FELLOWSHIP SUMMARY REPORT

GENERAL DATA

"**Antimicrobial resistance in Atlantic salmon (*Salmo salar* L.) from salmon aquaculture in Chile"**

- Tania Pérez Sánchez -

Theme II - Managing Risks in a Connected World
Antimicrobial Resistance and Food Safety

Host institution - Universidad Austral de Chile (Chile)
Instituto de Medicina Preventiva - Facultad de Ciencias Veterinarias
Host collaborator – PhD Carmen Lopez-Joven

Dates of fellowship – 24/03/2018 to 05/05/2018

I consent that this report could be posted on the Co-operative Research Programme's website
Tania Pérez Sánchez

1. Objectives and importance of this research project

Aquaculture is an increasingly important activity that requires large-scale production facilities, high-density animal populations, which provide ideal conditions for the emergence and spread of infections, thereby causing severe economic losses (Pérez-Sánchez *et al*., 2018). Antimicrobials have been commonly used as prophylactic and therapeutic agents to control these infections, although its extensive use has generated the selection, persistence and spread of antimicrobial-resistant bacteria (Cabello *et al*., 2013).

Antimicrobial resistance (AMR) has become a major public health concern, because antimicrobial-resistant bacteria may compromise the effectiveness of antimicrobial therapy. In fact, several studies have been conducted to assess the emergence and prevalence of AMR in clinically relevant pathogens and other studies have also paid attention to antibiotic resistance genes (ARGs) released into the environment (Marti *et al*., 2014). Although there is a clear evidence that the use of antimicrobials could have negative effects on the environment as the normal microbiota can be altered and enriched with ARGs (Proia et al., 2016), limited information is available on the role of farmed animals in the ecology and evolution of AMR.

Knowledge and scientific evidence on the complex ecology of AMR and interactions between bacterial communities, pathogens and commensals, the food chain and the environment is needed (http://effort-against-amr.eu). This research project was thus designed to achieve a deeper knowledge about AMR in Chilean aquaculture that it is not only important for veterinary purposes, but also for human health. Chile is the second largest producer of farmed salmon and trout in the world after Norway (FAO, 2014). And taking into account, the emergence of this kind of resistance among pathogenic bacteria is considered one of the biggest threats to Public Health, in this research project we tried to understand some aspects related to this relevant issue. Specifically, this investigation was proposed to determine risk factors associated with this resistance in salmon Chilean aquaculture, after the analysis of AMR in farmed salmon.

2. Achievement of the Objectives

The main objective of this research project was to study the presence and diversity of ARGs in intestinal samples of Atlantic salmon (*Salmo salar*). In order to achieve this goal, the following specific objectives were:

- *To compare the presence and diversity of ARGs in fish intestinal samples from different locations.*

  First of all, a salmon farm from southern Chile with antibiotic treatment was chosen to obtain the samples. Intestinal mucus from Atlantic salmon (*Salmo salar* L.) were selected to study ARGs. Genomic DNA was then extracted using the DNeasy Blood & Tissue Kit (Qiagen, USA) and the final concentration was determined using a spectrophotometer (MaestroGen).

Complementarily, total RNA was precipitated using TRIzol Reagent. Then, RNA samples were stored in 75% ethanol at – 80ºC to gain some insights on immune response. Additionally, intestinal samples were stored for future analyses.

According to the experience of the researchers of the Chilean University and the antibiotics that are intensively used in Chilean fish farms, real-time PCR (qPCR) assays were carried out to quantify genes conferring resistance to β-lactams (bla_{CTXM}), florfenicols (*floR*), sulfonamides (*sulI*) and tetracyclines (*tetA*). In addition, *16S rRNA* and *EF-1* were added in this study.

Each gene (*floR, tetA, sulI,* bla_{CTXM}*, 16S, EF-1*) was amplified using a specific primers set. After each qPCR assay, a dissociation curve was constructed by increasing the temperature from 65 to 95 °C in order to confirm the specificity of the amplified products. In this point, we had some technical problems as the sensitivity of qPCR was not good, so copy number of each gene studied could not be quantified. We think that the main reason of this technical problem was one of the reagent (MasterMix) used (MasterMix brand was not the same as the thermocycler recommendations).

Although previous results were not useful, we will improve qPCR reactions to verify preliminary results. Then, we will do the data analysis. Unfortunately, 6 weeks was a limited time, but we have good DNA samples to gain some insights about the presence and diversity of ARGs in fish intestinal samples.

Additionally to the objectives proposed for this project and taking into account that the concentration and quality of DNA extracted from mucus intestinal samples was good, interactions between bacterial communities could be analyzed. This research could be conducted by molecular and bio-informatics tecnologies (massive sequencing *16S*).

- *To determine risk factors associated with antimicrobial resistance in salmon aquaculture.*

  In veterinary medicine, the use of antimicrobials has improved animal health, welfare and the efficiency of production. However, the overuse leads to selection of bacteria resistant against the substance used. When we proposed this project, we thought that if we could determine the occurrence and abundance of ARGs, it would be possible to establish some guidelines of intervention strategies related to the use of antibiotics. For example, the withdrawal period expressed as degree-days for aquatic species (set by each substance in accordance is meat for human consumption) should consider also the presence of AMR in fish.

  These criteria could be extended to the rest of species produced for consumption.

3. Major achievements

In a summarized way, the development of this research project has achieved the following goals:

- *Understanding antimicrobial resistance in Chilean aquaculture*
  - Selection of resistant genes according to therapeutic strategies
  - Presence and quantification of resistant genes

- *Ecological consequences of antibiotic intervention strategies*
  - Antimicrobials used as prophylactic measures have negative impacts in the environment
  - Impact of antimicrobial resistance in the food chain

4. Follow-up work

During this fellowship we discussed with the collaborator researchers involved in this project about the need of writing a review or opinion article about the use of antibiotics in Chilean and Spanish salmonids aquaculture to lay the groundwork for this project and future research. This manuscript could be published in a peer-reviewed scientific journal. Recently, we published an opinion article about the biological approaches for disease control in aquaculture in *Trends in Microbiology* - a topic related to antimicrobial resistance - so this journal could be an option for this publication.

Also, we have planned to write a manuscript with the results of the occurrence and abundance of ARGs in salmon samples, once we could repeat qPCR reactions both in the host institution (Universidad Austral de Chile) and in the home university (Universidad de Zaragoza).

Recently, we are thinking about the possibility of applying for a project of the INNOGLOBAL programme to cooperate internationally. This programme is for small and medium size enterprises (SMEs) and there is a Spanish SME (Pentabiol) working in animal nutrition interested in the development of a product for salmonids that could reduce the use of antibiotics. These days we got in touch with the national project coordinator in Spain (CDTI) to know if the Universidad Austral de Chile could be the international partner to process with the application.

5. Importance of the research project for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society

Chile is one of the countries where salmon aquaculture is an exponentially growing industry, but due to few available vaccines, the continuous high prevalence of bacterial infections and the presence of antimicrobial-

resistant pathogens and the emergence of new pathogens has stimulated the use of large quantities of antimicrobials (Asche et al., 2010; Ibieta et al., 2011; Millanao et al., 2011; Cabello et al., 2013).

With this project we tried to understand the impact of antimicrobial resistance in the food chain to establish specific measures aimed at reducing the emergence, spread and transmission of this phenomenon. The importance of the results is not only for this area where we have performed the first analysis, but understanding the impact of the use of antimicrobial agents it is beneficial for society.

World Health Organization (WHO) has classified the emergence and spread of antibiotic resistant bacteria as one of the biggest threats to Public Health in the 21st century. According to a report from the UK Review on Antimicrobial Resistance (AMR), 700,000 people die every year from infections due to resistant organisms, with numbers expected to rise to over 10 million by 2050 (http://amr-review.org) if steps are not taken.

We think if we could perform similar analysis with the methodology developed for this project, it would be possible to know when resistance appears, what kind of antibiotics has a negative impact with the aim of planning intervention strategies.

Thanks to the opportunity to do this research in Chile together with the researchers involved, we got in contact with one of the main companies of animal nutrition for aquatic species (Skretting, Puerto Montt, Chile) that it is interested in the study we have started, so we could repeat this investigation in different sites whether in Chile or Spain.

6. Relevance of the Project according to the objectives of CRP and research Theme

In Theme II of the Co-operative Research Programme - *Biological Resource Management for Sustainable Agricultural Systems* - we found *Antimicrobial resistance* as one of the risks that should be managed in a connected world. The emergence and spread of antimicrobial resistant pathogens has raised concerns for Public Health.

Antimicrobials are used to treat infectious diseases not only in human medicine but also in animal husbandry like aquaculture. These agents in salmon are usually administered with food, therefore the additional quantity of food that is not consumed by fish or fish feces containing unabsorbed antimicrobials and secreted metabolites in the water and sediment, often retain their antimicrobial activity and can remain for variable periods of time (Buschmann *et al*., 2012).

One of the pillars of this Co-operative Research Programme in Theme II – *Managing risks in a connected world* is *Food Safety*, and this project falls also within this issue. Actions aimed at ensuring that food is as safe as possible are needed.

Scientific community has proved that the extensive use of antibiotics in animal feeding causes crossed resistance to a wide spectrum of substances. This crossed resistance is one of the current global medical challenges, then Food Safety Agencies are continuously restricting the use of antimicrobials in animal feeding

Knowledge on the ecology and transfer of AMR throughout the food chain remains incomplete and scientific approaches are needed to provide this evidence (http://effort-against-amr.eu). In this study we analyze antimicrobial resistance to determine risk factors associated with this resistance in salmon Chilean aquaculture with the objective of ensuring that food (from production to consumption) is as safe as possible.

This proposal also relates to Theme I - *Managing natural capital for the future* - in which aquaculture and fisheries are considered, so we think that with this fellowship we have started a collaboration (Chile and Spain) to develop a multidisciplinary investigation.

7. Satisfaction

This fellowship has been a great opportunity for my professional career, both as a fish veterinarian and as a researcher. Chile is a country where aquaculture is one of the most important economic activities and knowing the production system with the help of the researchers, particularly the host collaborator (Dr. Lopez Joven), at the Austral University has been a fantastic experience.

The Chilean laboratory and our research group in Spain have lot of points in common and thanks to this *OECD* fellowship we are sure that this collaboration will continue in the future. Our research group in Spain has been focused on the search of alternatives to the use of antimicrobial agents, mainly in aquaculture, and we consider that these better friendly practices to manage diseases could be studied in salmon Chilean aquaculture. Also, these strategies are easier to develop in Chile than in Europe, as oral vaccines are the usual way to prevent diseases, so we have emerged different possibilities of study that hopely we could put in practice throughout new projects.

My expectations for this stay at the Austral University have been conformed and the participation in this co-operative programme has been an excellence opportunity for my research. Also, as a fish vet I would highlight the possibility of knowing the main diseases that salmon aquaculture has to deal as well as the different ways to prevent and treat pathologies that could be useful for Spanish trout farms. Although we had some technical problems that have been previously pointed out, we would try to arrange in the following weeks.

8. Advertising the Co-operative Research Programme

I learnt about the Co-operative Research Programme in my university (University of Zaragoza – Spain), specifically in our online news journal. After that, in the webpage (http://www.oecd.org), I could learn all the steps needed for the application.

From my point of view, the programme would be more visible if in social networks information directly related to OECD fellowships and the specifications of this programme will be published.

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