Monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals


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Summary
The World Organisation for Animal Health (OIE) Aquatic Animal Health Code recommends that programmes for the monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals be initiated by the appropriate authorities. This paper discusses the classes of bacteria to be studied in such programmes and the methods of sample collection to be employed. It also discusses the susceptibility test protocols appropriate for use in such programmes, the interpretive criteria that should be applied to the data they generate and the form in which the output of such programmes should be reported.

The authors argue that it is essential that all monitoring and surveillance programmes should employ standardised and internationally harmonised susceptibility test methods to the greatest extent possible. With respect to bacteria capable of infecting aquatic animals, it is recommended that the set of consensus-based standards and guidelines published by the Clinical and Laboratory Standards Institute be adopted as the basis for international harmonisation of test protocols, as they are significantly more developed than any alternatives. It is further recommended that, for the purpose of evaluating antimicrobial resistance trends, such as emerging resistance, the data generated by these protocols should be interpreted by the application of epidemiological cut-off values.

However, as yet, internationally agreed cut-off values have been produced for only one species. Thus, for many species, authorities will be obliged to set their own local and laboratory-specific cut-off values. It is recommended that laboratories use a statistical and standardised method of establishing such local cut-off values.

Internationally harmonised standard test protocols and interpretive criteria have, to a large extent, been developed to monitor antimicrobial resistance in bacterial species capable of infecting humans. These methods can also be applied to microorganisms capable of infecting humans that are isolated from aquatic animals reared for human consumption or for sale as companion animals.

Keywords
Introduction

This paper was produced by the members of the World Organisation for Animal Health (OIE) ad hoc Group on the Responsible Use of Antimicrobial Agents in Aquaculture. This ad hoc Group was convened to develop a suite of chapters for the Aquatic Animal Health Code (Aquatic Code) to provide guidance for OIE Member Countries to appropriately address the selection and dissemination of resistant microorganisms and antimicrobial resistance determinants from the use of antimicrobial agents in aquatic animals.

Chapter 6.4. of the OIE Aquatic Code (28) recommends that countries should initiate monitoring and surveillance of antimicrobial resistance in microorganisms associated with aquatic animals. The implementation of such programmes is considered necessary to:

i) establish baseline data on the prevalence of antimicrobial-resistant microorganisms and determinants

ii) collect information on antimicrobial resistance trends in relevant microorganisms

iii) explore the potential relationship between antimicrobial resistance in aquatic animal microorganisms and the use of antimicrobial agents

iv) detect the emergence of antimicrobial resistance mechanisms

v) conduct risk analyses as relevant to aquatic animal and human health

vi) provide recommendations on human health and aquatic animal health policies and programmes

vii) provide information to facilitate prudent use, including guidance for professionals prescribing the use of antimicrobial agents in aquatic animals.

The aim of this paper is to address in detail the appropriate methods for monitoring and surveillance of antimicrobial resistance in microorganisms isolated from aquatic animals. It discusses the microbial groups that should be studied, and the appropriate sampling procedures, susceptibility test protocols and interpretive criteria to be used.

Relevant microorganisms

There are three groups that need to be considered when designing programmes for the monitoring and surveillance of antimicrobial agent resistance in microorganisms isolated from aquatic animals:

– microorganisms, present in or on aquatic food animals or their products, that are capable of infecting humans or other terrestrial animals

– microorganisms relevant to studies of the impact of antimicrobial agent use in aquaculture on the microflora of the wider aquatic environment.

This paper will concentrate on the first of these groups. The other groups will be briefly discussed at the end of the paper.

Monitoring and surveillance of non-zoonotic microorganisms isolated from aquatic animals

Developing a strain set

The most efficient method of assembling a strain set for any monitoring or surveillance programme is to select strains from those that have been isolated by diagnostic laboratories servicing the aquaculture industry in the area to be covered by the programme.

A major aim of monitoring or surveillance programmes is to generate data on the susceptibilities of the microorganisms that are the most frequent target of antimicrobial agent therapy in the aquaculture industry operating within the area covered by the programme. Variations in the species of aquatic animals reared and their environmental conditions mean that these microorganisms vary from area to area. Different authorities will, therefore, have to address different microorganisms.

It is suggested that each authority establish a list of microorganisms that are found in its area, based on data obtained by laboratories involved in the investigation and diagnosis of disease epizootics in aquatic animals in the region. These laboratories could also represent the most cost-effective source for collecting and assembling an adequate strain set for inclusion in any monitoring or surveillance programme.

It is, however, reasonable to assume that the intensity of microbiological investigations of any epizootic by a front-line diagnostic laboratory will vary. Epizootics that respond adequately to therapy by the first antimicrobial agent chosen may result in the collection and susceptibility determination of only a very few, if any, isolates. Those epizootics, however, which do not respond adequately to the first therapy will frequently involve the isolation and susceptibility determination of a greater number of strains.
Thus, when generating a strain collection for monitoring or surveillance programmes from strains isolated by frontline diagnostic laboratories, there is potential for over-representation of resistant strains among those studied by these laboratories.

**Laboratory in vitro susceptibility testing methods**

A variety of laboratory methods are available for in-vitro susceptibility testing. It is essential that any monitoring or surveillance programme use standardised and internationally recognised protocols governing its performance, when such methods are available.

Laboratory methods for determining antimicrobial agent susceptibility can be classified into two main categories. The first category includes methods designed to determine the minimum concentration of an agent required to inhibit the growth, in laboratory media, of a bacterium. Minimum inhibitory concentration (MIC) methods include agar dilution, and macro or micro broth dilution. The second category contains methods that establish the size of the inhibition zone produced by placing a disc containing an antimicrobial agent on a lawn of the test bacterium (disc diffusion tests). Comparative studies suggest that both these methods can generate useful data (3).

If MIC methods are chosen, care should be taken in selecting the range of dilutions to be included in any test. For the purposes of monitoring or surveillance programmes, it may not be necessary to use a range of dilutions that cover the full range of susceptibilities encountered in the species being studied. It is, however, important to cover the full range of MICs needed for the quality control organism in situations where relevant acceptable ranges have been set.

For microbial species or groups for which internationally recognised interpretive criteria have been set, the dilution range should be sufficient to allow workers to determine whether the MIC of a test strain is above or below the cut-off value. (See ‘The need for harmonisation and standardisation of test conditions used for susceptibility testing in monitoring and surveillance programmes’, below.) When conducting studies on such species or groups, useful data can be obtained from studies that employ only a range of dilutions that cover the established breakpoint or cut-off value. For example, if the cut-off has been set at ≤1 μg/ml, adequate information could be obtained from an MIC assay that includes a range of dilutions from 0.25 μg/ml to 4 μg/ml.

However, when studies involve microbial species for which internationally recognised interpretive criteria have not been set, the design of these studies must be sufficient to allow the setting of a local cut-off value that can be applied to the data generated in the study. Such cut-off values, however, cannot be established if the susceptibilities of a significant number of strains are ‘off range’ and can only be recorded as less than (or greater than) a certain value. In order to set such local cut-off values, it is necessary to extend the range of dilutions employed to allow the establishment of a quantitative value of the susceptibility of all fully sensitive strains. For these strains, the minimum dilutions that must be included in any study cannot be established internationally but must be established experimentally by each laboratory.

It should be noted that many commercially available broth micro-dilution plates have been developed for terrestrial species. These do not include dilutions appropriate for examining microorganisms isolated from aquatic animals.

**The need for harmonisation and standardisation of test conditions used for susceptibility testing in monitoring and surveillance programmes**

The numerical values of the measures of in-vitro susceptibility are dependent on the specific values set for parameters of the protocols used to obtain them. Variations in parameters such as the medium used, its pH, the incubation temperature, the methods of inoculum preparation and the concentration or amount of agent used can all result in susceptibility tests that produce quantitatively different measures of susceptibility (3).

Thus, if a laboratory is involved in a monitoring and surveillance programme designed to detect changes in the susceptibility of the strains examined over time, it is essential to use a well-standardised test protocol that includes quality control requirements (see ‘Quality control’, below). Monitoring and surveillance programmes should also be designed so that the data generated can be compared with data produced in other laboratories covering other areas. Thus, there is a need for international harmonisation of the standardised test protocols used in all laboratories.

With respect to bacteria of human importance, the development of an internationally accepted standard set of test methods faced the problems posed by the prior existence of many national standard methods (14). The situation for bacteria associated with aquatic animals was, however, radically different. Before 2000, no standard methods had been developed. The first step in developing a single set of standardised test methods for these bacteria was taken by a group of scientists from 17 countries. They produced a set of provisional standard protocols (1) that were subsequently adapted and adopted by the Clinical and Laboratory Standards Institute (CLSI) and published as guideline documents M42-A (6) and M49-A (7). Although the development of these guidelines is incomplete, it is far in advance of any alternatives so far proposed or considered.
(see 'Current state of development of standardised test conditions', below).

Therefore, the use of protocols contained in currently available consensus-based guidelines for the development of monitoring and surveillance programmes, such as M42-A and M49-A, is essential.

Current state of development of standardised test conditions

Significant progress has been made in establishing standard test conditions to be used in in-vitro susceptibility tests, but they do not, as yet, cover the testing of all microbial species that might be isolated from aquatic animals (Table I). Currently, CLSI has specified standard test conditions for non-fastidious microorganisms that yield results when grown on or in unmodified Muller Hinton media at $22 \pm 2{^\circ}C$ or $28 \pm 2{^\circ}C$ within 48 h (6, 7). Standard test methods for MIC determinations of *Flavobacteria* will be published in the next edition of the CLSI guideline M49 (11).

The CLSI has also suggested potential modifications to the standard non-fastidious conditions that might be suitable for the majority of the other species encountered in aquatic animals. Although these potential modifications have not yet been accepted as standard methods, it is strongly recommended that, unless there are compelling reasons to the contrary, they should be given preference in designing any monitoring and surveillance programme.

Quality control

An essential element of the CLSI standardised test protocols is their inclusion of obligatory quality control requirements (8). In the CLSI approach, determinations of the susceptibility of at least one quality control reference strain must be performed in association with every determination of the susceptibility of test strains (6, 7). Only if the measure of the susceptibility obtained for the reference strain(s) lies within the specified acceptable ranges can a laboratory consider itself to be in compliance with the relevant CLSI guidelines.

<table>
<thead>
<tr>
<th>Category</th>
<th>Bacterial groups</th>
<th>Test conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1: non-fastidious bacteria</strong></td>
<td>Enterobacteriaceae</td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td><em>Aeromonas salmonicida</em></td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td>Mesophilic aeromonads</td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas</em> spp.</td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td><em>Plesiomonas</em> shigelloides</td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td><em>Shewanella</em> spp.</td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio</em> (non-halophilic)</td>
<td>Accepted</td>
</tr>
<tr>
<td><strong>Group 2: halophiles</strong></td>
<td>Obligate halophilic <em>Vibrionaceae</em> and <em>Photobacteriaceae</em></td>
<td>Suggested $^{(a)}$</td>
</tr>
<tr>
<td><strong>Group 3: gliding bacteria</strong></td>
<td><em>Flavobacterium columnare</em></td>
<td>Accepted (MIC only)</td>
</tr>
<tr>
<td></td>
<td><em>Flavobacterium psychrophilum</em></td>
<td>Accepted (MIC only)</td>
</tr>
<tr>
<td><strong>Group 4: Streptococci</strong></td>
<td><em>Lactococcus</em> spp.</td>
<td>Suggested</td>
</tr>
<tr>
<td></td>
<td><em>Vagococcus</em> salmoninarum</td>
<td>Suggested</td>
</tr>
<tr>
<td></td>
<td><em>Streptococcus</em> spp.</td>
<td>Suggested</td>
</tr>
<tr>
<td><strong>Group 5: other fastidious bacteria</strong></td>
<td>Psychrophilic <em>Aeromonas salmonicida</em></td>
<td>Suggested</td>
</tr>
<tr>
<td></td>
<td><em>Vibrio salmonicida</em> and <em>Monitella viscous</em></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td><em>Tenacibaculum maritimum</em></td>
<td>Suggested</td>
</tr>
<tr>
<td></td>
<td><em>Renibacterium salmoninarum</em></td>
<td>Suggested</td>
</tr>
<tr>
<td></td>
<td><em>Mycobacterium</em> spp.</td>
<td>Accepted</td>
</tr>
<tr>
<td></td>
<td><em>Nocardia seriolae</em></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td><em>Piscirickettsia salmonis</em> and other <em>rickettsia</em></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td><em>Francisella piscicida</em></td>
<td>No</td>
</tr>
<tr>
<td></td>
<td><em>Francisella asiatica</em></td>
<td>No</td>
</tr>
</tbody>
</table>

(a) ‘Suggested’ indicates that insufficient data were available to completely evaluate the suitability of the test conditions but that further work should concentrate on the use of these conditions.

MIC: minimum inhibitory concentration
When investigating the susceptibility of organisms for which test conditions have yet to be standardised, laboratories have no formal quality control procedure to apply. In this situation, it is recommended that they build quality control elements into their local test protocols that allow them to establish the consistency of their data over time. The most suitable way of achieving this is to select one strain to be included in every test series. In selecting a strain to serve in a local quality control procedure, it is important that the strain is stable in terms of its susceptibility. It is an added advantage if it is easily accessible to other laboratories. Although the type strain of the species being studied may be an attractive option, it should be noted that *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658 have been adopted as the reference strains for all the test conditions set so far for aquatic bacteria (6, 7, 11).

The need to harmonise and standardise the interpretive criteria used in susceptibility monitoring and surveillance analyses and reporting

Interpretive criteria are required to estimate resistance frequencies generated from the quantitative measures of susceptibility, which in turn are generated by *in-vitro* testing. As there is often a need to compare resistance frequencies established in different laboratories, it is necessary to address the standardisation and harmonisation of the interpretive criteria to be used.

Types of interpretive criteria

Two types of interpretive criteria – clinical breakpoints and epidemiological cut-off values – can be applied to *in-vitro* susceptibility data (18). Clinical breakpoints aim to categorise isolates on the basis of the probable outcome of applying specified therapies to epizootics in specific hosts, under specific environmental conditions. The general lack of relevant pharmacokinetic/pharmacodynamic and clinical correlation data, coupled with the diversity of the hosts and environmental conditions found in aquacultural epizootics, presents serious difficulties when setting experimentally validated clinical breakpoints. So far, provisional clinical breakpoints have been suggested for only two antimicrobial agents against one bacterial species (9). It should, however, be noted that, to make it easier to predict the clinical outcomes of the diverse treatments encountered in aquaculture, it might be necessary to adopt more than one clinical breakpoint for any particular bacterial species/antimicrobial agent combination.

Epidemiological cut-off values categorise isolates on the basis of their *in-vitro* susceptibilities only. These cut-off values are abbreviated to the acronyms ‘ECVs’ by CLSI and ‘ECOFFs’ by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), but it should be noted that neither organisation has standardised the procedures to be used in setting these values. Applying an epidemiological cut-off value allows us to place isolates into one of two categories. Isolates that generate susceptibility measures indistinguishable from those generated by fully susceptible members of their species (or group) are categorised as wild-type (WT). All other isolates are categorised as non-wild-type (NWT). The nomenclature WT/NWT has been adopted by both EUCAST (13) and CLSI (9).

According to EUCAST, a microorganism is defined as WT for a species by the absence of acquired and mutational resistance mechanisms to the drug in question (13). As the primary aim of monitoring and surveillance programmes is to gain information on the prevalence and emergence of antimicrobial-resistant microorganisms and antimicrobial resistance determinants, Silley et al. (19) and the OIE (28) have argued that epidemiological cut-off values are the most appropriate type of interpretive criteria for use in these programmes.

Estimating epidemiological cut-off values

In theory, ECVs are relatively easy to set. They represent the limits (the upper limit in the case of MIC data and the lower limit in the case of disc diffusion data) of the distribution of susceptibility measures for WT strains. These limits can be estimated by visual examination of *in-vitro* susceptibility data sets. However, Kronvall (15, 16) has developed a statistical method, normalised resistance interpretation (NRI), that allows us to estimate ECVs from these data using a standardised procedure. Using NRI when setting ECVs has the advantage of obviating distortions in the estimation of ECVs caused by strains that manifest low-level resistance (15). For this reason, and in the interests of transparency, it is recommended that, when laboratories set their own ECVs, they should use the NRI method (see ‘Should estimating epidemiological cut-off values be considered universal or local’, below).

With respect to the number of strains that are required to set an ECV, it should be noted that the precision of any estimate is a function of the log of the number of strains used to set it (17). A CLSI report (10) suggests that a plausible ECV could be set from as few as 30 MIC observations and a similar minimum has been suggested for disc diffusion observations (20).

Should estimating epidemiological cut-off values be species specific or can they be applied to a broader taxonomic group?

A central question that arises, when attempts are made to set or apply interpretive criteria for *in-vitro* susceptibility data, is the width of the taxonomic group to which a single ECV can be applied. If it is determined that ECVs can be
validly applied only to a narrow taxonomic group, such as an individual species, the amount of work needed to set all the criteria required for the diverse species encountered in aquaculture will be enormous. On the other hand, if it is found that ECVs can be applied to generic or multi-species groups, the workload will be greatly reduced.

So far, the only ECVs that have been set for aquatic microorganisms have been species specific (8). However, the data produced for *Vibrio* spp. and *Aeromonas* spp. (22, 24) suggest that ECVs could possibly be applied to wider taxonomic groups without an unacceptable loss of precision.

In aquaculture, there are insufficient data on which to base a decision about the appropriate taxonomic width of the microbial groups to which a single ECV should apply. Until more susceptibility data are available, it will not be possible to formulate, at an international level, an evidence-based decision as to the appropriate taxonomic width. Given this uncertainty, individual authorities will have to make their own local decisions as to the best option for the microbial groups which they are studying. These decisions should be considered as provisional and open to change as more data become available.

**Should estimating epidemiological cut-off values be considered universal or local?**

There is some debate as to whether the variations between laboratories for the data produced in disc diffusion studies are so great that applying a single, internationally agreed cut-off value will result in potentially serious differences in the various laboratories’ abilities to correctly categorise strains that manifest low-level resistance (15, 21). In terms of microorganisms from aquatic animals, this debate will only be resolved when more data become available from more laboratories.

What is certain, however, is that, with the exception of those for *A. salmonicida* (9), no consensus-backed ECVs have yet been set for any of the species or genera encountered in aquaculture. Faced with this situation, individual laboratories involved in monitoring or surveillance programmes may not have the option to use international, consensus-backed interpretive criteria. If they are to offer any interpretation of the data they produce for organisms other than *A. salmonicida*, they must generate and apply their own cut-off values, estimated locally from their own data. In this context, the use of NRI analysis, which represents a standardised protocol for setting local cut-off values, is strongly recommended.

**Inappropriate application of interpretive criteria**

It is important to remember that interpretive criteria are protocol specific. The protocols used to estimate them must be identical to those used to generate the data to which they are applied. It follows that, when attempting to interpret data obtained in studies of aquatic microorganisms, it is inappropriate to apply interpretive criteria that have been developed and validated for susceptibility test data obtained in other tests using different test conditions.

**Reporting data**

It has been argued in previous sections that the development of standardised and harmonised susceptibility tests for microorganisms isolated from aquatic animals is incomplete and is a work in progress. At the present stage of development, laboratories performing monitoring or surveillance programmes will be forced to make local decisions about the test conditions, quality control procedures and interpretive criteria to be applied to some of the microorganisms under study. This inevitable element of local decision-making has consequences for the manner in which the results of these programmes are published.

To ensure maximum international comparability, it is recommended that the results of monitoring and surveillance programmes always be reported as unprocessed quantitative data (28). That is, all data should be reported as frequencies of the distribution of zone sizes or MIC values. Reports should also provide details of the test protocols used in generating these data.

In addition, reports may include processed or interpreted summaries of the raw data. However, given the current lack of international consensus on the interpretive criteria to be used, interpretations should always give details of the local interpretive criteria and cut-off values applied by the laboratory or authority generating the report and, importantly, the protocols used to establish them.

**Microorganisms present in or on aquatic animals or their products that can infect humans or other animals**

Monitoring and surveillance programmes may be designed to quantify the frequency of antimicrobial agent resistance in microorganisms transmitted to humans via food derived from aquatic animals. As the microorganisms involved in this mode of transmission will all be capable of infecting humans, it can be assumed that standardised protocols for their susceptibility testing have already been largely established.
With respect to the design of sampling protocols, analysing food derived from aquatic animals shares many aspects with analysing food from terrestrial animals. The OIE Terrestrial Animal Health Code (29) recommends sampling protocols for monitoring and surveillance of antimicrobial resistance in food derived from terrestrial animals. Broadly, these sampling recommendations can also be applied to aquatic animal studies, provided that certain conditions specific to antimicrobial resistance in aquatic animals are given due consideration.

First, in aquatic animals, the prevalence of zoonotic microorganisms is considerably lower than in terrestrial animals. This may influence decisions on the intensity of sampling to be employed when investigating antimicrobial agent resistance in microorganisms present in various aquatic food products.

Secondly, the Terrestrial Code (29) recommends monitoring the resistance of some elements of the commensal intestinal microflora of terrestrial animals. However, it has been suggested that the intestinal microflora of aquatic animals show significant fish-to-fish and site-to-site variation (23). So far, it has not been possible to establish a bacterial group that is sufficiently and universally present in fish intestinal microflora that it can be assumed to function as an indicator organism. For this reason, the Aquatic Code (28) does not recommend routine monitoring of resistance frequencies in the microflora of healthy aquatic animals.

A third factor should be noted when examining data from aquatic food products sampled at the point of sale. Some antimicrobial-resistant microorganisms detected at the point of sale may have arisen as a consequence of antimicrobial agent use in aquaculture facilities. Others may have arisen from terrestrial contamination of the rearing water used by these facilities or from post-harvest contamination of their products. Thus, if the output of monitoring and surveillance programmes is intended to be used to assess the risks associated with using antimicrobial agents in aquaculture, resistance frequency data collected from point-of-sale sampling may, because of the potential confounding factors, need additional interpretation.

Microorganisms present in or on ornamental or pet fish that can infect humans

Antimicrobial agent use in the non-food, ornamental or companion animal/pet fish industry has been less well studied and its potential impact on the selection and dissemination of resistant microorganisms and resistance determinants is more poorly understood than that of aquatic food animals. Nevertheless, it has been suggested that the use of antimicrobial agents in this industry may be widespread and frequent (26), and recent studies (25) have shown high frequencies of antimicrobial resistance in microorganisms associated with these fish. Differences in the route of exposure between aquatic animals and humans would need to be taken into account when developing monitoring and surveillance programmes for ornamental or pet fish. Sampling of aquatic animals as well as their carriage water should be considered.

In terms of appropriate antimicrobial susceptibility testing methods, ornamental fish isolates can be studied in the same way as those from aquatic animals produced for human consumption. The comments made above, although made with respect to food-producing aquaculture, are also applicable to ornamental fish (see ‘The need to harmonise and standardise the interpretive criteria used in susceptibility monitoring and surveillance analyses and reporting’ and ‘Microorganisms present in or on aquatic animals or their products that can infect humans or other animals’).

Monitoring the impact of antimicrobial agents used in aquaculture on microorganisms in the wider aquatic environment

The development of a reservoir of resistant microorganisms or resistance determinants in the aquatic environment has been identified as a potential risk arising from the use of antimicrobial agents in aquaculture (27). The monitoring of these reservoirs would therefore appear desirable. However, the development and implementation of appropriate programmes have been significantly challenged by the ecological complexity of the environments concerned, as well as by the complexity of the biological pathways involved in the origin, persistence and movement of resistance determinants within these environments (2, 12).

A detailed treatment of these issues and their possible resolution is beyond the scope of this article and will not be attempted here. However, it could be argued that current understanding would be improved by greater knowledge of the movement of resistant determinants within the environmental microflora, rather than the movement of resistant microorganisms through the environment (4). This would, in turn, suggest that monitoring programmes designed for this purpose should employ molecular methods.
specifically aimed at quantifying these determinants, rather than the culture-dependent, in vitro susceptibility testing methods that have been discussed in this article. The pace of work in this area is encouraging and those considering the design of monitoring and surveillance programmes should take note of the development and application of new methods.

Conclusion

The collection of reliable data on the frequency of antimicrobial resistance is a necessary prerequisite to developing and regulating prudent and rational use of antimicrobial agents in aquaculture. There has been significant progress towards the development of standardised and harmonised methods appropriate for the monitoring and surveillance programmes that will be necessary to achieve these data. This paper has reviewed the current state of antibiotic susceptibility testing methods and provided guidance on conducting the investigations recommended in the OIE Aquatic Code (28).

La surveillance et le suivi de l’antibiorésistance chez les micro-organismes associés aux animaux aquatiques

P. Smith, V. Alday-Sanz, J. Matysczak, G. Moulin, C.R. Lavilla-Pitogo & D. Prater

Résumé

Le Code sanitaire pour les animaux aquatiques de l’Organisation mondiale de la santé animale (OIE) préconise que les autorités en charge de la santé des animaux aquatiques mettent en place des programmes de surveillance et de suivi de la résistance aux agents antimicrobiens chez les micro-organismes associés aux animaux aquatiques. Les auteurs du présent article font le point sur les catégories de bactéries à prendre en compte dans ces programmes et sur les modalités appropriées de prélèvement. Ils examinent également les protocoles de réalisation des tests de sensibilité conduits dans le cadre de ces programmes, les critères d’interprétation des données obtenues et la manière de présenter ces résultats dans les rapports de surveillance.

Les auteurs recommandent que dans la mesure du possible, les programmes de suivi et de surveillance fassent appel à des tests de sensibilité aux antibiotiques normalisés et harmonisés au plan international. En ce qui concerne les bactéries affectant les animaux aquatiques, les auteurs recommandent de s’inspirer de l’ensemble des normes et des lignes directrices élaborées par voie de consensus par le Clinical and Laboratory Standards Institute, qui pourraient servir de base pour l’harmonisation internationale des protocoles de tests, compte tenu de leur niveau de précision bien supérieur à celui d’autres propositions. Ils préconisent également d’appliquer des seuils limites épidémiologiques lors de l’interprétation des données produites par ces protocoles aux fins d’évaluation des tendances en matière d’antibiorésistance.

Néanmoins, à ce jour des seuils limites épidémiologiques n’ont été validés au plan international que pour une seule espèce. De ce fait, pour nombre d’autres espèces, les autorités devront établir leurs propres seuils limites, déterminés localement par des laboratoires individuels. Il est recommandé que les laboratoires utilisent...
Seguimiento y vigilancia de las resistencias a los agentes antimicrobianos en los microorganismos ligados a los animales acuáticos

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Resumen
En el Código Sanitario para los Animales Acuáticos de la Organización Mundial de Sanidad Animal (OIE) se recomienda a las autoridades competentes que pongan en marcha programas de seguimiento y vigilancia de las resistencias a los antimicrobianos en los microorganismos ligados a los animales acuáticos. Los autores explican las clases de bacterias que deben estudiarse en tales programas y los métodos de muestreo que conviene emplear. También exponen los protocolos de realización de antibiogramas que resulta apropiado utilizar en esos programas, los criterios de interpretación que hay que aplicar a los datos obtenidos y el modo en que se deben comunicar los resultados de los programas. Para los autores es esencial que en todos los programas de seguimiento y vigilancia se empleen, en la mayor medida posible, métodos normalizados e internacionalmente armonizados para realizar antibiogramas. Por lo que respecta a las bacterias capaces de infectar a los animales acuáticos, se recomienda que como base para armonizar los protocolos a escala internacional se utilice el conjunto de normas y directrices consensuadas que publica el Instituto de Estándares Clínicos y de Laboratorio, sensiblemente más elaborado que cualquier otra norma alternativa. Se recomienda asimismo que, a la hora de determinar las tendencias que siguen las resistencias a los antimicrobianos, como en el caso de una resistencia que empieza a surgir, se utilicen valores umbral epidemiológicos para interpretar los datos generados con esos protocolos. Sin embargo, hasta ahora solo se han consensuado valores umbral a escala internacional en el caso de una especie. Por tal motivo, para muchas especies las autoridades se verán obligadas a definir sus propios valores umbral locales y
específicos de un laboratorio. Para fijar esos valores umbral de carácter local se recomienda a los laboratorios que empleen un método estadístico y normalizado. Los protocolos de prueba y los criterios de interpretación internacionalmente armonizados responden en buena medida al objetivo de seguir de cerca las resistencias a los antimicrobianos en especies bacterianas capaces de infectar al ser humano. Estos métodos también se pueden aplicar a microorganismos capaces de infectar al hombre que se aíslan a partir de animales acuáticos producidos para el consumo humano o para su venta como animales de compañía.

**Palabras clave**

**References**


