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ORIGINAL ARTICLE

No evidence for a relationship between farm or transformation process locations and antibiotic resistance patterns of *Pseudomonas* population associated with rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Aims: Study the relationship between antibiotic resistance patterns of *Pseudomonas* isolated from farmed rainbow trout fillets and farm or transformation process locations.

Methods and Results: *Pseudomonas* strains were isolated from rainbow trout sampled in two differently located farms and filleted in laboratory or in a processing factory. One hundred and twenty-five isolates were confirmed as belonging to *Pseudomonas* using CFC selective media, Gram staining, oxidase test and quantitative polymerase chain reaction methods. Fifty-one isolates from separate fish fillets were further identified using MALDI-TOF mass spectrometry, and the minimal inhibitory concentrations (MIC) of 11 antibiotics were also determined by microdilution method. Most of the isolates belonged to the *Pseudomonas fluorescens* group (94.1%), and no relationship was established between antibiotic resistance patterns and sampling locations (farms or filleting areas). Multiple resistance isolates with high MIC values (from 64 μg ml^{-1} to more than 1024 μg ml^{-1}) were identified.

Conclusions: Antibiotic resistance patterns found in *Pseudomonas* isolates were not influenced by farms or transformation process locations. Seven isolates were found highly resistant to four different antibiotic classes.

Significance and Impact of the Study: This study does not provide evidence of a relationship between farm or transformation process locations on antibiotic resistance patterns of *Pseudomonas* population.

KEYWORDS

antimicrobials, aquaculture, pseudomonads, rainbow trout, resistance

Introduction

Fish farms are interfaces between aquatic environments and food-producing animals whose bacterial ecosystems are exposed to effluents from human activities (e.g. effluents from wastewater treatment plants, pastures) and to antibiotics used for the treatment of fish diseases (Woolhouse & Ward, 2013). Therefore, fish foodstuffs have been considered as potential reservoirs of antibiotic-resistant bacteria. However, the contamination sources—such as farming practices (including environmental contamination associated with the farming environment and the slaughtering process)—and the filleting processing types have rarely been considered. In Europe, rainbow trout is one of the most produced farmed fish; its production must comply with strict regulations, especially on the use of antibiotics. Limited to a few classes, these molecules can only be used for the treatment of a fish disease following a veterinary prescription. In France, the six antibiotics with marketing authorizations for the sole treatment of fish diseases are florfenicol, flumequine, oxolinic acid, oxytetracycline (OTC), sulphadiazine and trimethoprim. Antibiotic resistance genes have been described in bacterial ecosystems associated with rainbow trout samples, in skin, gut (Muziasari et al., 2017) and fillets samples (Helsens, Calvez, Bouju-Albert, et al., 2020), suggesting that these antibiotics——whether from effluents or fish treatment—play a role in the selective pressure related to the emergence of antibiotic-resistant bacteria.

The genus *Pseudomonas* is considered as the most diverse and ecologically important bacterial group. It has an outstanding ability to survive in different types of habitats (soil, freshwater and marine environments) and to grow well at cold temperatures (as low as 4°C). As such, *Pseudomonas* are frequently found in aquatic samples (Akinbowale et al., 2007; Gordon et al., 2006; Miranda & Zemelman, 2002), and in the skin and gut microbiota of farmed rainbow trout (Lowrey et al., 2015; Wong et al., 2013; Zhang et al., 2018). As a result of their ability to persist in processing environments, they can be found in the bacterial communities of fresh fish fillets (Helsens, Calvez, Bouju-Albert, et al., 2020; Langsrud et al., 2016; Møretrø et al., 2016). The *Pseudomonas* genus is composed of more than 200 ubiquitous species (Peix et al., 2009, 2018) classified into several groups, such as the *P. fluorescens* group which is composed of more than 50 species (Garrido-Sanz et al., 2017). Various species can cause fish diseases, like *Pseudomonas anguilliseptica* (Wiklund & Bylund, 1990), *P. aeruginosa* (Algammal, Mabrok, et al., 2020) and *Pseudomonas putida* (Altinok et al., 2006), but non-human pathogenic *Pseudomonas* species have also been described in samples from aquatic environments and animals (Boutin et al., 2014; Luczkiewicz et al., 2015). Among bacterial pathogens, *P. aeruginosa* is recognized as the main cause of septicaemia in freshwater fishes (Roberts, 2012). Its pathogenicity is often due to several virulence genes as *oprL* or *toxA* coding for outer-membrane protein and exotoxin A respectively (Algammal, Mabrok, et al., 2020). *Pseudomonas fluorescens* is scarcely involved in diseases in French aquaculture but some cases have been reported in several fish species whom histopathological aspects have been described (Miyazaki et al., 1984). More recently, authors have identified several iron-acquisition proteins involved in bacterial virulence (Sun & Sun, 2015). At least, an experimental infection of rainbow trout carried out with *P. fluorescens* has shown a mortality rate of 40% at an infectious dose of 10^8 CFU per ml (Dinçtürk & Tanrıkul, 2021).

Bacterial multidrug resistance increase globally and is considered as public health threat (https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance). Antibiotic-resistant *Pseudomonas* isolates have been found in samples from diseased farmed fish such as catfish (Nguyen et al., 2014), *Oreochromis niloticus* and *Clarias gariepinus* (Algammal, Mabrok, et al., 2020), in influents and effluents of wastewater treatment plants (Luczkiewicz et al., 2015), in river water (Gordon et al., 2006) and in sediment from rainbow trout farms (Akinbowale et al., 2007; Ishida et al., 2010). Furthermore, they possess mobile genetic elements and can transfer antibiotic resistance determinants to various non-pathogenic and pathogenic *Pseudomonas* species (Cazares et al., 2020; Kottara et al., 2018; Molina et al., 2014; Yomoda et al., 2003) and to pathogenic species from other genera like *Escherichia coli* (Dominguez et al., 2019). Several previous studies revealed the emergence of multidrug-resistant bacterial pathogens from different origins especially fish, warranting proper use of the antimicrobial agents in both health and veterinary sectors (Abolghait et al., 2020; Algammal, Mohamed, et al., 2020; El-Sayed et al., 2019; Yang et al., 2018).

In a context admittedly characterized by the presence of antibiotic-resistant *Pseudomonas* species in aquatic samples, this work aimed at determining whether the location of farm on a same river and the transformation process area (laboratory vs. factory facility) has an effect on the antibiotic resistance patterns of *Pseudomonas* isolates sampled from rainbow trout fillets. The isolates were tested against a selection of antibiotics including those used for the treatment of fish diseases in France, and various other antibiotics of clinical interest in human therapeutics. The following results constitute a first approach toward the characterisation of antibiotic-resistant *Pseudomonas* isolates according to the sampling location of farmed rainbow trout.

MATERIALS AND METHODS

Fish and fillet sampling

Rainbow trouts were sampled from two farms (A and B) located on the same river in Brittany. Farm A was located at the upstream of an urban area and the other one at the downstream. The farm owners were recruited on a voluntary basis according to the experimental design of Helsens, Calvez, Prévost, et al. (2020). In this previous study, a questionnaire filled by farmers identified that only Farm B had used an antibiotic treatment, either one OTC treatment 27 weeks before the sampling date. Twenty-eight fish were randomly collected from the raceway environment of each farm; 14 were filleted in the laboratory, and the other 14 were filleted in a processing factory. All fish and fillets were individually packed in plastic bags and stored on melted ice (0°C) prior to processing in laboratory (6 h of handling). In laboratory, each fillet was portioned under sterile conditions to obtain at least 30 g of tissue with the most uniform possible flesh-to-skin ratio. Thirty grams from one fillet from one rainbow trout were considered as one sample. Each fish sample (1–28) was encoded with two letters indicating the original farm (A or B), and the filleting location (R for raceway/laboratory; F for factory) The 56 samples were vacuum packed individually in plastic bags and stored at −20°C until analysis.

Total mesophilic bacteria count and *Pseudomonas* isolation

Each 30-g sample was transferred to a 63-µm stomacher bag (Interscience) with 50 ml of phosphate buffer saline containing 5% TWEEN® 80 (Sigma-Aldrich). Bacterial suspensions were obtained by the rinsing method according to Helsens, Calvez, Bouju-Albert, et al. (2020). The filtered rinse solution was collected from the stomacher bag for enumeration of total viable mesophilic aerobic count (TVMC) as an indicator of fish fillet quality and good manufacturing processing (Özyurt et al., 2015).

Enumeration was performed using a 10-fold serial dilution of the rinse solution (10^{-1}, 10^{-2}) spread onto brain heart infusion (BHI) agar plates (Difco™, VMR USA) and incubated at 20°C for 48 h. Colonies were initially quantified using eCOUNT™ (Heathrow Scientific).

Presumptive *Pseudomonas* strains were isolated according to the ISO 13720 standard (International Organization for Standardization, 2010). For each sample, 400 µl of the fillet rinse solution were collected and spread onto two plates of selective cephalothin sodium fusidate cetrimide (CFC) agar (Merck Millipore), and then incubated at 25°C for 48 h. A maximum of three yellow and other light-coloured colonies were selected *per* sample and tested for their Gram and oxidase properties. The bacteria that appeared as Gram-negative rods and showed a positive reaction for oxidase were considered as presumptive *Pseudomonas* spp. Each selected isolate was grown on BHI agar (BHI, VWR Chemicals Prolabo) to obtain pure cultures. For each isolate, one colony was inoculated in 10 ml of BHI broth and incubated overnight at 25°C. Then, each bacterial suspension was cryopreserved by adding 20% of glycerol, and stored at −80°C.

DNA extraction and confirmation of the *Pseudomonas* genus by quantitative polymerase chain reaction

The isolates were recovered on BHI agar plates and incubated at 30°C for 24 h. One colony was sub-cultured in BHI broth overnight at 25°C under stirring at 180 rpm. One millilitre of this culture was centrifuged at 6000 *g* for 5 min in a 1.5-ml microtube. The supernatant was removed, and the cell pellet was suspended in 100 µl of sterile water and boiled at 95°C for 3 min in a water bath. The boiled cell suspension was centrifuged at 6000 *g* for 5 min, and the supernatant containing DNA was transferred into a new microtube. These DNA samples were stored at −20°C until use. Each DNA sample was diluted 100-fold in a total water volume of 500 µl and used as a template for quantitative polymerase chain reaction (qPCR) amplification.

qPCR amplification was performed on 143 isolates to confirm their *Pseudomonas* affiliations. Primers Pse 435F (5′-ACTTTAAGTTGGGAGGAAGGG-3′) and Pse 449R (5′-ACACAGGAAATTCCACCACCC-3′) targeting the 16S rRNA V3–V4 region were used to amplify a specific 251-bp fragment (Bergmark et al., 2012). The reaction was carried out in a final volume of 20 µl containing 10 µl of 2 X Sso Advanced universal SYBR Green Supermix (Bio-Rad), 0.2 µl of each primer at 50 µM, 4.6 µl of water, and 5 µl of 1/100 diluted DNA sample. A *P. fluorescens* CIP 69.13T DNA sample was used as positive control, and water was used as a no-template control. The amplification reaction was conducted using a Bio-Rad CFX connect real-time PCR detection system (Bio-Rad). The cycling parameters were as follows: initial denaturation step at 98°C for 3 min, 39 cycles of 98°C for 10 s and 60°C for 30 s. A melting curve from 55 to 95°C was determined after the last amplification cycle and at a temperature transition rate of 0.5°C/5s. Quantification cycles (Cq) values were automatically obtained from the Biorad CFX Manager software program.

Identification of the *Pseudomonas* group

Among all the *Pseudomonas* isolates confirmed by qPCR, 51 isolates were randomly chosen to represent each fish fillet positive for this genus. The isolates were recovered on MH agar plates and sent at ambient temperature to LABOCEA lab (https://www.labocea.fr/) for MALDI-TOF analysis. Each isolate was deposited in a 96-well plate and recovered with 1 μl of 70% formic acid for fast extraction. One microlitre of co-crystallizing matrix solution containing α-cyano-4-hydroxycinnamic acid (HCCA) solubilized in acetonitrile (50%, v/v), ultra-pure water (47.5%, v/v) and trifluoroacetic acid (2.5%) was added to each deposit. Once dry, each MALDI target was introduced in the mass spectrophotometer for analysis. The spectra were generated and analysed by a MALDI-TOF Microflex™ system (BrukerDaltonics, Germany) using the flexControl and MBT Compass software programs. The criteria for successful identification were within a confidence score of ≥2.0 for species confirmation and ≥1.7 for genus confirmation.

Antimicrobial susceptibility test

Minimum inhibitory concentrations (MICs) were determined by the microdilution method in cation-adjusted Mueller-Hinton broth (CAMHB) (Oxoid) according to the MIC guidelines of the Clinical and Laboratory Standards Institute (CLSI M07-A10 [2015], M100S-26th [2016], VET03/VET04-S2 [2014]). The 51 *Pseudomonas* isolates identified by MALDI-TOF mass spectrometry were tested against 11 antibiotics purchased from Sigma-Aldrich (Merck) and listed in Table 1. Six molecules subjected to the European marketing authorisation for the treatment of fish bacterial infections were tested: florfenicol (1–128 μg ml^{-1}), flumequine (2–256 μg ml^{-1}), oxolinic acid (0.0625–8 μg ml^{-1}), OTC (0.5–64 μg ml^{-1}), and the sulfadiazine/trimethoprim association (0.5/9.5 to 64/1216 μg ml^{-1}). In the absence of interpretive criteria for sulfadiazine/trimethoprim, sulfamethoxazole was used to detect phenotypic resistance to sulfonamides. Four antibiotics used in human treatment were tested according to the CLSI M100 referential: colistin (1–128 μg ml^{-1}), piperacillin (8–1024 μg ml^{-1}), gentamicin (2–256 μg ml^{-1}) and ceftazidime (4–512 μg ml^{-1}). Azithromycin was chosen to represent macrolides and was used from 8 to 1024 μg ml^{-1}. The concentrations corresponded to the CLSI recommendations (2016) for non-Enterobacteriaceae bacteria including *Pseudomonas* spp. non-*aeruginosa* for piperacillin, ceftazidime, gentamicin, colistin and the trimethoprim-sulfamethoxazole association (CLSI M100). For oxolinic acid, OTC and florfenicol, the recommended concentrations for *Aeromonas salmonicida* were tested according to the CLSI VET 03 reference database. No indication was available for azithromycin and flumequine, so the concentrations were chosen according to Boss et al. (2016) with azithromycin on *P. aeruginosa* and to Scarano et al. (2018) with flumequine on *Aeromonas* spp. Eight isolates presenting highly resistant profiles for some antibiotics were tested at higher concentrations (Table 1).

Stock solutions of antibiotics corresponding to 20× concentrations of the maximum concentrations tested in the MIC experiments were prepared in their appropriate diluent solutions and kept at 4°C. They were extemporaneously diluted to 2× in CAMHB. One hundred microlitres of the 2× solutions were placed in the first well of a 96-U-well plate. Fifty microlitres were sampled and serially diluted in 50 μl of CAMBH in the following 7 wells in order to test eight concentrations for each antibiotic. Bacterial suspensions were prepared from colonies cultured on Mueller Hinton agar plates at 30°C for 24 h for the *Pseudomonas* spp. isolates and at 37°C for the quality control (QC) *E. coli* ATCC 25922. The suspensions were adjusted at an optical density at 600 nm

TABLE 1 Antibiotic concentrations (μg ml^{-1}) tested on the 51 isolates of *Pseudomonas* spp

	***Pseudomonas* spp.**	**Highly resistant profile *Pseudomonas* spp.**	***Escherichia coli* ATCC 25922**
Oxytetracycline	0.5–64	32–512	0.25–4
Oxolinic acid	0.062–8	4–128	0.016–0.25
Florfenicol	1–128	64–1024	0.5–8
Trimethoprim/sulfamethoxazole	0.5/9.5–64/1216	32/608–512/9728	0.031/0.594–0.5/9.5
Flumequine	2–256		0.062–1
Colistin	1–128	64–1024	0.125–2
Piperacilline	8–1,024		0.250–4
Ceftazidime	4–512		0.031–0.5
Gentamicin	2–256		0.062–1
Azythromycin	8–1024		

(OD_{600}) of 0.13–0.14 for the *Pseudomonas* spp. isolates and at 0.1 OD_{600} for the QC to obtain suspensions containing 10^8 CFU per ml. The suspensions were diluted to 10^6 CFU per ml in CAMBH, then 50 μl were distributed in each well containing the antibiotic to be tested. The positive control was a mix of 50 μl of CAMBH and 50 μl of inoculum suspension. The negative control contained 100 μl of CAMBH.

The 96-U-well plates were sealed with Parafilm®. *Pseudomonas* spp. plates were incubated at 28°C for 24–28 h, and the QC plate was incubated at 35°C for 16–20 h, both under stirring at 180 rpm. The bacteria in the inoculum suspension were counted on BHI agar plates. MICs were determined as the lowest concentrations with no bacterial or hardly any bacterial development compared to the positive control.

No MIC breakpoints were available for OTC, florfenicol, flumequine and oxolinic acid. For this reason, we calculated ECOFF (Epidemiological Cut-Off) values using ECOFF Finder software (Turnidge et al., 2006). These values determined within the *Pseudomonas* population isolated in our study were used to check for the presence of isolates possessing atypical (or non-wild-type) phenotypes compared to the wild-type population.

Statistical analyses

Nonparametric data were analysed using a Wilcoxon Mann–Whitney test in the R environment (version 4.0.3). The e1071 package (Meyer et al., 2021) was used for statistical analyses. The ggplot2 (Wickham, 2016) and ggpubr (Kassambara, 2020) packages were used for the graphic design of the statistical test. A multiple correspondence analysis (MCA) was performed using the FactoMineR 2.4 and factoextra 1.0.7 packages to visualise and summarise the dataset built with *Pseudomonas* isolates as individuals and the active variables that described them (e.g. antibiotic resistance phenotypes). The aim was to assess the relationship between the antibiotic resistance patterns and the sampling locations of *Pseudomonas* isolates (farm and filleting locations). Within categorical variables, phenotypes for antibiotics with available clinical breakpoints were defined as follows: (i) susceptible: strains susceptible at the standard dose, and also "intermediate" strains newly proposed as susceptible when exposed to increased doses of antibiotics (European Committee on Antimicrobial Susceptibility Testing [EUCAST], 2019), (ii) resistant and (iii) highly resistant strains that we defined with MICs at least 32-fold the ones of the clinical breakpoint. For antibiotics without a clinical breakpoint, two phenotypes (wild type; atypical/non wild type) were defined according to the calculated ECOFF values of the total population. The number of resistances to different antibiotics were used as quantitative variables, and two illustrative variables (farm and filleting location) were also considered in the MCA. Confidence ellipses (95%) were built to exhibit significant differences between the farming/filleting locations and the antibiotic resistance patterns of *Pseudomonas* isolates.

RESULTS

Total aerobic bacteria count and *Pseudomonas* isolation

The measurement of total viable mesophilic aerobic bacteria is mainly used as an indicator of fish fillet quality and is very helpful to determine the presence of spoiler bacteria when values are beyond 7 log CFU per g. In this study, TVMCs ranged from 2.19 log CFU per g (farm A) to 3.33 log CFU per g (farm B) in rainbow trout fillet samples (Figure 1). A significant difference ($p < 0.0001$) was highlighted between the two farms whatever the filleting location (laboratory or factory). On CFC medium, 154 isolates with yellow and other light-coloured colonies phenotypes, Gram-negative aspects and oxidase activities were considered as presumptive *Pseudomonas* (80 isolates from farm A; 74 from farm B). Then, 54 isolates (farm A) and 71 isolates (farm B) were confirmed as belonging to the *Pseudomonas* genus by qPCR. After these confirmations, 51 out of 56 fish fillet samples were considered as *Pseudomonas*-positive. We randomly chose one isolate *per* fish fillet, distributed as follows: 12 and 13 isolates from fish samples from farm A (and 14 and 12 from farm B) filleted in the laboratory or in the factory respectively. These 51 isolates were further analysed for identification and assessment of the MICs of different antibiotics.

Identification of *Pseudomonas* isolates using MALDI-TOF mass spectrometry

Conventional approaches (phenotypical and biochemical characterization, selective media and PCR) did not allow us to determine the affiliation of the *Pseudomonas* isolates within the different phylogenetic groups or many species. However, molecular approaches (as MLST or whole genome sequencing) for group or species identification of *Pseudomonas* isolates are available. In our study, we have chosen to use a faster approach based on the identification of proteins profiles of each isolates compared to a database of known micro-organisms. The 51 *Pseudomonas* isolates were identified using MALDI-TOF mass spectrometry associated with the Bruker database (Table 2). All isolates were confirmed as belonging to the *Pseudomonas* genus

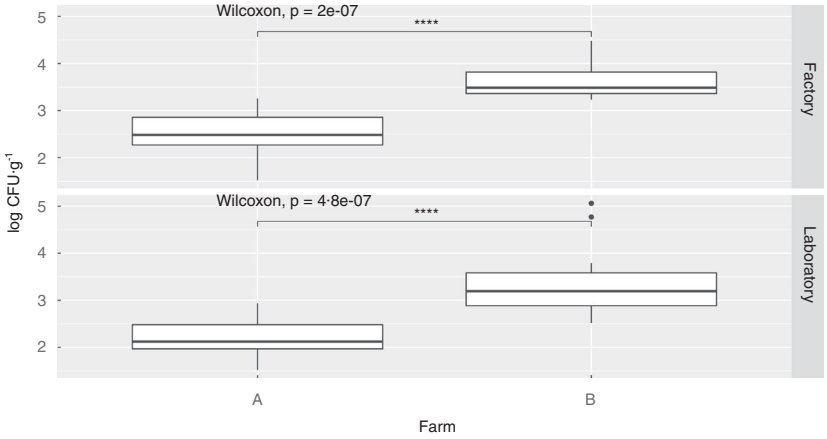

FIGURE 1 Enumeration of total viable mesophilic aerobic bacteria (log CFU per g) on fish fillets sampled in two farms (A and B) and according to the filleting location (laboratory versus factory). Significant TVMC differences between the farms were tested using a non-parametric Wilcoxon test ($\alpha = 0.05$)

TABLE 2 Distribution of *Pseudomonas* isolates from two different farms and filleting locations

	FARM A (*N*)		FARM B (*N*)		
Group affiliation	**Laboratory**	**Factory**	**Laboratory**	**Factory**	**Total (%)**
P. fluorescens	11	12	13	12	94.1
P. lutea	1	0	1	0	3.9
P. syringae	0	1	0	0	2.0

Note: N, number of isolates.

TABLE 3 Distribution of the MICs of 11 antimicrobial drugs for *Pseudomonas* isolates from two rainbow trout farms and filleting locations

Antimicrobial drug	Number of isolates with a MIC (μg.mL⁻¹) of													Interpretation (%)		
	< 0.5	1	2	4	8	16	32	64	128	256	512	1,024	> 1,024	S	I	R/NS
Oxytetracycline	8	16	20	2	0	0	0	3	0	2	0	0	0	-	-	-
Florfenicol	0	0	0	1	3	4	14	20	4	0	2	0	3	-	-	-
	< 1	1	2	4	8	16	32	64	128	256	512	1,024	> 1,024			
Colistin	3	0	37	1	1	2	5	0	0	0	1	0	1	76.5	2.0	21.6
	-	< 2	2	4	8	16	32	64	128	256	512	1,024	> 1,024			
Flumequine	-	3	0	12	29	5	2	0	0	0	0	0	0	-	-	-
Gentamicin	-	51	0	0	0	0	0	0	0	0	0	0	0	100.0	0.0	0.0
	-	-	< 4	4	8	16	32	64	128	256	512	1,024	> 1,024			
Ceftazidime	-	-	13	0	18	6	9	4	1	0	0	0	0	60.8	-	39.2
	-	-	-	< 8	8	16	32	64	128	256	512	1,024	> 1,024			
Piperacilline	-	-	-	23	0	21	4	3	0	0	0	0	0	86.3	13.7	0.0
Azithromycin	-	-	-	5	0	2	11	5	12	11	5	0	0	-	-	-
	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128	> 128			
Oxolinic Acid	0	0	0	1	8	25	15	2	0	0	0	0	0	-	-	-
	0.5/9.5	1/19	2/38	4/76	8/152	16/304	32/608	64/1,216	128/2,432	256/4,864	512/9,728	> 512/9,728	-			
Trimethoprim / Sulfamethoxazole	4	17	9	14	2	0	0	0	2	1	0	2	-	58.8	-	41.2

Note: Vertical dark lines: breakpoints used with the following sequence of interpretation: S = susceptible; I = susceptible, increased exposure; R = resistant/NS = non-susceptible; Vertical white lines: calculated ECOFF values using the ECOFF Finder program with 95% endpoint. Grey areas, range of antimicrobial concentrations used during the tests.

with a confidence score greater than 1.7, and 33 isolates (64.7%) were confirmed at the species level with a score ≥2.0. Most of the isolates belonged to the *P. fluorescens* group ($n = 48/51$), and to a far lesser extent to the *Pseudomonas lutea* ($n = 2/51$) and *Pseudomonas syringae* ($n = 1/51$) groups.

Antibiotic resistance of the *Pseudomonas* isolates

The MICs of 10 antibiotic compounds were assessed on all 51 *Pseudomonas* isolates (Table 3). As regards antibiotics commonly used in aquaculture, the

trimethoprim/sulfamethoxazole association was the only one with a clinical breakpoint. Based on this, 21 *Pseudomonas* isolates out of 51 were considered resistant. The MICs of other compounds without a clinical breakpoint available (OTC, florfenicol, flumequine and oxolonic acid) were highly variable. The different antibiotic susceptibility phenotypes highlighted variable proportions of isolates resistant to compounds of critical interest in human medicine, such as ceftazidime ($n = 20/51$) or colistin ($n = 11/51$). Conversely, all *Pseudomonas* isolates were highly sensitive (<2 μg ml^{-1}) to gentamicin.

Among all isolates, 16 (31.4%) were sensitive to four different antibiotic classes (β-lactamines, aminoglycosides, sulphonamides and polymixins) with available clinical breakpoints. Twenty-four (47.1%), nine (17.6%) and one (2.0%) isolates were resistant to one, two and three antibiotic classes respectively. The ECOFF values of our *Pseudomonas* population varied for antibiotics without available breakpoints (Figure S1). For this population, calculated ECOFF values were: 8 μg ml^{-1} for OTC, 32 μg ml^{-1} for flumequine, 256 μg ml^{-1} for florfenicol and 512 μg ml^{-1} for azithromycin. Five isolates displayed atypical (or non-wild-type) resistance phenotypes to OTC and florfenicol. The respective MICs of OTC and florfenicol for this atypical resistant sub-population found in both farms ranged from 64 to 256 μg ml^{-1} and from 512 μg ml^{-1} to more than 1024 μg ml^{-1}. These isolates were also resistant to the trimethoprim/sulfamethoxazole association, with MICs ranging from 128/2432 μg ml^{-1} to more than 512/9728 μg ml^{-1}. Two more isolates were highly resistant to colistin at a MIC of 512 μg ml^{-1} in farm B, compared to 3 isolates at 32 μg ml^{-1} maximum in farm A. Thirteen isolates were resistant to ceftazidime in farm B with MICs of 16–128 μg ml^{-1}, and 7 in farm A with MICs of 16–64 μg ml^{-1}. Finally, the analysis of antibiotic resistance patterns based on both breakpoint and ECOFF values revealed that eight (15.7%), five (9.8%) and one (2.0%) were resistant to two, three and four antibiotic classes respectively (Table 4). Among these subpopulations of fourteen isolates, the main patterns were composed by resistance to (i) trimethoprim/sulfamethoxazole association and ceftazidime (6 out of 14 multiple resistant isolates), (ii) colistin associated with ceftazidime or trimethoprim/sulfamethoxazole (3 out of 14 isolates) and (iii) those described above with high MIC values (OTC, florfenicol, trimethoprim/sulfamethoxazole).

TABLE 4 Profiles of the 14 multiple antibiotic resistant isolates within the *Pseudomonas* population ($N = 51$) based on both breakpoint and ECOFF values

Isolate ID	Phylogenetic group affiliation	Multiple resistance patterns	Number of resistance[a]
Farm A—laboratory filleting			
A03RPs3-08	*P. fluorescens*	OTC-FFC-TMP/SXT-CAZ	4
A12RPs2-30	*P. fluorescens*	OTC-FFC-TMP/SXT	3
A14RPs3-37	*P. fluorescens*	OTC-FFC-TMP/SXT	3
Farm A—factory filleting			
A13FPs3-75	*P. fluorescens*	TMP/SXT-CAZ	2
Farm B—laboratory filleting			
B03RPs3-86	*P. fluorescens*	TMP/SXT-COL-CAZ	3
B04RPs1-87	*P. fluorescens*	COL-CAZ	2
B05RPs3-90	*P. fluorescens*	TMP/SXT-CAZ	2
B07RPs3-98	*P. fluorescens*	TMP/SXT-CAZ	2
B11RPs3-110	*P. fluorescens*	TMP/SXT-CAZ	2
Farm B—factory filleting			
B03FPs1-121	*P. fluorescens*	TMP/SXT-CAZ	2
B05FPs3-125	*P. fluorescens*	TMP/SXT-COL	2
B21FPs3-128	*P. fluorescens*	OTC-FFC-TMP/SXT	3
B26FPs3-140	*P. fluorescens*	OTC-FFC-TMP/SXT	3
B14FPs1-144	*P. fluorescens*	TMP/SXT-CAZ	2

Abbreviations: CAZ, ceftazidime; COL, colistin; FFC, florfenicol; OTC, oxytetracycline; TMP/SXT, trimethoprim/sulfamethoxazole association.

[a]To different antibiotic classes.

Relationship between the sampling locations of the fish fillets and the antibiotic resistance patterns of the *Pseudomonas* population

In order to assess the relationship between the sampling locations of the fish fillets (farm or filleting facility), we performed a MCA on 5 out of 10 active variables (Figure 2). As partly described above, all *Pseudomonas* isolates were susceptible or shown as having wild-type phenotypes to five antibiotic compounds (oxolinic acid, flumequine, piperacilline, gentamicin and azithromycin). The relevance of these active variables was considered low in explaining differences between the antibiotic resistance patterns of the isolates. The MCA biplot showed that the total variance of our data was mainly explained by the first and second dimensions (62.5%, the contributions of each of the six dimensions are presented in Table S1). The variables significantly involved ($p < 0.001$) in the first dimension were the resistance patterns with high MICs of OTC, florfenicol and trimethoprim/sulfamethoxazole ($R^2 = 0.99$). For the second dimension, the variables more significantly ($p = 0.002$) related to its construction were the resistance phenotypes when exposed to sulfamethoxazole ($R^2 = 0.62$), ceftazidim ($R^2 = 0.44$) and colistin ($R^2 = 0.28$). The ellipse-coloured biplot highlighted that the antibiotic resistance patterns of the *Pseudomonas* isolates from each farm (A in red, B in green) overlapped with a 95% CI, confirming a weak association between resistance phenotypes and farm location. The confidence ellipses of the antibiotic resistance patterns of the *Pseudomonas* isolates also overlapped when laboratory filleting (purple) and factory filleting (blue) were taken into account.

DISCUSSION

The total viable mesophilic aerobic bacteria count (TVMC) is considered as an indicator of fish fillet quality and good manufacturing practices (Özyurt et al., 2015). Several studies performed on rainbow trout fillets in different countries have shown TVMCs ranging from 1.543 log CFU per g to 6.3 log CFU per g (Oraei et al., 2011; Özyurt et al., 2015; Pao et al., 2008; Pullela et al., 1998; Ucak et al., 2020). Ozyurt et al. (2015) defined that TVMCs ranging from 3.06 log CFU per g to 3.31 log CFU per g could indicate a high fish quality which are in agreement of our values. These results are informative of fillet freshness: they suggest that the bacterial communities are representative of those associated with rainbow trout in its environment. However, the rainbow trout fillets from farm B were significantly more contaminated than the fillets from farm A. The different TVMCs could be explained by the release of effluents upstream of farm B, in an urbanized area including two wastewater treatment plants, as previously described using *tuf* gene-targeted qPCR (Helsens, Calvez, Bouju-Albert, et al., 2020). Among aerobic mesophilic bacteria communities, several genera and species have been isolated from rainbow trout fillet, for example, *Micrococcus*, *Corynebacterium*, *Aeromonas* or *Pseudomonas* (Nedoluha et al., 2001). The present study confirms the presence of *Pseudomonas* associated with rainbow trout fillets, in accordance with the ubiquitous character of this genus in the environment (Pirnay et al., 2005; Sazakli et al., 2005). We identified *P. fluorescens* as the main phylogenetic group of the population using MALDI-TOF mass spectrometry. This approach was recently confirmed as accurate for phylogenetic group or sub-group identification within this genus in freshwater ecosystems and different fish species (Kačániová et al., 2019; Mulet et al., 2020). Our findings are in agreement with previous studies in which the *P. fluorescens* group was the most isolated one on fillets of different fish species (Duan et al., 2019; Shehata et al., 2020).

Pseudomonas in rainbow trout can cause diseases, such as infections by *P. putida* or the newly identified species *P. tructae* (Altinok et al., 2006; Oh et al., 2019). On the contrary, other species like *P. fluorescens* seem to be involved in the inhibition of rainbow trout colonization by other pathogenic micro-organisms (Gonzalez-Palacios et al., 2019; Korkea-aho et al., 2012; Liu et al., 2015). We did not isolate any pathogenic *Pseudomonas*, and the health status of the farms was good based on historical data provided by the farmers (no diseased fish sampled). Even if non-pathogenic *Pseudomonas* isolates can have a protective effect on fish, the isolates from the two farms showed various antibiotic resistance profiles and could be a concern in terms of animal/human health. In this study, we first identified that no relationship could be established between the farm or the factory processing locations and the *Pseudomonas* antibiotic resistance patterns. Indeed, similar multiple antibiotic resistance patterns were widespread on fish fillets sampled in both farms whatever the proximity to urban wastewaters. Moreover, no changes in antibiotic resistance patterns were occurred between filleting at the factory (high amount of fishes filleted per day) and at the laboratory, suggesting that factory would not be involved in potential enrichment of antibiotic-resistant bacteria. This result is supported by previous work carried out in the same farms by Helsens, Calvez, Bouju-Albert, et al. (2020), who hypothesized that the microbiota of fresh farmed rainbow trout was mainly influenced by the river environment. *Pseudomonas* is ubiquitous in many environments, and it is probably present in the river that hosts both farms. Nevertheless, to confirm these results, it would be interesting to compare the resistance patterns to

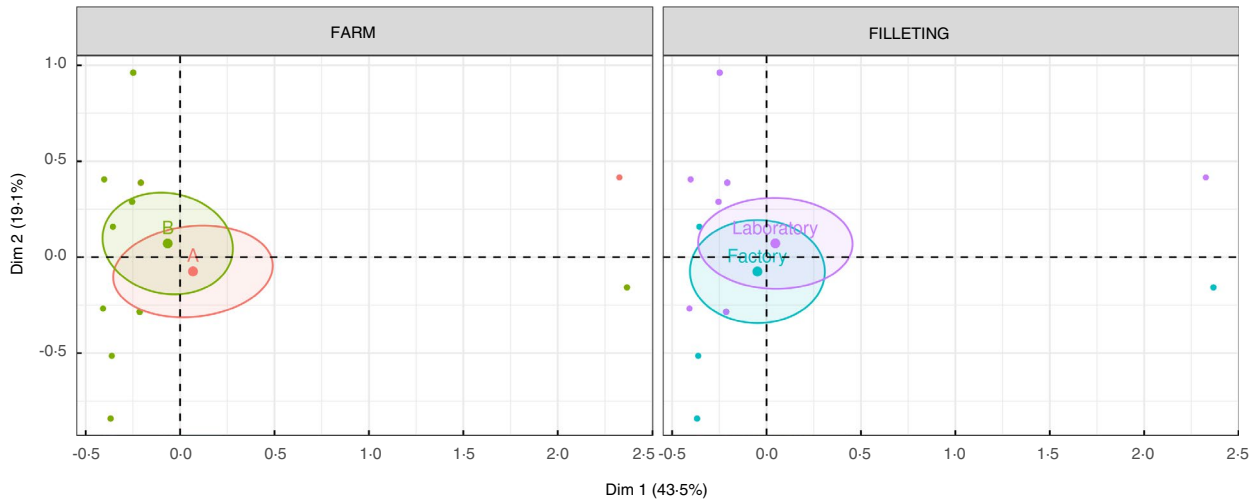

FIGURE 2 Graphical representation of the multiple correspondence analysis. The statistical data analysis by MCA did not show any relationship between the antibiotic resistance profiles of the *Pseudomonas* strains isolated from two farms (A and B) or according to the sampling location of fish fillets (laboratory or factory). Five active variables (resistance patterns to oxytetracycline, florfenicol, ceftazidime, colistin and trimethoprim/sulfamethoxazole) and four illustrative variables (farm A, farm B, laboratory, factory) were used. The first and second dimensions represent 62.5% of total inertia

the same antibiotics of *Pseudomonas* isolated from fillets of rainbow trout farmed in other rivers.

The data for each antibiotic confirmed results from other studies reporting a predominance of gentamicin-susceptible *Pseudomonas* isolated from rainbow trout farms (Akinbowale et al., 2007; Duman et al., 2019). Populations isolated from Australian rainbow trout but also from other freshwater and marine fish showed that a great proportion of isolates were resistant to different classes of antibiotics (Akinbowale et al., 2006, 2007). Among 44 *Pseudomonas* isolates, Akinbowale et al. (2007) revealed that 90.9% were resistant to florfenicol, with MICs of 32–128 μg ml^{-1} according to the recommendations of the CLSI. Similarly, we determined that 86.3% ($n = 44/51$) of the *Pseudomonas* isolates were resistant to this range of MICs, while 5.9% ($n = 3/51$) had the highest MICs (>1024 μg ml^{-1}). The resistance phenotypes related to this antibiotic appear to be widespread in different countries; the atypical resistance profiles observed in our study could be due to its use in France. This hypothesis is supported by another study in Chilean salmon farms where this antibiotic is highly used for bacterial infection treatment (Miranda & Rojas, 2007). These authors demonstrated that the MICs of isolates from two farms ranged from 256 μg ml^{-1} to more than 1024 μg ml^{-1}. While we evidenced resistance to trimethoprim/sulfamethoxazole, several authors isolated *Pseudomonas* strains from different fish species that were fully sensitive to this antibiotic association, using dilution broth or agar diffusion methods (Akinbowale et al., 2007; Türe & Alp, 2016). A study performed on 316 *Pseudomonas* strains isolated from contaminated waters showed a wide, species-dependent distribution of resistance phenotypes to trimethoprim/sulfamethoxazole association (Camiade et al., 2020). These data showed a percentage of resistant *Pseudomonas* (49.4%) similar to the one observed in our study (41.2%). Likewise in recent study performed in Chilean salmon farms, the authors highlighted a great proportion of *Pseudomonas* isolates resistant to several antibiotic classes like sulphonamides (Dominguez et al., 2019). Their MICs were similar to and even higher than those observed in our study. These authors identified several isolates belonging to the *Pseudomonas* genus and carrying the *sul* and *dfr* genes encoding enzymes which confer sulphonamide resistance—dihydropteroate synthase and dihydrofolate reductase enzymes. They also demonstrated that resistance to antibiotics was attributable to the acquisition, spreading and maintenance of class-1 integrons. Among 25 isolates carrying one or two plasmids, ten were able to co-transfer resistance to sulphonamides, trimethoprim and other classes (florfenicol, OTC) to donor bacteria (Dominguez et al., 2019).

We isolated five *Pseudomonas* isolates resistant to OTC (from 64 to 256 μg ml^{-1}), and also resistant to florfenicol and the trimethoprim/sulfamethoxazole combination, corresponding to 9.8% of the isolates, while all 44 *Pseudomonas* strains isolated from rainbow trout in Australia were sensitive to OTC (Akinbowale et al., 2007). A study on antibiotic resistance in 30 trout farms in Turkey yielded a proportion of 30% of OTC resistance out of 20 isolated *Pseudomonas* isolates; among them, seven carried at least one of the *tet*A, *tetB*, *tetC* or *tetD* resistance genes. However, none of them was involved in a multidrug resistance profile (Capkin et al., 2017). Likewise,

Türe and Alp (2016) found that 20% of the species isolated from fish raised in sea-water or freshwater farms were resistant to OTC from which only 15 *Pseudomonas* isolates were isolated out of 240. Among these 15 isolates, four were multi-drug resistant, but none of them was resistant to OTC, and none carried the *tetB* gene (Türe & Alp, 2016). Interestingly, although OTC is commonly used in fish farming in most countries, the resistance profiles of the five isolates in our study were not in accordance with the studies of Türe and Alp (2016) and of Capkin et al. (2017). Deeper investigations of our relevant isolates would be necessary to understand the resistance profiles of these ones and to confirm the hypothesis of resistance genes carried by plasmids, integrons or phages.

Ceftazidime—a third-generation cephalosporin—is considered as a critically important antimicrobial for human medicine by the World Health Organization (2019). Knowledge on resistance phenotypes within the *Pseudomonas* population is mainly supported by *P. aeruginosa* because of its interest in human medicine. A collection of 499 *Pseudomonas* isolates through an urban water cycle was recently built by Butiuc-Keul et al. (2021). The authors showed that the percentages of ceftazidime-resistant isolates were higher in hospital effluents and in one drinking water source (*ca*. 20.0%) than in wastewater treatment plant influent or effluent (*ca*. 12.0% and 4.0% respectively). These intriguing results suggest that ceftazidime resistance phenotypes within the *Pseudomonas* population are more related to found species than to proximity to a contamination source. Out of 316 *Pseudomonas* strains isolated from contaminated environments, only 2.5% were resistant to ceftazidime (Camiade et al., 2020). Two out of five isolates were found resistant to ceftazidime in a *Pseudomonas* population isolated from fin fishes (Carol et al., 2013). We identified 20 out of 51 *Pseudomonas* isolates (39.2%) resistant to this antibiotic in the two farms. Based on the high diversity of resistance phenotypes to ceftazidime within the *Pseudomonas* population probing of whether this variability could be related to bacterial species or to antibiotic use.

Polymyxins such as colistin have also been listed as critically important antimicrobials for human medicine (World Health Organization, 2019). To our knowledge, few studies have investigated resistance phenotypes to this antibiotic class within the *Pseudomonas* population, except for *P. aeruginosa*. Among *Pseudomonas* isolates from blood stream infections, two out of 19 isolates were resistant to colistin, with maximum MICs of 8 $\mu g\ ml^{-1}$ (Varghese et al., 2020). In the same way, 40 *P. aeruginosa* isolates were all found susceptible to colistin, with MICs of 0.25–2 $\mu g\ ml^{-1}$ (Zhang et al., 2020). In our study, most *Pseudomonas* isolates (76.5%) were considered susceptible to this antibiotic. Among the resistant sub-population, eight isolates were resistant, with MICs of 4–32 $\mu g\ ml^{-1}$, and two were highly resistant (MICs of 512 and >1024 $\mu g\ ml^{-1}$ respectively). These two phenotypes were not associated with resistance to other antibiotic classes and did not seem to be among clinical isolates of *P. aeruginosa* involved in human infections. Based on the results discussed above, we could hypothesize that "environmental" *Pseudomonas* populations could be a reservoir of highly colistin-resistant isolates. Considering the critical importance of this antimicrobial, it is recommended to further characterise the genetic elements involved in this phenotypic resistance in order to determine whether it would be transferable to pathogenic bacteria for fish and humans.

In conclusion, rainbow trout fillets from two French farms harboured *Pseudomonas* isolates mainly belonging to the *P. fluorescens* group. This study provided data on antibiotic resistance levels and patterns of *Pseudomonas* non-*aeruginosa* isolated from rainbow trout fillets. No evidence for a relationship of farm or filleting processing locations has been highlighted on antibiotic resistance patterns found in *Pseudomonas* population. This study confirmed that *Pseudomonas* spp. associated with rainbow trout fillets are reservoirs of antibiotic resistance traits and revealed that these isolates were widespread in the environment independently of proximity of fish farms to human or animal inputs. Among these multiple antibiotic resistance isolates, seven were highly resistant to antibiotics used in aquaculture (OTC, florfenicol and sulphonamides) and in human medicine (colistin). To better understand their role on the spreading of antibiotic resistance to pathogenic bacteria for rainbow trouts (e.g. *A. salmonicida* subsp. *salmonicida* or *Flavobacterium psychrophilum*) or human (e.g. *Pseudomonas aeruginosa*), it would be of interest to further study this population and characterize their genetic contents by a whole genome approach.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Abolghait, S.K., Fathi, A.G., Youssef, F.M. & Algammal, A.M. (2020) Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. *International Journal of Food Microbiology*, 328, 108669.

Akinbowale, O.L., Peng, H. & Barton, M.D. (2006) Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *Journal of Applied Microbiology*, 100, 1103–1113.

Akinbowale, O.L., Peng, H., Grant, P. & Barton, M.D. (2007) Antibiotic and heavy metal resistance in motile aeromonads and pseudomonads from rainbow trout (*Oncorhynchus mykiss*) farms in Australia. *International Journal of Antimicrobial Agents*, 30, 177–182.

Algammal, A.M., Mabrok, M., Sivaramasamy, E., Youssef, F.M., Atwa, M.H., El-kholy, A.W. et al. (2020) Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor *oprL* and *toxA* virulence genes and *blaTEM*, *blaCTX-M*, and *tetA* antibiotic-resistance genes. *Scientific Reports*, 10, 15961.

Algammal, A.M., Mohamed, M.F., Tawfiek, B.A., Hozzein, W.N., El Kazzaz, W.M. & Mabrok, M. (2020) Molecular typing, antibiogram and PCR-RFLP based detection of *Aeromonas hydrophila* complex isolated from *Oreochromis niloticus*. *Pathogens*, 9, 238.

Altinok, I., Kayis, S. & Capkin, E. (2006) *Pseudomonas putida* infection in rainbow trout. *Aquaculture*, 261, 850–855.

Bergmark, L., Poulsen, P.H.B., Al-Soud, W.A., Norman, A., Hansen, L.H. & Sørensen, S.J. (2012) Assessment of the specificity of *Burkholderia* and *Pseudomonas* qPCR assays for detection of these genera in soil using 454 pyrosequencing. *FEMS Microbiology Letters*, 333, 77–84.

Boss, R., Overesch, G. & Baumgartner, A. (2016) Antimicrobial resistance of *Escherichia coli*, enterococci, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* from raw fish and seafood imported into Switzerland. *Journal of Food Protection*, 79, 1240–1246.

Boutin, S., Sauvage, C., Bernatchez, L., Audet, C. & Derome, N. (2014) Inter individual variations of the fish skin microbiota: host genetics basis of mutualism? *PLoS One*, 9, e102649.

Butiuc-Keul, A., Carpa, R., Podar, D., Szekeres, E., Muntean, V., Iordache, D. et al. (2021) Antibiotic resistance in *Pseudomonas* spp. through the urban water cycle. *Current Microbiology* 78(4), 1227–1237.

Camiade, M., Bodilis, J., Chaftar, N., Riah-Anglet, W., Garderes, J., Buquet, S. et al. (2020) Antibiotic resistance patterns of *Pseudomonas* spp. isolated from faecal wastes in the environment and contaminated surface water. *FEMS Microbiology Ecology*, 96, fiaa008.

Capkin, E., Ozdemir, S., Ozturk, R.C. & Altinok, I. (2017) Determination and transferability of plasmid-mediated antibiotic resistance genes of the bacteria isolated from rainbow trout. *Aquaculture Research*, 48, 5561–5575.

Carol, G.R., Jeyasanta, K.I., Mani, A.E. & Patterson, J. (2013) Prevalence of *Pseudomonas* sp in fin fishes and their antibiotic susceptibility. *Journal of Pure and Applied Microbiology*, 7, 677–681.

Cazares, A., Moore, M.P., Hall, J.P.J., Wright, L.L., Grimes, M., Emond-Rhéault, J.G. et al. (2020) A megaplasmid family driving dissemination of multidrug resistance in *Pseudomonas*. *Nature Communications*, 11, 1370.

Dinçtürk, E. & Tanrıkul, T.T. (2021) *Yersinia ruckeri* and *Pseudomonas fluorescens* co-infection in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Aquaculture Research*, 52(10), 1–9.

Dominguez, M., Miranda, C.D., Fuentes, O., de la Fuente, M., Godoy, F.A., Bello-Toledo, H. et al. (2019) Occurrence of transferable integrons and *sul* and *dfr* genes among sulfonamide-and/or trimethoprim-resistant bacteria isolated from Chilean salmonid farms. *Frontiers in Microbiology*, 10, 748.

Duan, S., Zhou, X., Xiao, H., Miao, J. & Zhao, L. (2019) Characterization of bacterial microbiota in tilapia fillets under different storage temperatures. *Journal of Food Science*, 84, 1487–1493.

Duman, M., Saticioglu, I.B. & Altun, S. (2019) Molecular characterization and antimicrobial resistance profile of fecal contaminants and spoilage bacteria that emerge in rainbow trout (*Oncorhynchus mykiss*) farms. *Bioscience of Microbiota Food and Health*, 38, 41–48.

El-Sayed, M., Algammal, A., Abouel-Atta, M., Mabrok, M. & Emam, A. (2019) Pathogenicity, genetic typing, and antibiotic sensitivity of *Vibrio alginolyticus* isolated from *Oreochromis niloticus* and *Tilapia zillii*. *Revista de Medicina*, 170, 80–86.

EUCAST. (2019) EUCAST: new S, I and R definitions [WWW Document]. https://www.EUCAST.org/newsiandr/.

Garrido-Sanz, D., Arrebola, E., Martínez-Granero, F., García-Méndez, S., Muriel, C., Blanco-Romero, E. et al. (2017) Classification of isolates from the *Pseudomonas fluorescens* complex into phylogenomic groups based in group-specific markers. *Frontiers in Microbiology*, 8. https://doi.org/10.3389/fmicb.2017.00413

Gonzalez-Palacios, C., Fregeneda-Grandes, J.-M. & Aller-Gancedo, J.-M. (2019) Biocontrol of saprolegniosis in rainbow trout (*Oncorhynchus mykiss* Walbaum) using two bacterial isolates (LE89 and LE141) of *Pseudomonas fluorescens*. *Journal of Fish Diseases*, 42, 269–275.

Gordon, L., Giraud, E., Ganière, J.-P., Armand, F., Bouju-Albert, A., de la Cotte, N. et al. (2006) Antimicrobial resistance survey in a river receiving effluents from freshwater fish farms. *Journal of Applied Microbiology*, 102, 1162–1176.

Helsens, N., Calvez, S., Bouju-Albert, A., Rossero, A., Prévost, H. & Magras, C. (2020) Comparison of stomaching versus rinsing for recovering bacterial communities from rainbow trout (*Oncorhynchus mykiss*) fillets. *Journal of Food Protection*, 83, 1540–1546.

Helsens, N., Calvez, S., Prévost, H., Bouju-Albert, A., Maillet, A., Rossero, A. et al. (2020) Antibiotic resistance genes and bacterial communities of farmed rainbow trout fillets (*Oncorhynchus mykiss*). *Frontiers in Microbiology*, 11, 1–17.

International Organization for Standardization. (2010) Meat and meat products: enumeration of presumptive *Pseudomonas* spp. International Organization for Standardization.

Ishida, Y., Ahmed, A.M., Mahfouz, N.B., Kimura, T., El-Khodery, S.A., Moawad, A.A. et al. (2010) Molecular analysis of antimicrobial resistance in Gram-negative bacteria isolated from fish farms in Egypt. *Journal of Veterinary Medical Science*, 72, 727–734.

Kačániová, M., Klūga, A., Kántor, A., Medo, J., Žiarovská, J., Puchalski, C. et al. (2019) Comparison of MALDI-TOF MS Biotyper and 16*S* rDNA sequencing for the identification of *Pseudomonas* species isolated from fish. *Microbial Pathogenesis*, 132, 313–318.

Kassambara, A. (2020) ggpubr: "ggplot2" based publication ready plots. https://cran.r-project.org/web/packages/ggpubr/index.html

Korkea-aho, T.L., Papadopoulou, A., Heikkinen, J., von Wright, A., Adams, A., Austin, B. et al. (2012) *Pseudomonas* M162 confers protection against rainbow trout fry syndrome by stimulating immunity. *Journal of Applied Microbiology*, 113, 24–35.

Kottara, A., Hall, J.P.J., Harrison, E. & Brockhurst, M.A. (2018) Variable plasmid fitness effects and mobile genetic element dynamics across *Pseudomonas* species. *FEMS Microbiology Ecology*, 94. https://doi.org/10.1093/femsec/fix172

Langsrud, S., Moen, B., Møretrø, T., Løype, M. & Heir, E. (2016) Microbial dynamics in mixed culture biofilms of bacteria surviving sanitation of conveyor belts in salmon-processing plants. *Journal of Applied Microbiology*, 120, 366–378.

Liu, Y., Rzeszutek, E., van der Voort, M., Wu, C.-H., Thoen, E., Skaar, I. et al. (2015) Diversity of aquatic *Pseudomonas* species and their activity against the fish pathogenic Oomycete Saprolegnia. *PLoS One*, 10, e0136241.

Lowrey, L., Woodhams, D.C., Tacchi, L. & Salinas, I. (2015) Topographical mapping of the rainbow trout (*Oncorhynchus mykiss*) microbiome reveals a diverse bacterial community with antifungal properties in the skin. *Applied and Environment Microbiology*, 81, 6915–6925.

Luczkiewicz, A., Kotlarska, E., Artichowicz, W., Tarasewicz, K. & Fudala-Ksiazek, S. (2015) Antimicrobial resistance of *Pseudomonas* spp. isolated from wastewater and wastewater-impacted marine coastal zone. *Environmental Science and Pollution Research International*, 22, 19823–19834.

Meyer, D., Dimitriadou, E., Hornik, K., Weingessel, A. & Leisch, F. (2021) e1071: misc functions of the Department of Statistics, probability Theory Group (Formerly: E1071), TU Wien.

Miranda, C.D. & Rojas, R. (2007) Occurrence of florfenicol resistance in bacteria associated with two Chilean salmon farms with different history of antibacterial usage. *Aquaculture*, 266, 39–46.

Miranda, C.D. & Zemelman, R. (2002) Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. *Science of the Total Environment*, 293, 207–218.

Miyazaki, T., Kubota, S.S. & Miyashita, T. (1984) A histopathological study of *Pseudomonas fluorescens* infection in tilapia. *Fish Pathology*, 19, 161–166.

Molina, L., Udaondo, Z., Duque, E., Fernández, M., Molina-Santiago, C., Roca, A. et al. (2014) Antibiotic resistance determinants in a *Pseudomonas putida* strain isolated from a hospital. *PLoS One*, 9, e81604.

Møretrø, T., Moen, B., Heir, E., Hansen, A.Å. & Langsrud, S. (2016) Contamination of salmon fillets and processing plants with spoilage bacteria. *International Journal of Food Microbiology*, 237, 98–108.

Mulet, M., Montaner, M., Roman, D., Gomila, M., Kittinger, C., Zarfel, G. et al. (2020) *Pseudomonas* Species Diversity Along the Danube river assessed by *rpoD* gene sequence and MALDI-TOF MS analyses of cultivated strains. *Frontiers in Microbiology*, 11, 2114.

Muziasari, W.I., Pitkänen, L.K., Sørum, H., Stedtfeld, R.D., Tiedje, J.M. & Virta, M. (2017) The resistome of farmed fish feces contributes to the enrichment of antibiotic resistance genes in sediments below Baltic sea fish farms. *Frontiers in Microbiology*, 7, 2137.

Nedoluha, P.C., Owens, S., Russek-Cohen, E. & Westhoff, D.C. (2001) Effect of sampling method on the representative recovery of microorganisms from the surfaces of aquacultured finfish. *Journal of Food Protection*, 64, 1515–1520.

Nguyen, H.N.K., Van, T.T.H., Nguyen, H.T., Smooker, P.M., Shimeta, J. & Coloe, P.J. (2014) Molecular characterization of antibiotic resistance in *Pseudomonas* and *Aeromonas* isolates from catfish of the Mekong Delta, Vietnam. *Veterinary Microbiology*, 171, 397–405.

Oh, W.T., Kim, J.H., Jun, J.W., Giri, S.S., Yun, S., Kim, H.J. et al. (2019) Genetic characterization and pathological analysis of a novel bacterial pathogen, *Pseudomonas tructae*, in rainbow trout (*Oncorhynchus mykiss*). *Microorganisms*, 7, 432.

Oraei, M., Motalebi, A.A., Hoseini, E. & Javan, S. (2011) Effect of gamma irradiation and frozen storage on microbial quality of Rainbow trout (*Oncorhynchus mykiss*) fillet. *Iran Journal of Fisheries Science*, 10, 75–84.

Özyurt, G., Ozkutuk, A.S., Simsek, A., Yesilsu, A.F. & Erguven, M. (2015) Quality and shelf life of cold and frozen rainbow trout (*Oncorhynchus mykiss*) fillets: effects of fish protein-based biodegradable coatings. *International Journal of Food Properties*, 18, 1876–1887.

Pao, S., Ettinger, M.R., Khalid, M.F., Reid, A.O. & Nerrie, B.L. (2008) Microbial quality of raw aquacultured fish fillets procured from Internet and local retail market. *Journal of Food Protection*, 71, 1544–1549.

Peix, A., Ramírez-Bahena, M.H. & Velázquez, E. (2009) Historical evolution and current status of the taxonomy of genus *Pseudomonas*. *Infection, Genetics and Evolution*, 9, 1132–1147.

Peix, A., Ramírez-Bahena, M.H. & Velázquez, E. (2018) The current status on the taxonomy of *Pseudomonas* revisited: an update. *Infection, Genetics and Evolution*, 57, 106–116.

Pirnay, J.P., Matthijs, S., Colak, H., Chablain, P., Bilocq, F., Van Eldere, J. et al. (2005) Global *Pseudomonas aeruginosa* biodiversity as reflected in a Belgian river. *Environmental Microbiology*, 7, 969–980.

Pullela, S., Fernandes, C.F., Flick, G.J., Libey, G.S., Smith, S.A. & Coale, C.W. (1998) Indicative and pathogenic microbiological quality of aquacultured finfish grown in different production systems. *Journal of Food Protection*, 61, 205–210.

Roberts, R.J. (2012) *Fish pathology*. 4th edition. UK: Wiley-Blackwell.

Sazakli, E., Leotsinidis, M., Vantarakis, A. & Papapetropoulou, M. (2005) Comparative typing of *Pseudomonas* species isolated from the aquatic environment in Greece by SDS-PAGE and RAPD analysis. *Journal of Applied Microbiology*, 99, 1191–1203.

Scarano, C., Piras, F., Virdis, S., Ziino, G., Nuvoloni, R., Dalmasso, A. et al. (2018) Antibiotic resistance of *Aeromonas* spp. strains isolated from *Sparus aurata* reared in Italian mariculture farms. *International Journal of Food Microbiology*, 284, 91–97.

Shehata, H.R., Mitterboeck, T.F. & Hanner, R. (2020) Characterization of the microbiota of commercially traded finfish fillets. *Food Research International*, 137, 109373.

Sun, Y. & Sun, L. (2015) *Pseudomonas fluorescens*: iron-responsive proteins and their involvement in host infection. *Veterinary Microbiology*, 176, 309–320.

Türe, M. & Alp, H. (2016) Identification of bacterial pathogens and determination of their antibacterial resistance profiles in some cultured fish in Turkey. *Journal of Veterinary Research*, 60, 141–146.

Turnidge, J., Kahlmeter, G. & Kronvall, G. (2006) Statistical characterisation of bacterial wild-type MIC value distributions and the determination of epidemiological cut-off values. *Clinical Microbiology & Infection*, 12, 418–425.

Ucak, I., Khalily, R., Carrillo, C., Tomasevic, I. & Barba, F.J. (2020) Potential of propolis extract as a natural antioxidant and antimicrobial in gelatin films applied to rainbow trout (*Oncorhynchus mykiss*) fillets. *Foods*, 9, 1584.

Varghese, A., Udayalaxmi, J., Rao, P. & Suman, E. (2020) Blood stream infections caused by non-fermenting gram negative bacilli, clinical correlation, MIC for colistin, gene detection. *Journal of Pure and Applied Microbiology*, 14, 2017–2025.

Wickham, H. (2016) *ggplot2: elegant graphics for data analysis*. New York: Springer-Verlag.

Wiklund, T. & Bylund, G. (1990) *Pseudomonas anguilliseptica* as a pathogen of salmonid fish in Finland. *Diseases of Aquatic Organisms*, 8, 13–19.

Wong, S., Waldrop, T., Summerfelt, S., Davidson, J., Barrows, F., Kenney, B.B. et al. (2013) Aquacultured rainbow trout (*Oncorhynchus mykiss*) possess a large core intestinal microbiota that is resistant to variation in diet and rearing density. *Applied and Environment Microbiology*, 79, 4974–4984.

Woolhouse, M.E. & Ward, M.J. (2013) Sources of antimicrobial resistance. *Science*, 341, 1460–1461.

World Health Organization (2019) *Critically important antimicrobials for human medicine*. 6th edition. Geneva: World Health Organization.

Yang, Y., Miao, P., Li, H., Tan, S., Yu, H. & Yu, H. (2018) Antibiotic susceptibility and molecular characterization of *Aeromonas hydrophila* from grass carp. *Journal of Food Safety*, 38, e12393.

Yomoda, S., Okubo, T., Takahashi, A., Murakami, M. & Iyobe, S. (2003) Presence of *Pseudomonas putida* strains harboring plasmids bearing the Metallo- -lactamase gene bla_{IMP} in a Hospital in Japan. *Journal of Clinical Microbiology*, 41, 4246–4251.

Zhang, X., Ding, L., Yu, Y., Kong, W., Yin, Y., Huang, Z. et al. (2018) The change of teleost skin commensal microbiota is associated with skin mucosal transcriptomic responses during parasitic infection by *Ichthyophthirius multifillis*. *Frontiers in Immunology*, 9, 2972.

Zhang, Y., Zhao, C., Wang, Q., Wang, X., Chen, H., Li, H. et al. (2020) Evaluation of the *in vitro* activity of new polymyxin B analogue SPR206 against clinical MDR, colistin-resistant and tigecycline-resistant Gram-negative bacilli. *Journal of Antimicrobial Chemotherapy*, 75, 2609–2615.

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