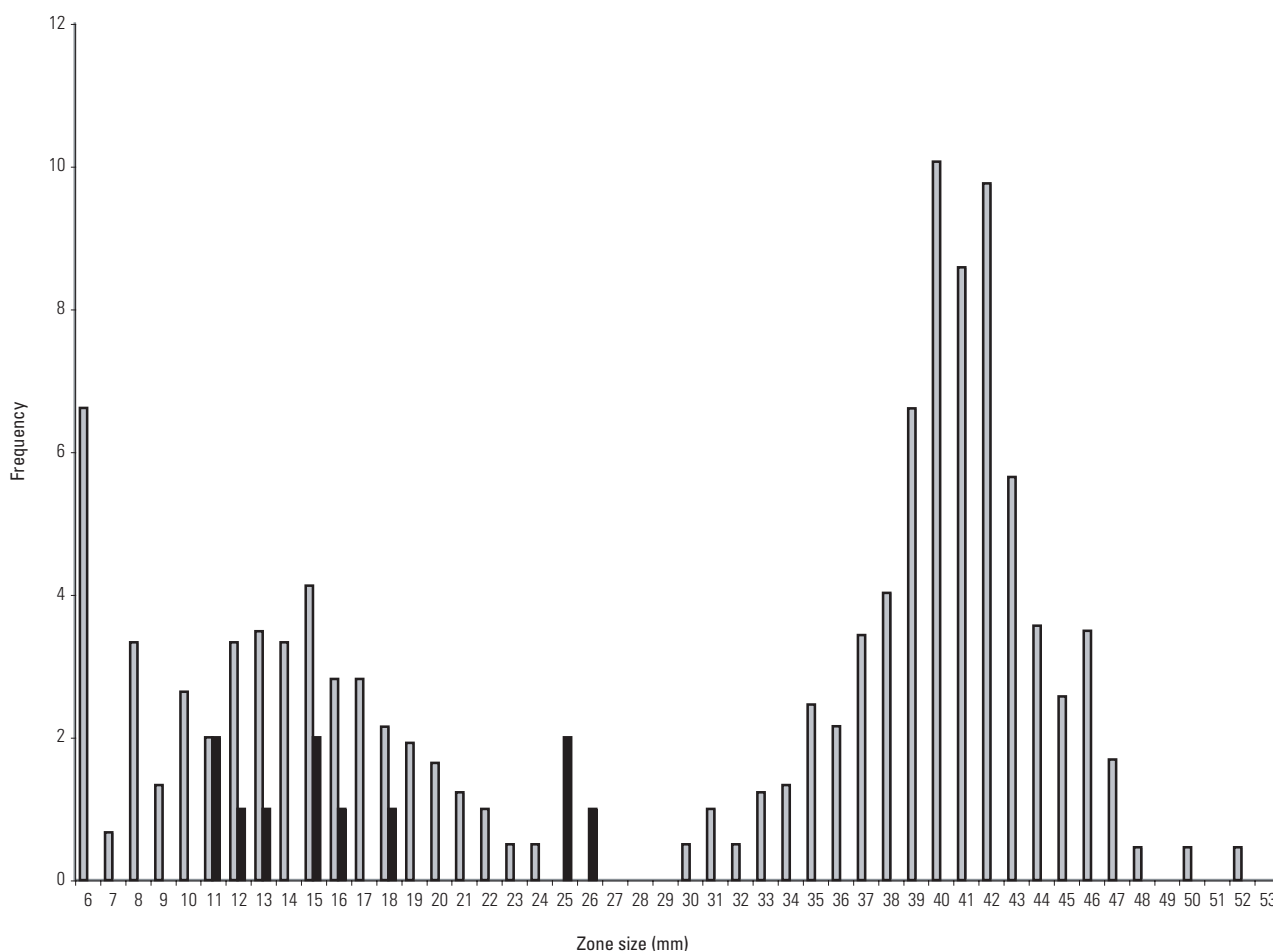


Fig. 2
Histogram showing the distribution of zone size obtained by 2 µg oxolinic acid discs against 323 strains of *Aeromonas salmonicida* (grey bars) and the breakpoints currently being used (black bars)

The *A. salmonicida* data were obtained from Miller and Reimschuessel (70) and Smith *et al.* (98). The breakpoints were from Smith (92)

probability, the majority of antimicrobial treatments in global aquaculture are not informed by any susceptibility testing at all. If we accept that promoting the rational and prudent use of antimicrobials in aquaculture is an important goal, one of the main tasks will be to increase the number of laboratories that can perform susceptibility testing. This increase in testing will have to be achieved in areas with very limited scientific and technical infrastructure. This places an obligation on us to develop susceptibility testing and interpretive criteria procedures that are robust, reasonably error-free, but essentially simple.

The central problem that needs to be addressed in setting ECO is that of inter-laboratory variation in the numerical measures of susceptibility generated by disc diffusion testing. This phenomenon has been well studied in hospital laboratories (56, 57, 59) and has been identified in aquaculture studies (42, 71, 75, 98) and is inherent in the breadth of the acceptable ranges for control strains in M42-A (23). The three ways in which ECO could be set all represent different approaches to overcoming the problem of inter-laboratory variation.

Rigorous standardisation

In this approach the aim is to eliminate or sufficiently reduce inter-laboratory variation by rigorous standardisation of the test protocols and the inclusion of strict quality control (QC) requirements. The advantage of this approach is that it would allow species and drug-specific but laboratory-independent ECO values to be set internationally. Miller and Reimschuessel (70), who implicitly adopt this approach, have published a set of ECO values for four agents against *A. salmonicida*. The disadvantages are associated with the fact that, as these values are protocol-specific, they can only be applied by laboratories that are in compliance with the strict QC requirements of that protocol. The QC requirement specified in M42-A was originally developed for hospital laboratories with high throughputs. For laboratories handling only a few strains a year, they would represent a significant increase in work and, therefore, in the cost of analysis. A further criticism of this approach is that it cannot completely eliminate inter-laboratory variation and the degree of residual variation results in a lack of precision in the ECO values it generates. This lack of precision raises questions as to the ability of the laboratory-independent ECO values to allow the regular detection of strains with low-levels of resistance (57, 95).

Normalised resistance interpretation

This approach was developed for human medicine by Kronvall and his co-workers (44, 57, 58) and has been applied in studies of aquatic bacteria (29, 83, 95, 98). The assumption underlying this approach is that inter-laboratory variation cannot be adequately reduced by standardisation. It therefore involves the use of a standard

method (normalised resistance interpretation [NRI]) of generating laboratory-specific ECO values. NRI assumes that the distribution of zone sizes for WT strains is normal and calculates the ECO as the mean of these zone sizes minus 3 (or 2.5) times its standard deviation. One important aspect of this approach is that, as the ECO values arrived at are protocol-independent, there is no obligation for laboratories to perform the detailed QC testing required by the rigorous standardisation approach. The NRI approach was designed to facilitate the detection of low-level resistances. However, the central disadvantage of this approach is that the NRI calculations, which must be performed in each testing laboratory, require both some statistical sophistication and a considerable body of data for each species and agent combination.

Internal standardisation (Stokes method)

In this approach, originally developed over 50 years ago (102), the zone sizes of test strains are measured on the same agar plate as those for a fully susceptible, WT, control strain (20). Results are then recorded as the differences in zone sizes between the test and control strains. The differences recorded between the zones recorded for WT clinical isolates and the control strain should be normally distributed and their spread should reflect only the degree of intra-laboratory variation in the performance of the test. In this approach, NRI analysis would be employed to set ECO values from the distribution of the differences for a set of test strain and WT strain combinations. An initial (unpublished) study of the application of this approach to the susceptibility of *A. salmonicida* to florfenicol resulted in the recommendation that any clinical isolate whose zone radius was 3 mm smaller than that measured for the test strain on the same plate, should be classified as NWT. The advantage of this approach is that the ECO values can be generated by a central laboratory and are laboratory-independent, protocol-independent and may well be species- and agent-independent as well. The major disadvantage of this method would be that each testing laboratory would have to have a set of relevant control strains available for testing.

Summary

- a) We have standard test protocols for performing disc diffusion susceptibility tests on bacteria associated with diseases in aquaculture.
- b) We do not, as yet, have any validated way of determining the clinical significance of the data generated by these tests.
- c) There are reasonable grounds for assuming that not all the recommendations currently being made by laboratories are error-free.
- d) At the present state of our knowledge, it would appear that setting epidemiological cut-off values rather than clinical breakpoints would represent the most effective

method of reducing our error rate in the immediate future.

e) There is an urgent need to establish a standard approach to the setting of epidemiological cut-off values.

Detecting resistance in bacteria isolated from the vicinity of aquaculture operations

Frequencies of resistance

There have been many reports of the frequencies of resistance in bacteria present in the vicinity of aquaculture operations. There are a few of these that present well-designed studies and where the data obtained were appropriately analysed, but many others can be criticised either on the basis of the methods used to generate data or on the basis of the methods used to interpret their significance. Still others produced data on the frequencies of resistance but because they were undertaken to investigate different problems, the data cannot be used to estimate the impact of antimicrobial use. In an article of this length it would not be possible to provide a detailed analysis of each of the papers that have been published. However, the recent review by Cabello (22) requires that some comments be made. In this review the arguments the author made were drawn after an extraordinarily uncritical, partial, or in many cases totally illegitimate, reading of the primary source papers. The following set of observations are, therefore, provided as a guideline to the questions that must be asked of papers that have been published in this area before they can be taken as providing evidence relating to the impact of antimicrobial use on resistance frequencies in environmental bacteria.

Were the media and cut-off values appropriate?

The frequencies of resistance detected in any study are a function of the media and the interpretive criteria employed. It must be accepted that we lack any agreed consensus as to the media or cut-off values we should use in these studies. Thus, at present, there are no 'correct' methods. However, it must also be accepted that factors such as the inclusion of seawater or divalent cations in test media will influence measures of susceptibility to agents such as oxytetracycline and the quinolones (90). Equally, studies where breakpoints developed for human pathogens are uncritically applied to data generated using totally different test protocols must be treated with some caution.

Were adequate control samples collected and analysed?

Resistance to antimicrobials has been found in bacteria present in environments that were thought not to have been directly impacted by human activities (5, 6, 15, 17, 18, 30, 45, 50, 66, 68); consequently, the demonstration of resistant bacteria in the vicinity of aquaculture operations cannot be taken as evidence of an impact of any antimicrobial use in that operation.

Was adequate attention paid to innate resistances?

No antimicrobial agent has a spectrum of action that covers all bacterial groups, and therefore there are bacterial species that must be considered as innately resistant to some agents. Resistances are considered as innate when they result from the fundamental structure and organisation of the cell rather than being the result of a mutation or the acquisition of a specific gene. For example, most aeromonads must be considered as resistant to amino-penicillins and first-generation cephalosporins (101). Equally, both the pseudomonads (63) and to a lesser extent the vibrios (47) have significant innate resistances.

Was linkage demonstrated or investigated between antimicrobial use and resistance frequencies?

Many studies undertaken to investigate frequencies of resistant bacteria in aquaculture operations were not designed to investigate the linkage between the frequencies of these bacteria and the administration of antimicrobial agents. Studies of this type include those of Alcaide *et al.* (2), Miranda and Zemelman (73), Rhodes *et al.* (80) and Sandaa *et al.* (85).

Was any account taken of the factors other than antimicrobial use that have been demonstrated to give rise to increased frequencies of antimicrobial resistance?

Evidence has been presented that nutrient enrichment of aquatic environments, even in the absence of any antimicrobial agent, may result in a detection of an increased frequency of resistant bacteria (49, 107). This phenomenon may be related to the reports of elevated frequencies of resistance in the outflow of aquaculture operations that are unrelated to any use of these agents (11, 51, 67, 73).

Was any investigation undertaken of the footprint?

Guardabassi *et al.* (39) demonstrated a significant elevation of oxolinic acid resistance in a stream receiving effluent from a farm using this agent. Recent work from France (35, 37, 74) has, however, suggested that both the spatial and temporal footprint of elevated frequencies of resistance to this agent might be relatively small. Both the area and the time period over which elevated frequencies of resistance can be detected are relevant parameters, but also ones which have rarely been reported.

Summary

a) The use of antimicrobials in aquaculture will result in some of the agents entering the extra-farm environments.

b) The presence of these agents in the environment has the potential to exert selective pressure for the emergence of elevated frequencies of resistance. It should, however, be noted that environmental factors may significantly reduce the biological activity of these agents (35, 78).

c) Generating experimental data quantifying the extent of selection involves careful consideration of method, interpretive criteria and experimental design. Any attempt to generate an overview of this issue is complicated by the variation in these parameters in various published studies.

Negative consequences of antimicrobial use in aquaculture

Antimicrobial use in aquaculture can have both positive and negative effects, often simultaneously. Both the positive and negative consequences are easy to identify, but they have proved significantly more difficult to quantify. The negative consequences associated with residues either in the environment or in foodstuffs will not be addressed here. Those associated with the emergence of bacteria resistant to antimicrobials can be considered as those that impact on farmers themselves and those that have an impact on public health.

Negative consequences experienced within aquaculture

There have been few recent, large-scale studies linking the patterns of use of antimicrobial agents in aquaculture to the frequency of clinical resistance in bacteria associated with aquatic animal disease. Even if any such studies had been attempted their conclusions would have been compromised by the lack of harmonisation in the laboratory methods used to determine 'resistance'. However, despite the problems associated with detecting 'resistance', there are no grounds for questioning the conclusion arrived at by Smith *et al.* (96) that the emergence of resistance in the bacteria that are the target of antimicrobial therapy is the most significant negative consequence of the use of these agents in aquaculture. At least in theory, this should provide a self-regulating negative feedback loop that would limit the use of antimicrobials. The efficiency of this negative feedback is, however, dependent on the quality of information in the system. It will only work when farmers have access to laboratory susceptibility testing and when the data from this testing is interpreted by the application of valid criteria.

Negative consequences experienced in human and public health contexts

The most significant public health risks associated with increased frequencies of resistance resulting from the use of antimicrobial agents in aquaculture can be considered under two headings (111):

- those associated with the selection of resistant variants of bacteria capable of inducing infections in humans that would require antimicrobial therapy
- those associated with the movement of genes encoding resistance from bacteria in the aquatic environment to those in the terrestrial environment that are capable of infecting humans or other land-based animals.

Selection for resistance in bacteria associated with human disease

Because of the fundamental similarities of the animals involved and the environments in which they live, bacteria capable of playing a role in human disease are frequently encountered in land-based agriculture. It has been assumed that the major risks associated with the use of antimicrobials in land-based agriculture are those consequent on the selective enrichment of resistant variants of zoonotic bacteria (41). There is still an active debate as to the size of this risk, with some arguing that it is relatively small (21, 108) and others that it might be significant (9). Bacteria capable of infecting humans are encountered much less frequently in aquaculture than in agriculture. Thus, whatever the risks to public health associated with the selection of resistant variants of zoonotic bacteria by the use of antimicrobial agents in agriculture, those arising from the selection of such bacteria by aquacultural use must be considered as very significantly smaller (91).

The WHO/FAO/OIE (111) expert working group identified two groups of bacteria that might be encountered in aquaculture and might also be capable of infecting humans. The presence of members of one group, that included enteric pathogens such as the *Salmonella*, would result from contamination of aquaculture by human or animal wastes. The second group was composed of aquatic bacteria and the expert group specifically mentioned *Vibrio parahaemolyticus* and *V. cholerae*. It should be noted, however, that the validity of some of the evidence used by the expert group to identify the significance, in this context, of *V. cholerae* (7) has recently been questioned (93).

Selection for transferable resistances

After their initial examination of the possibility of performing a risk analysis of the impact on human health associated with antimicrobial agent use in aquaculture, the WHO/FAO/OIE expert working group (111) offered this conclusion: 'The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in bacteria in aquatic environments from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens.'

There are ample data, recently reviewed by Sørum (100), demonstrating that genes encoding resistance to antimicrobials and capable of transfer (or being transferred) to terrestrial bacteria have been regularly detected in bacteria associated with disease of aquatic animals. It is an entirely reasonable assumption that the use of antimicrobial agents in aquaculture has been one of the major factors leading to the enrichment of these genes.

There are also ample data demonstrating that transferable resistance genes are present in the bacteria found in the vicinity of aquaculture operations (72, 80, 86). Rather surprisingly, there are few papers that have linked, in a convincing manner, the use of antimicrobials in aquaculture with an increase in the frequency of occurrence of these transferable genes. However, in the absence of any specific data, prudence must suggest that we should assume that such a linkage does, in fact, exist.

The available data, therefore, support the hypothesis that a reservoir of transferable resistance genes will develop as a consequence of the use of antimicrobials in aquaculture. What is less certain is the size of this reservoir and its public health significance (91).

Movement of transferable resistances between terrestrial and aquatic microflora

Molecular studies have demonstrated that the genes involved in resistance in bacteria associated with aquaculture are significantly similar to those that have been detected in terrestrial bacteria associated with human and land-based animal disease. Amongst other workers this phenomenon has been documented by Bolton *et al.*, Kim *et al.*, and Sørum (16, 55, 100). These similarities strongly suggest that these genes can move between bacteria in these two environments. This conclusion is supported by laboratory studies, such as those of Kruse and Sørum (61), and Sandaa and Enger (84), which have demonstrated that these genes can be transferred from aquatic bacteria to terrestrial bacteria with relatively high efficiencies.

Demonstration of a molecular similarity of the genes involved in resistance in human and aquacultural bacteria suggests that these genes can move between these two groups of bacteria, but does not necessarily inform us as to the dominant direction of the flow. The difficulty of establishing the direction of movements of genes can be illustrated by the debate over the role of aquacultural use of florfenicol in the emergence of *floR* encoded florfenicol resistance in *Salmonella enterica* serovar Typhimurium DT 104. Sequence analysis (16) has demonstrated that the gene found in *Salmonella* is very closely related to that detected in *Pasteurella piscicida* (now renamed *Vibrio damsela*) isolated from Japanese aquaculture (53). Angulo (7), Angulo and Griffin (8), Ribot *et al.* (81) and more recently Cabello (22) have argued that this similarity

indicates that this gene first emerged in Japanese aquaculture and that it was subsequently transferred to the salmonella. The primary evidence for this argument appears to be based on the date at which the relevant sequences were submitted to the GenBank (<http://www.ncbi.nlm.nih.gov/sites/gquery>) database. A more rigorous reading of the relevant literature reveals that in Japanese aquaculture *floR* first emerged in *Vibrio damsela* in 1992 (52, 53), approximately two years after the introduction of this agent. Ribot *et al.* (81) have, however, reported that the *floR* gene was present in a *Salmonella enterica* serovar Typhimurium DT 104 isolated in America in 1985 and Cloeckaert *et al.* (25) have demonstrated that it was present in a plasmid first detected in a strain of *klebsiella* isolated from a human patient in Paris in 1969. Thus, the *floR* gene was circulating in bacteria associated with humans at least a quarter of a century before it was first detected in a bacterium associated with aquaculture. On the balance of probabilities it would appear that this was a case where a gene encoding resistance, initially enriched by human use of antimicrobials, eventually found its way into aquatic bacteria, resulting in a compromising of the therapy of disease in fish.

Thus, although it is possible that the enrichment of the frequencies with which these genes occur in the aquatic environment may influence their frequencies in those bacteria associated with human disease, the reverse is also possible. The mobility of genes could result in the frequency of resistance in bacteria associated with disease in aquatic animals being influenced by the use of these agents in human and veterinary therapies.

The importance of formal risk analysis

It has been clear for over thirty years that use of antimicrobial agents in aquaculture could impact on the management of infections of humans (109). What we did not know then and still do not know is whether such an impact has occurred or, if it has, how significant any impact has been or might be (91). It is in the context of this failure that attention has turned to formal risk analysis (111).

Limitations on the application of risk analysis to antimicrobial use in aquaculture

With respect to some hazards, risk analysis has been successful in estimating the size of the risk, in determining appropriate methods of managing that risk and also in identifying the data required for a more sophisticated understanding of that risk. However, in addressing the

risks associated with antimicrobial use in aquaculture the WHO/FAO/OIE expert working group (111) concluded that 'a quantitative risk assessment on antimicrobial resistance in aquaculture is difficult to perform due to lack of data and the many different and complex pathways of gene flow.'

Even if a more modest target of a qualitative risk assessment had been accepted, it has been argued that the task would have encountered serious difficulties (3). In addition to the shortage of data and the complexities of the exposure pathways that are required to explore the movement of transferable genes, these difficulties would arise from the huge diversity of the activities included under the term aquaculture (94).

The shortage of data

The WHO/FAO/OIE expert working group (111) identified the types of data that are required for any risk analysis. The major thrust of this article has, however, been to stress that not only do we lack data but we also do not have any consensus as to how the required data should be collected. Calling for more data is ultimately no more than political window-dressing, unless those data are associated with detailed specifications as to the methods that should be used to collect them. If we lack validated consensus methods for assessing resistance in a single isolate how can we expect to generate useful data by collating data from laboratories all of which are using different test protocols and different criteria of interpretation?

The complexity of exposure pathways

Risk analysis has been most successful in situations where it has been possible to develop clear exposure pathways. As has been recognised (111), the complexity of the factors that influence gene flow between bacteria in the aquatic and terrestrial environments has the automatic consequence that simple and direct exposure pathways cannot be developed. According to Snary *et al.* (99), the identification of the risk pathway is the first essential step in any risk analysis. Any difficulty in formulating an appropriate risk pathway will significantly limit the gains that can be expected from risk analysis.

It must be considered entirely possible that the risk pathways required to adequately represent the selection, maintenance and movement of genes as they move from aquaculture to an infected human are so complex that risk analysis is no longer an appropriate tool.

The diversity of aquaculture

Aquaculture encompasses the rearing of a huge variety of animals in widely varying environments by a set of fundamentally dissimilar husbandry techniques (32, 94). If risk analysis is to provide any insight, serious

consideration should be given to approaching this problem by first identifying, as a test case, a specific area of aquaculture where, on *a priori* grounds, the risk is thought to be most significant. A detailed and formal, and possibly even a quantitative, risk analysis might then be possible.

The outcomes of risk analysis

The two most valuable outcomes that can be expected from any risk analysis are the identification of rational, science-based risk management strategies and the identification of the future requirements for additional data.

Designing optimal risk management strategies

An important reason for taking a risk analysis approach is that it should allow the identification of the key areas where intervention could minimise the risk. The identification of these key areas would then allow the design of effective risk management strategies. To the extent that risk analysis can provide some estimate of the size or significance of a risk, it will also provide the basis for a cost-benefit analysis of any intervention. The development of risk analysis of antimicrobial use in aquaculture (111), has not reached the state where it could provide any science-based rationale for the adoption of any risk management strategies. However, there is a general consensus that some action to manage the risk should be taken. Our difficulty in responding to this need is not only a function of our lack of any reasonable estimate of the size of the risk we are trying to manage, but also of the fact that we have no way of predicting the probable success or even the consequence, of any management strategy we might adopt.

One management strategy that is currently being promoted involves the development of different sets of antimicrobial agents for different areas of use. Despite its apparent simplicity or maybe because of it, not all researchers are confident that this is the correct, or even an effective approach (88). Currently, this strategy has led to the production of a list of 'critically important antimicrobials' for human use (112). In response, the OIE has also generated a list of critically important antimicrobials for veterinary use that cover both agriculture and aquaculture (http://www.oie.int/eng/en_index.htm). Unfortunately, no risk analysis has been performed to justify this approach, nor has any explicit presentation of the theoretical understanding of resistance gene ecology that underlies this approach been provided. Thus, it is not clear how conflicts between the human and veterinary lists could be resolved. There is a clear risk that decisions to reserve certain antimicrobials for use in one or other of these sectors could be taken on the basis of the power politics of the respective international agencies rather than on any science-based examination of the dynamics of the

situation. It should also be noted that there are particular problems for aquaculture in any attempt to move towards a reservation of specific antimicrobials for different sectors. The economics of antimicrobial use in aquaculture coupled with the cost of obtaining marketing authorisation for their use in this industry has the consequence that no antimicrobial would ever be developed specifically for an aquacultural application. For the foreseeable future the only antimicrobials available for use in aquaculture will be those that were initially developed for use in humans or other land-based animals.

In this difficult situation, modelling approaches to gene flow and the ecology of resistance in the microbial world may have some value. One such modelling approach, produced by American scientists (89), predicts that the contribution of non-human use of antimicrobials to the frequency with which resistance is encountered in human therapy would be inversely proportional to the levels of resistance that already exist in human pathogens. Thus, this model would predict that the maximum impact of non-human use of an agent would be when the levels of resistance to that agent in human pathogens were low. Conversely, it would predict that when resistance to an agent is frequently encountered in human pathogens, non-human use of the agent would only have a minor impact on that frequency. Clearly, the predictions of this model would suggest a totally different management strategy than that implicit in the 'critical important antimicrobials' approach (112). It is important here to note that models of gene flow cannot be the product of a risk analysis but must, rather, be developed before a risk analysis can be performed. Thus, development and validation of such models is an urgent priority.

Identifying data requirements

It is important to note that our failure to be able to answer the questions raised by the demonstration of transferable resistance in aquatic bacteria by Watanabe *et al.* in 1971 (109) is not a function of the lack of research effort in this area. It is rather a function of the failure to identify and to clearly formulate the appropriate questions that that research should address (91). One of the most significant outcomes of any formal risk analysis, whether it is quantitative or qualitative, is that it should allow specific research targets to be identified. In this context it is worth noting that, even if risk analysis is not entirely successful in assessing a risk, the investigation and formal delineation of exposure pathways may still be a valuable method of improving the targeting of research efforts.

Conclusions

Any consideration of resistance to antimicrobials resulting from their use in aquaculture encounters major problems

associated with the diversity of aquaculture, the complexity of the issues involved and our fundamental lack of data. It must also address economics. Any progress will cost money and, if that money is to be spent wisely, we must attempt to prioritise the areas where investment in research and data acquisition will be most productive. The arguments developed in this article indicate three areas where progress is urgently required.

The very limited scientific, technical and educational services available to the majority of world aquaculture producers is the major issue that is hindering the promotion of prudence and rationality in the use of antimicrobials in aquaculture. However, any attempt to improve this situation cannot be undertaken without the simultaneous development of the science that would inform these services. Critical in this regard will be the development of valid methods of assessing resistance in target bacteria and the acquisition of the data that would allow the design of therapies that are more efficacious and that reduce the emergence of resistance.

It will be essential that we improve our monitoring and surveillance of the use of antimicrobials in aquaculture and of the consequences of this use. Aarestrup (1) has discussed the issues raised by the design of monitoring programmes for the use of antimicrobials in land-based agriculture. A reading of this discussion reveals that we are a long way from being able to design, let alone implement, a similar programme for aquacultural use. Again, a critical issue would be the lack of any harmony in the laboratory methods used to identify resistance and to quantify the frequencies of resistance that result from antimicrobial agent use in aquaculture. Until this and other significant issues are addressed, any call for improved monitoring and surveillance will remain purely aspirational and largely meaningless.

We urgently need to develop science-based management strategies that will allow us to minimise the impact of bacterial resistance, selected by the aquacultural use of antimicrobials, both on the control of diseases encountered in aquaculture itself and in those encountered in humans and land-based agriculture. Currently, the potential of risk analysis to provide these strategies is being explored. It must be recognised that this exploration has so far suggested that the complexity of the problem is such that it may be beyond the scope of this approach. Serious consideration should be given to applying risk analysis to a more defined sub-set of antimicrobial use in aquaculture. Even if this more limited target was accepted, it is clear that difficulties would be encountered in developing the exposure or risk pathways that it would require. In this situation we have a clear obligation to investigate the possibility that modelling approaches to gene flow and gene ecology might have significant value. ■

Résistance aux antimicrobiens chez les animaux aquatiques

P. Smith

Résumé

L'antibiothérapie représente l'une des réponses les plus efficaces pour gérer les urgences zoonosaires dues à des agents pathogènes infectieux. Toutefois, l'utilisation d'agents antimicrobiens a pour effet potentiel d'accroître la résistance des bactéries à ces agents, ce qui a un impact négatif sur le recours ultérieur à ces produits pour lutter contre les maladies infectieuses affectant les espèces aquacoles. Par ailleurs, l'enrichissement de bactéries résistantes ou de gènes codant pour la résistance risque d'avoir une incidence négative sur l'action des antimicrobiens utilisés chez l'homme ou chez les animaux terrestres. À ce jour, les tentatives d'appliquer une méthode formelle d'analyse des risques à ce problème se sont soldées par un échec, en raison de l'extrême diversité qui caractérise l'aquaculture et de la pénurie globale d'informations pertinentes sur le sujet. L'auteur insiste néanmoins sur le fait que ce ne sont pas seulement les données nécessaires à la conduite d'un tel exercice qui font défaut ; nous manquons aussi, et surtout, de méthodes validées pour collecter ces informations. Au niveau le plus fondamental, nous manquons de méthodes validées pour décider si une bactérie isolée sur un site aquacole doit ou non être classée parmi les organismes résistants. À défaut d'une évaluation du risque digne de ce nom, les tentatives actuelles de gestion du risque sont axées sur l'élaboration de listes d'agents antimicrobiens d'importance cruciale pour chaque type d'utilisation. L'auteur estime qu'il est urgent de réaliser des études sur l'écologie des gènes et de modéliser les flux géniques dans l'environnement, afin de pouvoir ensuite évaluer cette démarche de gestion du risque, anticiper ses conséquences et concevoir les stratégies les plus appropriées.

Mots-clés

Agent antimicrobien – Analyse du risque – Antimicrobien d'importance cruciale – Aquaculture – Épreuve de sensibilité aux médicaments – Résistance – Santé publique.



La resistencia a los antimicrobianos en acuicultura

P. Smith

Resumen

La administración de un tratamiento antimicrobiano adecuado es una de las medidas de gestión más eficaces para afrontar emergencias relacionadas con epizootias infecciosas. El uso de tales agentes, sin embargo, podría inducir la proliferación de resistencias bacterianas, lo que después dificultaría el uso de los mismos agentes para luchar contra enfermedades infecciosas en acuicultura. También existe la posibilidad de que la proliferación de bacterias resistentes (o de los genes que codifican las resistencias) perjudique el uso de antimicrobianos para controlar ciertas enfermedades en el hombre o en animales terrestres. Las tentativas de aplicar a este problema métodos

homologados de análisis de riesgos no han fructificado por la enorme diversidad de las actividades de acuicultura y la escasez general de datos sobre la cuestión. Una de las principales ideas que aquí defiende el autor es, sin embargo, que no sólo carecemos de los datos que tal proceso exigiría, sino también, y ante todo, de métodos validados para reunir esos datos. Y lo más importante de todo es que ni siquiera disponemos de métodos validados para determinar si se debe o no calificar de "resistente" a una bacteria aislada en una explotación acuícola. A falta de toda evaluación de riesgos digna de tal nombre, lo que ahora mismo se intenta es gestionar los riesgos elaborando listas de antimicrobianos de importancia básica destinadas a los diversos usuarios de estos agentes. En opinión del autor, para que estemos en condiciones de evaluar este método de gestión de riesgos, predecir sus consecuencias o definir estrategias más adecuadas, lo indispensable y urgente es disponer de estudios de ecología génica y modelos de flujo génico en el medio ambiente.

Palabras clave

Acuicultura – Agente antimicrobiano – Análisis del riesgo – Antimicrobiano de importancia básica – Método experimental para determinar la susceptibilidad – Resistencia – Salud pública.



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