In vitro antimicrobial sensitivities of *Flavobacterium* spp. isolates recovered from UK farmed fish

Ramón Pérez y Pérez de la Ossa[1], Kim D Thompson[1], Alexandra Adams[1], Rodney Card[2] & David W. Verner-Jeffreys[3]

[1]*Aquatic Vaccine Unit, Institute of Aquaculture, University of Stirling, Stirling, UK*

[2] *AHVLA, Weybridge Laboratory, Weybridge, Surrey, UK*

[3]*Cefas Weymouth Laboratory, Weymouth, Dorset, UK*

Correspondence: david.verner-jeffreys@cefas.co.uk

Abstract

In the UK, *Flavobacterium spp.,* particularly *F. psychrophilum,* are now recognised as one of the most important group of bacterial pathogens affecting cultured rainbow trout (*Oncorhynchus mykiss*) [1] and, increasingly in Atlantic salmon (*Salmo salar*) hatcheries. The only control method currently available is the use of antibiotics, particularly florfenicol. A preliminary survey was undertaken to help determine whether the *Flavobacterium* spp. circulating in UK fish farms are developing increased tolerance to antibiotics.

A total of 97 *Flavobacterium* spp. isolates from diseased fish were analysed for tolerance to 41 antimicrobials, using a combination of disc diffusion and broth microdilution protocols. All testing followed guidelines from the Clinical and Laboratory Standards Institute [2–3] with minor modifications for testing Flavobacteriaceae, specifically *F. psychrophilum,* as recommended by Hesami et al. [4]. Three control strains, *F. psychrophilum* reference strain ATCC 1947, *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* were also included in parallel in all testing. Isolates were characterised based on a combination of biochemical and phenotypic testing methods, including MALDI-TOF-MS. Preliminary results indicated that the bacteria tested included isolates that could be unambiguously characterised as *F. psychrophilum,* as well as a range of other Flavobacteriaceae, including *Chryseobacterium* spp.

Organisms displayed tolerance to a wide variety of the antimicrobials tested, including all those commonly used in UK rainbow trout and Atlantic salmon culture (per cent tolerant isolates in parentheses), oxytetracycline (41%) (Fig. 1a), oxolinic acid (28%), amoxicillin (52%) (Fig. 1b), sulphadiazine/trimethoprim (16%) (Fig. 1c) and florfenicol (21%).

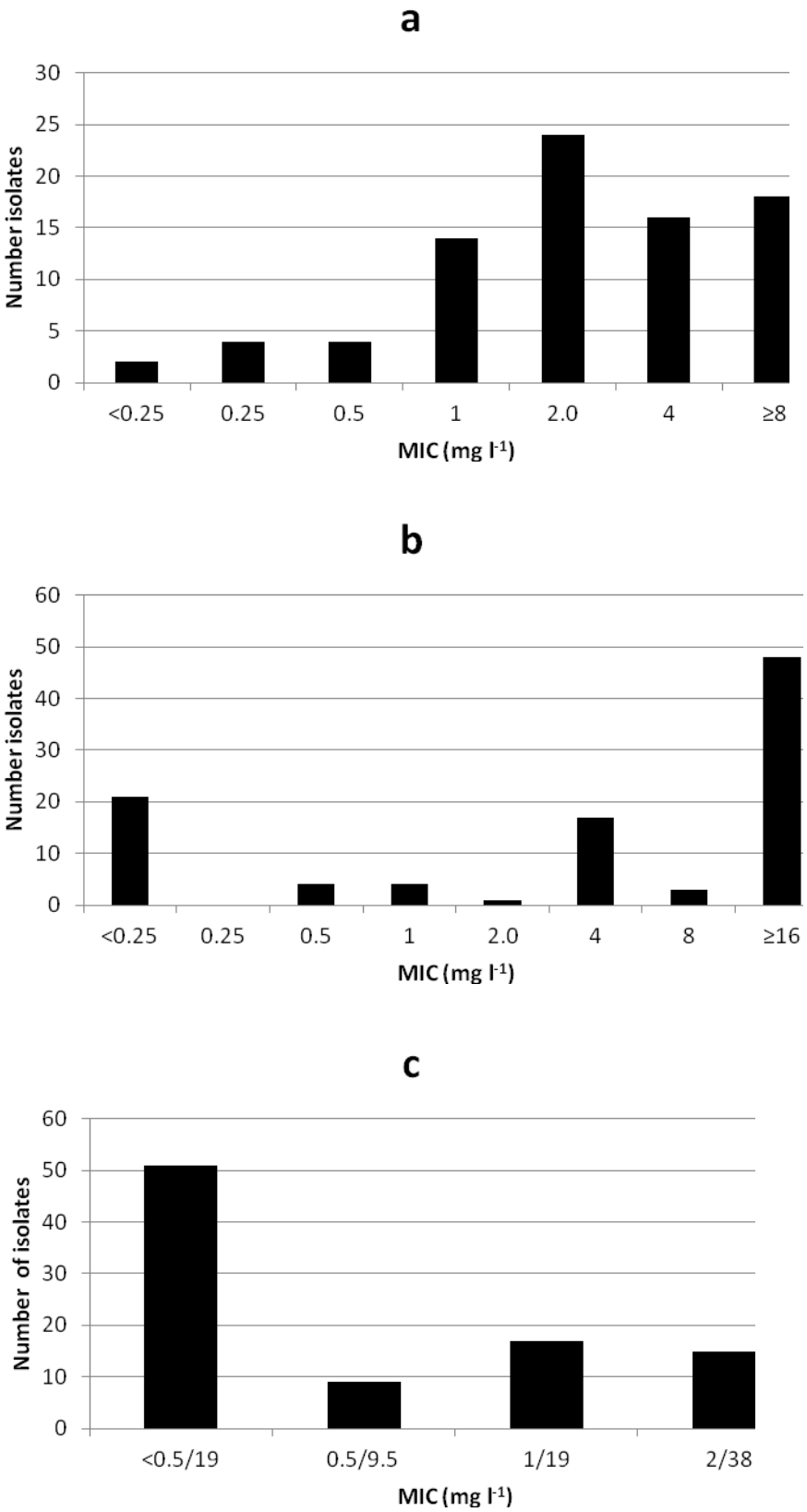

Figure 1a–c. Distribution of minimum inhibitory concentrations (MIC) of oxytetracycline (a), amoxicillin (b) and trimethoprim/sulphadiazine (c) in 97 *Flavobacterium* spp. isolates.

The minimum inhibitory concentrations (MIC) for 36 *Flavobacterium* spp. isolates that were classified as tolerant *in vitro* to florfenicol (grew in at least 2 mg l^{-1} florfenicol), were also determined using custom prepared both microdilution plates (Fig. 2). It was shown that 4 of these isolates were very resistant to this antimicrobial and were able to grow in 128 mg l^{-1}.

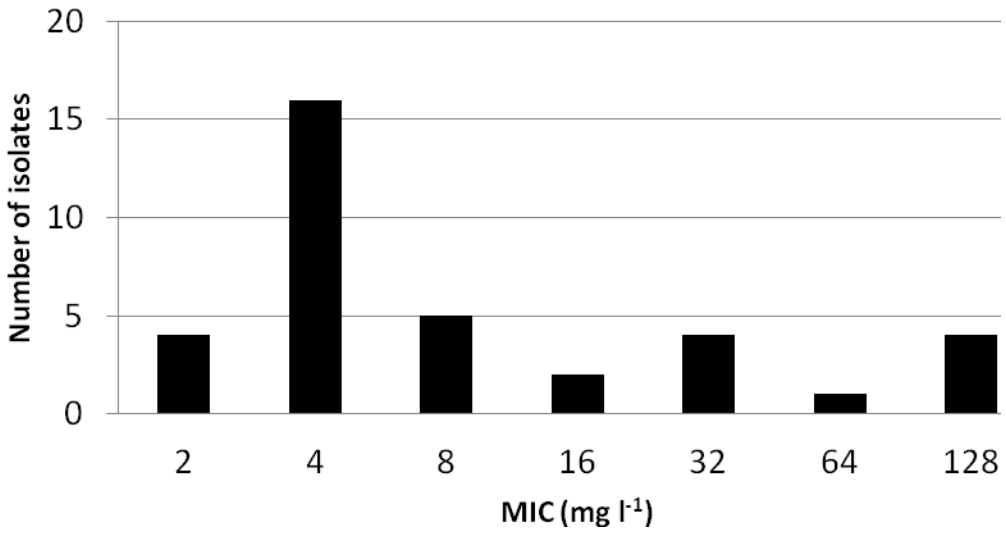

Figure 2. Distribution of MIC values of florfenicol in 36 *Flavobacterium* spp. isolates able to grow in at least 2 mg L^{-1} florfenicol.

The finding of high tolerance to all the antimicrobials commonly used to treat farmed salmonids in the UK is noteworthy. However, further work is now needed to determine the clinical significance of these observations. High levels of tolerance did not necessarily correlate with observed treatment problems, in the stocks of fish the bacterial isolates were recovered from. It was also noteworthy that the organisms tested, most of which were reportedly recovered from diseased fish, appear to have been phenotypically and genetically quite diverse. Follow up studies are needed to accurately determine what the organisms responsible for disease in farmed UK salmonids are. In particular, the role of Flavobacteriaceae other than *F. psychrophilum* in disease problems needs to be better determined. Further work is also needed to determine the molecular mechanisms responsible for isolates ability to grow in the presence of high concentrations of key antimicrobials, such as florfenicol.

References

[1] Madsen L., Møller J.D., Dalsgaard I., 2005. *Flavobacterium psychrophilum* in rainbow trout, *Oncorhynchus mykiss*, hatcheries - studies on broodstock, eggs, fry and environment. Journal of Fish Diseases, 28:39–47

[2] Clinical and Laboratory Standards Institute. (2004). Methods for Broth dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Proposed Guideline, M49-P. CLSI, Wayne, PA, USA.

[3] Clinical and Laboratory Standards Institute (2004) Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Proposed Guideline, M42-P. CLSI, Wayne, PA, USA.

[4] Hesami S., Parkman J., MacInnes J., Gray J., Gyles C., Lumsden J., 2011. Antimicrobial Susceptibility of *Flavobacterium psychrophilum* Isolates from Ontario. Journal of Aquatic Animal Health, 22:39–49