



Correct Diagnostics: Prerequisite for Prudent and Responsible Antimicrobial Administration

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Abstract

Since bacterial diseases have an adverse impact on the profitability of aquaculture, causing direct and indirect losses, this review paper is assessing the importance of accurate diagnostics in prudent and responsible administration of antimicrobials. Diagnostics and treatment of bacterial diseases in aquaculture are inevitable factors in their responsible management and consequently contribute to the reduction of antimicrobial use (AMU) and prevention of antimicrobial resistance (AMR) development. To mitigate and prevent the losses, fast and accurate recognition and detection of bacterial pathogens are the main prerequisites. Monitoring programmes in all stages of production, from broodstock to fattening units, are needed to avoid long diagnostic processes and enable fast commencement of diagnostic procedures and responsible AMU. Moreover, preventive measures to reduce the risk of bacterial infection includes good aquaculture practices (GAP) and biosecurity measures, in the absence of specific immunoprophylaxis, or vaccination, against endemic bacterial diseases. Antimicrobial use may be considered as therapeutic, metaphylaxis, prophylaxis, and growth promotion. Antimicrobials are most often administered through bio-enrichment of fish larvae or shrimp post larvae and medicated feed. The efficacy of the treatment via medicated feed depends on the rapid diagnosis and commencement of treatment, selection of appropriate antimicrobials, proper dosage, and duration of treatment. To prevent possible mistakes in AMU, it is necessary to avoid prophylactic use of antimicrobials, medication of viral infections, and repeated use of the same medicines.

Keywords: AMR, AMU, aquaculture, disease management

Introduction

Diseases have become the main constraint to aquaculture growth, impacting both economic and socio-economic development in many countries (Subasinghe et al., 2001; Bondad-Reantaso et al., 2005). The annual economic losses due to diseases are estimated to be billions of dollars. Among causative agents, bacteria can survive in the aquatic environment independently of their hosts and became the major obstacle to the cultivation of freshwater and marine fish species as well as crustaceans. The global economic impact of bacterial diseases on the aquaculture sector likely ranges from hundreds of millions to billions of dollars annually (Subasinghe, 2005) due to direct (mortalities, diagnostics and treatment costs) and indirect losses (cost of wasted

feed, removal of dead animals, retarded growth and lower feed conversion ratio (FCR).

Very rarely it is possible to control bacterial diseases in aquaculture by eradication and targeted antimicrobial chemotherapy remains vitally important for the treatment of some bacterial diseases (Smith et al., 2003). Hence, diagnostics and treatment of bacterial diseases in aquaculture are inevitable factors to its responsible management. The effective control and treatment of bacterial diseases require rapid, reliable, and highly sensitive diagnostic methods (Haenen and Zrnčić, personal communication). Clinical aspects of the outbreak, post-mortem examination, and histopathology are the primary methods used in diagnostics, but they often lack specificity and the pathogen is difficult to detect in animals without

clinical signs of the disease. Cultivation of pathogenic bacteria is a widely used method, but it is time-consuming and there are some non-cultivable, fastidious bacterial pathogens. Thus, it takes almost 10 days, from the occurrence of first signs of disease to the end of diagnostics procedure and sensitivity testing. However, this period is too long, and the losses may become enormous (Buller, 2004).

Generally, bacterial diseases are controlled by feeding infected fish with antibiotic-medicated feed, based on sensitivity testing. However, this practice may be ineffective because sick fish lose appetite. In addition, frequent use of antimicrobial compounds has led to the development of resistance to antimicrobial compounds in pathogens, posing serious challenges to both aquatic animal health and human health (Cunha, 2000).

Appropriate use of antimicrobials will cure some sick animals, speed up the recovery of the population, improve the welfare of treated animals, and prevent the spread of the bacterial infection to other animals (Kemper, 2008). Appropriate use of antimicrobials is dependent on the proper diagnosis, based not only on the detection of the pathogen but also on information such as farm history and outbreak or event history, followed by a visual examination of the aquatic animals with and without clinical signs before taking samples for laboratory tests. Prudent and responsible use of antimicrobials to minimise the risk of resistance is a challenging duty for aquatic animal health experts.

This paper emphasises the contribution of diagnostics for the responsible management of bacterial diseases in aquaculture. Moreover, the administration of antimicrobial compounds should be based on accurate diagnostics and carried out in a way that enables effective treatment and consequently promotes the reduction of antimicrobial use (AMU) and the prevention of the development of antimicrobial resistance (AMR).

Impact of Bacterial Diseases on the Profitability of Aquaculture

Bacterial disease outbreaks have an adverse impact on the profitability of the aquaculture facility, regardless if the disease occurs in the hatchery or on-growing facilities. Direct losses caused by the disease are mortalities, which vary according to the pathogen and category of the affected population. The fry and juveniles are usually more susceptible to bacterial infections and mortalities, which may reach up to 35 to 40 % of the population whereas mortalities in the older population may reach 15 to 25 % (Varvarigos, 2003). Costs for antimicrobial substances and diagnostic procedures should be included in the direct cost, as well as losses caused by disease re-occurrence. Indirect losses include the adverse effects on growth, which are expected to be severe due to the prolonged loss of appetite and the long and

drastic reduction of the feeding rate as a management response that will eventually increase the FCR. Additional indirect costs comprise of labour for the daily removal, transport, and sanitary disposal of dead fish. Extra costs for additional disinfection in the hatchery and of the equipment used for feeding, removal of dead fish, may also be added to the indirect costs due to the disease outbreak. The labour cost and time required to prepare the medicated feed daily is yet another additional cost caused by a bacterial disease outbreak. There is also the significant, but unquantifiable, psychological burden on the fish farmers.

Role of Diagnostics in Bacterial Disease Management

Immediate management of an outbreak on the aquaculture farm

When there is an outbreak of bacterial disease in the aquaculture facility, the main prerequisite for mitigation of losses is fast and accurate recognition and detection of the bacterial pathogen. Sensitive and specific methods for the detection of the bacterial pathogen are very important factors of the health monitoring program. Diagnostic skills should be continuously improved upon regardless of diagnostic capacity. Different actors in the disease recognition process have different diagnostic capacities (Bondad Reantaso et al., 2001) – level 1 consisting of environmental changes determination (water temperature, pressure, oxygen saturation, eutrophication, etc.), gross signs observation (changes in behaviour of the aquatic organism, pattern of the feeding, external signs,) and necropsy findings; level 2 consisting of general bacteriological and histological methods; and level 3 consisting of DNA based methods and spectrophotometric methods – are equally important for an accurate diagnosis.

Standard diagnostic procedure for immediate disease control begins when the farmer notices changes in the appearance of the farmed shoal and informs health specialists. They then commence the diagnostic procedure by identifying changes in the environment, clinical appearance of the affected population, and, together with results of the necropsy, inform the choice of bacteriological media and the procedure to be performed (Christofilogiannis, 2013). After obtaining pure bacterial cultures, sensitivity testing to approved and indicated antimicrobials should be carried out. Results of the testing using standardised protocols will advise on the choice of antimicrobials. The next step is an evaluation of the affected biomass that will enable the quantification of the required medicated feed. The farmer will order the medicated feed from an approved feed mill or, under the supervision of a health specialist, will prepare the medicated feed on

the farm. Treatment against the bacterial disease should last at least 10 days. The diagnostic procedure starts with notifying the first signs of the disease, followed by sampling sick fish and seeding the bacterial plates. The time needed to complete the diagnosis and susceptibility testing which is the basis for appropriate use of antimicrobials mixed in medicated meal lasts at least 6 to 7 days or even longer, in the case of slow-growing or fastidious bacteria (Buller, 2004).

Health management plans (HMP)

As it is obvious from the above described bacterial disease management procedure, that losses may rise to the level where they begin to pose a serious threat to the sustainability of the production. To mitigate the losses caused by a disease outbreak, reduce the risk of propagation of the pathogens, and to allow improvement of the treatment efficacy, it is useful to create and implement a health management plan, which includes several requirements:

1. Knowledge of the technical procedure including optimal ecological condition and normal appearance and behaviour at all stages of the farmed species during all steps of the cultivation – from broodstock to market size fish.
2. Knowledge of the ecological and environmental conditions favourable for a disease outbreak.
3. Knowledge of the clinical appearance of the particular bacterial disease, post-mortem signs, and histopathological changes in affected tissues, as primary methods for diagnostics often lack specificity and it is difficult to detect the pathogen in the animals without clinical signs of the disease. It is important to recognise the first changes to set up suspicion.
4. Effective control and treatment of bacterial diseases require rapid, reliable and highly sensitive and specific diagnostic methods; therefore, the health expert needs to choose the most appropriate diagnostic procedure. Cultivation of pathogenic bacteria is a widely used method, but time-consuming and there are some hardly cultivable, fastidious bacterial pathogens. If there is a possibility to implement immunological, protein-based, and molecular methods, all mentioned limitations might be solved.
5. In order to start the diagnostic procedure timely, it is most important to notify the suspicions of the disease quickly.

A health management plan should consist of several equally important components (Le Breton and Sourd, 2011):

1. Reducing bacterial pathogen pressure by implementing the following:
 - 1.1. Good aquaculture practices (GAPs) including the separation of generations, favourable stocking densities, proper feeding, feed quality, reducing stress by providing farmed animals appropriate light, protection from the predators, water exchange.
 - 1.2. Sanitation practices implementing appropriate cleaning and disinfection of farming units, equipment, containers, boats, nets.
 - 1.3. Biosecurity measures which include an introduction of certified stocks, awareness of the disease history on the farm, control of animal movement between and within farms, movement of people and vehicles, control of birds, predators, removal of dead fish.
2. Health monitoring programmes, which aim for early detection of the pathogen in all stages of the production. This should start with broodstock health monitoring and although biosecurity measures have been implemented, the vertical transmission of different bacterial diseases should be considered. For example, bacterial kidney disease caused by *Renibacterium salmoninarum* can be transmitted through fertilised eggs in salmonids (Pascho et al., 2002), or *Photobacterium damsela* subsp. *piscicida* (Romalde et al., 1999) in marine fish. Health monitoring in the hatchery should be carried out because sometimes biosecurity measures can fail and an infection may appear. Regular testing of fry, the most susceptible life stage, is needed. It should be kept in the mind that transport from the hatchery to on-growing units is a lot of work and the latent infections could occur after transportation. Disease monitoring, clinical inspection, and sampling followed by diagnostic testing should be performed during the on-growing period based on the knowledge of predisposing factors/periods for endemic diseases occurrence. In the absence of specific immunoprophylaxis (vaccination against endemic bacterial pathogens), losses can be mitigated only by prudent and responsible use of antimicrobials, and the key to successful treatment is continuous monitoring and early diagnosis.

Administration of Antimicrobials

Antimicrobial compounds are defined as substances that can kill or inhibit the growth of microorganisms (Romero et al., 2012). According to the document

issued jointly by FAO/OIE/WHO (WHO, 2004), the use of antimicrobials can be divided into (1) Therapeutic, antimicrobial treatment of established infections; (2) Metaphylaxis, a term used for group-medication procedures aimed to treat sick animals while medicating others in the group to prevent disease; (3) Prophylaxis, the preventative use of antimicrobials in either individuals or groups to avoid the development of infections; and (4) Growth promotion use, when an antimicrobial agent is used as a feed supplement in food animals to promote growth and enhance feed efficiency. When applying this scheme of antimicrobial use in aquaculture, it should be emphasised that the majority of antibiotic treatments in aquaculture are administered to populations (Smith, 2012). In fish farms and crustacean grow-out facilities, antibiotics are most often administered orally through a medicated feed. Prophylactic treatment is an administration of antibiotics to the population without observed clinical symptoms of the disease. Largely, therapeutic treatment is administered to the population where not all specimens are infected and uninfected specimens are treated prophylactically. However, the terms used for individual treatment cannot be correctly used for treatments of populations. When treatments are given to populations that contain infected individuals, it should be classified as metaphylactic.

Methods for Application of Antimicrobials

There are six different application methods (Austin and Austin, 2007) comprising of baths and dips, topical application, injection, and oral application via medicated feed or through bio-enrichment. Each method has its advantages and disadvantages depending on the targeted use, as well as potential environmental impacts (Rodgers and Furones, 2009):

1. Baths and dips are not as effective as some other treatment methods, particularly for systemic infections due to poor absorption of the antimicrobials used.
2. Topical application is usually necessary only for more valuable individual fish, such as ornamental varieties or broodstock, to treat ulcers or injuries.
3. Injection is more effective than using medicated feed but practically they are usually used only for valuable individuals. Injections are stressful and, before administering in the sick fish, anaesthesia is required. Injection application may be intraperitoneal or intramuscular.
4. Administration through bio-enrichment of live feed organisms, most often *Artemia* or rotifers, is done either directly or indirectly for fish larvae and shrimp postlarvae.

5. Medicated feed is a preferred method for antimicrobial administration, more often commercially prepared as sinking or floating pellets.

Many bacterial diseases of fish or crustaceans can be successfully treated with medicated feed. Medicated feeds are prepared by the incorporation of antimicrobial substances into the feed via powdered premix containing active ingredients and carriers (up to 5 %) in the form of sinking or floating pellets. The feed and antimicrobial substances have to be mixed thoroughly to be evenly distributed in the pellets. Medicated feed should be always administered according to a veterinary prescription. The choice of antimicrobials should be based on good diagnosis and sensitivity testing. The dosage of the antimicrobial compound is determined by the ratio of the active ingredient and biomass of fish being treated, as well as on the daily feeding rate. Medicated feeds need to be stored under the appropriate conditions, otherwise, it will deteriorate, and the antimicrobial compound may lose its efficacy. A vitally important prerequisite for effective treatment is a fast commencement of the medicated feed administration after the first clinical signs of the disease. For instance, if treatment of vibriosis in sea bass starts on the first day after the appearance of symptoms, overall mortalities are about 1.5 % of the fish in the facility compared to mortalities of 16 % if the treatment is delayed by one week (Zarza, 2012).

There are a few antibacterial compounds licensed for use in aquaculture but their approval varies from country to country. To treat aquaculture animals efficiently and avoid the development of AMR, it is very important to avoid sub-dosing, taking into consideration several very important facts such as correctly calculating the active ingredient concentration, appropriately identifying biomass to treat, and the daily feeding ratio. The treatment should last at least 3 days after cessation of the symptoms of the disease but not less than 7 days. Treated fish should not be harvested for human consumption before expiring the withdrawal period.

When medicated feed is used on a farm it is necessary to follow several rules aiming to foster the efficacy of treatment. The aquaculture animals to be treated should be starved before medicated feed administration. The daily feeding ratio of medicated feed should be reduced to ensure that most of the animals eat it and it should be offered as the first daily meal or adapted to the age and number of daily meals. It is preferred to administer the medicated feed manually or through small air cannons in big cultivation units like off-shore cages with high biomass per unit.

Conclusion

Aquaculture is the fastest growing food-producing

sector globally and there are many health challenges associated with this growth (Brun, 2016). Climate change, movement of aquatic commodities, and industrialisation are facilitating the spread of diseases and making them a primary constraint to the cultivation of many aquatic species. Health management programmes including biosecurity measures, disease notification and reporting, vaccination, and appropriate disease treatment should be implemented. There is good availability of commercial vaccines for bacterial diseases and they have resulted in the reduced use of antimicrobial agents, although there is still a need for improvements in delivery methods and efficacy (Rodgers and Furones, 2009). However, the lack of commercially available vaccines for the global fish culture means that there is a perpetual reliance on antibiotics to treat bacterial infections (Crumlish, 2017). Furthermore, farmed shrimp species cannot be conventionally vaccinated as they lack the appropriate immune system (Rowley and Pope, 2012). This leads to the demand for antimicrobial use. To minimise the risk of AMR development, it is necessary to improve knowledge on how and when to use antimicrobials, to enforce better regulation and policy, and support capacity building in all aspects of the aquaculture production chain.

The role of rapid and accurate diagnostics in AMR prevention should be emphasised as it enables the prudent use of antimicrobials to better treat infection, slowing the rise of drug resistance by reducing the unnecessary use of the particular antibiotic. Ultimately, implementation of appropriate diagnostics is changing our approach to treat bacterial infections through targeted and precise therapy.

It is imperative to engage efforts in avoiding all possible mistakes in the use of antimicrobials, namely to start the treatment too late, administer inadequate dosage or select improper medicine, implement too short of a duration of treatment, use antimicrobials prophylactically, use of antibiotics for viral infections, or repeatedly use the same medicine.

Ultimately, we should be aware that continuous monitoring and early diagnosis is a key to successful treatment and that prevention is always better than cure.

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