Understanding Antibiotic Treatment Failures in Salmon Aquaculture

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Abstract

Antibiotic treatment failure can occur due to several reasons. In this paper, we summarise our research in Chile and review relevant literature to identify the issues that result in antibiotic treatment failure. The four basic issues we have found for explaining treatment failure include misdiagnoses, resistance, subtherapeutic antibiotic tissue concentrations in target organs, and insufficient treatment time for the elimination of the pathogen at the individual and population levels. Our hypotheses are based on salmonid aquaculture systems but likely apply to other aquaculture industries that use in-feed antibiotic treatments for bacterial infections. It is important to better understand the specific causes of treatment failure as they result in repeated treatments and increased pathogen exposure to subtherapeutic antibiotic levels. Both of these phenomena could increase the risk of antibiotic resistance over time.

Keywords: biosecurity, AMR, *Piscirickettsia salmonis*, Chile

Introduction

It is anticipated that by 2050, the world's population will be over 9.8 billion (United Nations, 2017) and the demand for food will increase accordingly. As aquaculture industries grow to meet global market demand, farms are becoming larger and more densely clustered which increases the potential for transmission of host-dependent pathogens, such as bacteria, and makes it more difficult to control the spread of pathogens within and between facilities. It is, therefore, critical to prevent infections and control outbreaks early in the disease process.

To prevent bacterial diseases on fish farms, producers can increase their biosecurity to minimise the likelihood of pathogen introduction and improve the fish's resistance to infection to increase the likelihood that fish do not succumb to disease if the biosecurity fails. Because failure to prevent pathogenic bacteria from infecting fish on a farm can result in heavy losses, farmers often use antibiotics to prevent losses and curtail the spread of infection within a farm. As

farms increase in size, the quantity of antibiotics required to control bacterial disease outbreaks also increases, which raises the risk of developing antimicrobial resistance (AMR). Judicious use of antibiotics is of critical importance, and ensuring they are administered in a way that effectively treats the entire population will reduce the risk of developing antibiotic resistance and optimise treatment efficacy. Identifying and addressing issues associated with treatment failure is essential for improved antibiotic use and for the development of good practice guidelines. The objective of this paper is to identify issues that may account for antibiotic treatment failure, describe mitigation strategies to resolve failures, and improve the efficacy of antibiotic treatments in salmon aquaculture based on recent research conducted in Chile.

Antibiotic Use in Salmonid Aquaculture

The amount of antibiotics used annually in aquaculture industries varies significantly, depending

on the region. Norway, for example, uses orders of magnitude less than other countries (NORM/NORM-VET, 2016). Antibiotic use per kilogram of fish produced in this country has declined dramatically since 1992 (NORM/NORM-VET, 2016). In 2016, the Norwegian industry reported antibiotics usage at approximately 0.13 $g.tonne^{-1}$ of fish harvested (NORM/NORM-VET, 2016). The predominant antibiotics used in Norway are florfenicol and oxolinic acid (NORM/NORM-VET, 2016). Chile, on the other hand, uses mostly oxytetracycline and florfenicol, but reports between 300 and 650 $g.tonne^{-1}$ of fish harvested, depending on the year (Lozano et al., 2018). Scotland is reporting a decline in the usage of antibiotics in salmon, but the industry is still using between 3- and 100-fold more than Norway, depending on the year (Burridge et al., 2008). The USA and Canada do not report antibiotic use in aquaculture. A publication in 2008 suggested the Canadian industry used antibiotics at approximately 115 $g.tonne^{-1}$ of salmon harvested (Burridge et al., 2008, 2010). In the United States, a modelling study conducted by Benbrook (2002), for the Northwest Science and Environment Policy Center, estimated the use of antibiotics in the salmonid industry comparable to the usage in Chile, although this was not confirmed with empirical data. With exceptions of the Norwegian and Scottish industries, the use of antibiotics per kilogram of salmon, across the industry globally, is on par, or in some cases higher, than other food production industries (Van Boeckel et al., 2015). Given the market is moving towards antibiotic-free products, it is essential to reduce the use of antibiotics without the negative impacts on animal welfare. The judicious use of antibiotics is also important for slowing down the development of AMR (Anderson and Hughes, 2014).

Scenario for This Assessment

Currently, the Chilean salmon aquaculture industry is one of the most developed in terms of tracking antibiotic use and treatment efficacy. All antibiotic treatments in the Chilean aquaculture industry are conducted under the supervision of veterinarians and must have laboratory bacterial confirmation before treatment. These are federal regulatory requirements and the data on antibiotic usage is maintained by the government.

The dominant reason for antibiotic use in salmon in Chile is *Piscirickettsia salmonis* infections during the saltwater grow-out phase of the production cycle (Rozas and Enríquez, 2014). Despite veterinary oversight, treatments are often not effective at reducing mortality associated with this intracellular bacterial pathogen. The poor responses to treatments have resulted in farmers treating crops of fish multiple times with antibiotics. Although antibiotic resistance is one of the possible reasons for treatment failure, a recent study suggests that most

isolates of *P. salmonis* are susceptible to the two most common antibiotics used in this industry: florfenicol and oxytetracycline (Henriquez et al., 2016).

Investigating Antibiotic Treatment Failure

In the salmon industry, similar to other aquaculture industries, almost all antibiotic treatments are administered as in-feed metaphylactic treatments. That is, antibiotics are administered at a population level once a bacterial disease is identified in a cage of fish or on a farm. Antibiotics are given to the entire population, which may include infected and not-yet-infected animals. Metaphylactic treatments are the preferred treatment method in aquaculture for several reasons. First, it is not possible to treat only animals that are infected in a population because all the animals are housed together and are not handled individually. Second, the uninfected animals share the same environment as the infected animals, so in cases of pathogens that are transmitted via the water, it is very likely that at least some subclinical fish are already infected by the time the treatment is administered, and that uninfected animals will become infected if they are not treated. Third, having multiple unsynchronised treatments on a farm, where pathogens are shared between cages, maintains pathogens within the area and may increase pathogen exposure to subtherapeutic levels of antibiotics when the fish have finished treatment and are metabolising the drugs. Last, the automated feeder systems used in large saltwater salmon farms do not easily permit the customisation of feed regimes to individual cages. Therefore, although metaphylactic treatments increase the overall use of antibiotics, they may be the only practical way to effectively target all sources of infection in aquatic animal populations.

The reason metaphylactic treatments fail on salmon farms is likely multi-factorial. We have identified four primary conditions when this could happen for *P. salmonis* treatments, but the rationale may apply to many other bacterial treatment scenarios as well (Fig. 1). First, antibiotic treatments will not work if there is a misdiagnosis and the fish are not infected with a bacterial pathogen. Antibiotics will also not perform well if the fish have a concurrent non-bacterial infection. This may result in the successful treatment of the bacterial issue, but the fish will continue to experience mortality due to the untreated co-infection. There are anecdotal reports of this in the salmon industry when fish are infected with both infectious pancreatic necrosis virus and *P. salmonis*. In these cases, antibiotic treatment failure is likely due to the viral co-infection.

It is also possible that the bacteria have a natural or acquired resistance to the antibiotic. AMR is on the rise globally in all hosts, including humans (Center for Disease Dynamics, Economics & Policy, 2015). In the
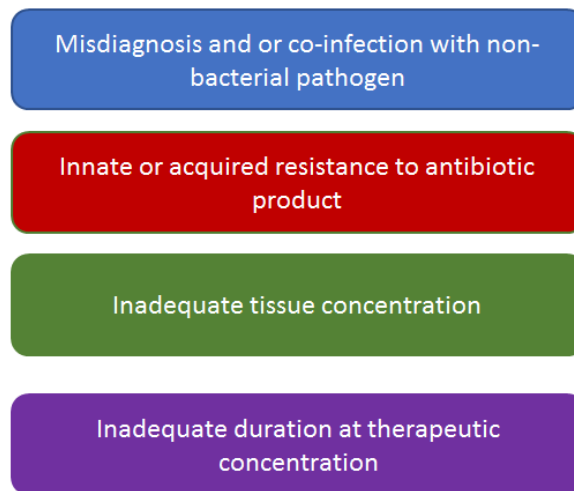
Fig. 1. Conditions for antibiotic treatment failure.

case of *P. salmonis,* AMR has been reported, but it tends to be the exception (Henriquez et al., 2016). Bacteria may also appear tolerant of an antibiotic if they are in a slow-growing or stationary growth phase (Pletnev et al., 2015). Products such as florfenicol and oxytetracycline inhibit peptidyltransferase reaction and protein synthesis respectively (Sekkin and Kum, 2011), so they require the bacteria to be replicating in order to be effective.

The other explanations for treatment failure are associated with sub-therapeutic tissue concentrations and insufficient contact time. The level of antibiotics in treated animals may not be adequate to be therapeutic, and/or the duration of the treatment may not be sufficient to eliminate the bacteria.

Recent studies have confirmed that the level of antibiotics in subclinical fish in treated pens is not always above the minimum inhibitory concentration (MIC) for *P. salmonis,* even on the last day of treatment (Price et al., 2018). Several reasons could account for low levels of antibiotics in tissues (Fig. 2). First, there could be insufficient levels of medication in the feed which could happen due to a feed mixing issue or a biomass miscalculation. The latter could happen because of the variation in fish weights. Some fish may also not be eating enough to receive the appropriate dose. Using in-feed medication to treat a population assumes that all animals will consume feed at a specific proportion of their body weight. Otherwise, it could result in an inadequate drug consumption and subtherapeutic tissue concentrations. This issue would be more problematic for drugs that have a short half-life, such as florfenicol (Martinsen et al., 1993), because they do not accumulate in tissues throughout the treatment. In the Price et al. (2018) study, the proportion of fish with levels of an antibiotic below the MIC was lower for florfenicol than for oxytetracycline.

Fish may also not consume sufficient feed to achieve a therapeutic dose because they are sick and reduce their feed consumption. If it takes a long time to diagnose and treat fish, then a higher proportion of the population will be off fed as the disease progresses. Although farmers in the salmon industry in Chile examine all the fish that die daily, it can take up to two weeks before the farmer initiates an antibiotic treatment on a farm after the initial suspicion of disease. This is due to the delay in obtaining a diagnosis from a laboratory and the time required to manufacture and deliver medicated feed to the farm, some of which are quite remote.

Fish may also not have adequate antibiotic tissue concentrations after treatment because they don't have access to sufficient feed, which can arise if the feeding strategy favours dominant fish. A wide variation in the size of fish from the same year class is one indication there may be hierarchical behavioural issues in a population. Addressing this behavioural issue when it first starts, before the occurrence of health issues, will reduce the negative impact of antibiotic consumption within the population.

The concentration of antibiotics may be sufficient in some tissues but inadequate in others, which could lead to poor treatment success. For example, in the case of florfenicol, the brain and skin often have lower concentrations than the visceral organs (Armstrong et al., 2005). This means that if these are the target organs for a pathogen, which is the case in chronic cases of piscirickettsiosis, then inadequate levels of antibiotics may occur even with a dose that provides therapeutic levels in visceral organs and the bacteria may not be effectively treated. In the case of *P. salmonis*, bacteria also hide within the tissue in granuloma-like lesions, which can further reduce exposure to therapeutic levels of antibiotics.

**Misdiagnosis and or co-infection
with non-bacterial pathogen**
- Fish have a non-bacterial infection
- Fish have both a bacterial and a non-bacterial infection

Innate or acquired resistance to antibiotic
- Natural resistance
- Acquired resistance
- Bacteria tolerant to antibiotic because slow replication phase

Inadequate tissue concentration
- Insufficient amount of antibiotic in the feed
- Specific target tissue not treated
- Fish off feed
- Bacteria hiding inside cells or tissues (granulomas)
- Inadequate dosing frequency for pharmacokinetics of antibiotic used

Inadequate duration at therapeutic concentration
- Large population of animals makes it difficult to consistently consume the appropriate dose to maintain a therapeutic drug concentration over time, especially for antibiotics with short half-lives
- Interruptions in therapy due to unforeseen events makes it difficult to maintain therapeutic concentration over time
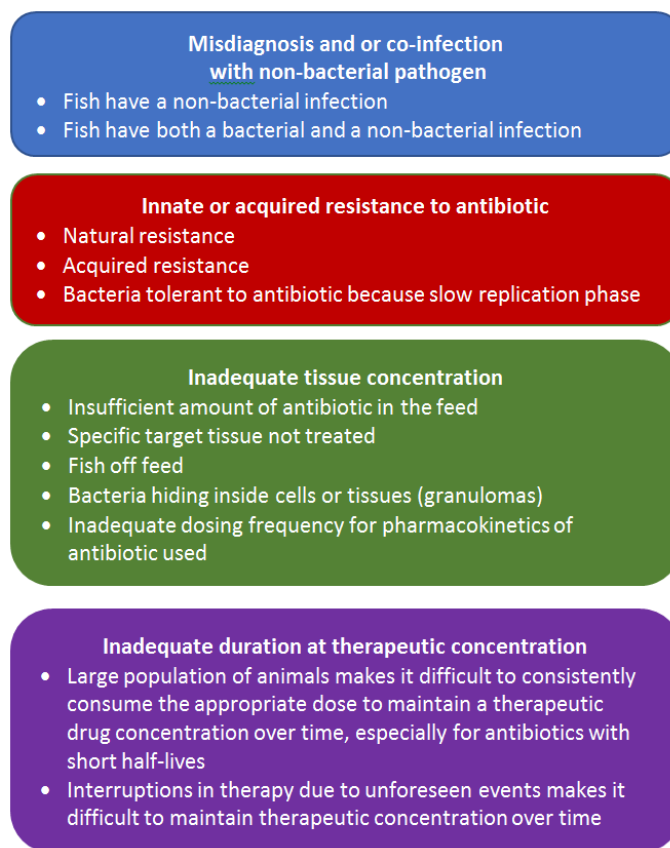
Fig. 2. Specific scenarios that could result in conditions leading to treatment failure.

Finally, even if a fish acquires adequate tissue concentrations of antibiotics, some products, such as oxytetracycline and florfenicol, are time-dependent antibiotics (Sekkin and Kum, 2011) and require drug concentrations to be maintained above the MIC of the bacteria to maximise efficacy. When treating individual animals, it is possible to achieve this contact time by ensuring the dose is taken at specific intervals based on the pharmacokinetics of the drug. For drugs with a short half-life that require frequent dosing, however, it may be difficult to ensure that all fish in a large population feed at precisely the correct interval to maintain tissue concentrations at or above the therapeutic dose. Additionally, the water temperature can further complicate the issue by affecting the pharmacokinetic properties of the antibiotic. No advice is provided on antibiotic labels to adjust for the effect of the water temperature on the tissue concentration and required contact time of products. Not considering this information may be another reason why some treatments are terminated before the entire population has been treated adequately.

On open net-pen farms, there are also instances when treatments are interrupted due to unforeseen reasons resulting in a drop in antibiotic tissue concentration. Reasons for treatment interruptions range from algal blooms to sea lice treatments, storms, predator attacks, and other events that can occur frequently. All these issues provide sources of variation for antibiotic treatments that can lead to inadequate therapeutic levels of drugs in all or a portion of a population.

Consequences of not treating the entire population

The first consequence of the inadequate treatment of the population is treatment failure. If infected fish receive inadequate treatment and remain in the population beyond the treatment period, they can serve as a source of re-infection for the other successfully treated fish on the farm. This is less so the case with acute bacterial diseases where fish succumb to infection within a week of exposure to the pathogen, as moribund fish that are not adequately treated would not remain in the population long enough to serve as a significant source of infection for other fish. Ensuring infected fish are either treated appropriately or removed from the population before the completion of the antibiotic treatment will reduce the frequency of treatment failure on farms.

As well as increasing the chances of treatment failure by inadvertently treating fish at subtherapeutic concentrations, producers may also be increasing the risk of AMR (Van Houweling and Gainer, 1978). If there is a large proportion of fish that do not achieve therapeutic levels of antibiotics for a sufficient period during treatment, then the bacteria may be directly exposed to subtherapeutic drug levels. Perhaps more

significantly, if a pathogenic bacteria is maintained within the fish population because of inadequate treatments and infected fish do not succumb to the disease before the end of the treatment, the infected fish can act as a source of bacteria to re-infect fish while they are metabolising the antibiotic at the end of their treatment period. The re-infection of fish during this critical period could be very significant on a salmon farm with a large number of fish. The period when antibiotic drug concentrations are low, but still detectable, would depend on the half-life of the product and could range from a few hours to days or weeks.

Addressing issues with antibiotic treatments in fish populations

The most effective way to address the issues surrounding metaphylactic antibiotic treatments in aquaculture is to reduce their use through good disease prevention strategies. When treatments are unavoidable, it is critical to ensure that the delivery of antibiotics, both dose and dosing interval, is closely monitored, and that the fish in the population achieve therapeutic concentrations at the site of the target organs for the required period to successfully eliminate the pathogen. Starting treatments early in the disease process, while the fish are still on feed, will improve treatment effect. Farmers should consider hierarchical behaviours within cages and should take into account the pharmacokinetics of different products so that all fish receive an adequate dose during feeding. A study presented at an industry meeting in Chile suggested the frequency of meals may play an important role in distributing feed more evenly within salmonid populations (unpublished). Finally, ensuring that there are no sources of bacteria in the population (i.e. infected fish) once the treatment has ended is key to reducing the exposure of pathogenic bacteria to subtherapeutic levels of drugs and re-infection. The latter requires consideration of population-level pharmacokinetics when determining the duration of treatments in large fish populations.

Conclusion

The long-term impacts of metaphylactic antibiotic treatments on bacterial communities, including pathogens, and animal health in aquaculture are unknown. The fact that the salmon industry, one of the most sophisticated and heavily regulated aquaculture industries globally, has issues with antibiotic treatment failures suggests that this problem is likely to exist in other aquaculture industries and that better guidelines for the use of antibiotics are required. Identification of specific reasons for the treatment failures for different scenarios, as well as factors associated with these failures, will enable veterinarians to take corrective measures. It will also help identify circumstances or practices leading to treatments that may increase the

risk of AMR. It is imperative to investigate the practices that maximise the efficacy of antimicrobial treatments while minimising AMR if we are to provide effective guidelines for practitioners and producers to mitigate this growing problem.

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