Veterinary Microbiology 142 (2010) 435–439

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Short communication

# Antimicrobial resistance and its genetic determinants in aeromonads isolated in ornamental (koi) carp (*Cyprinus carpio koi*) and common carp (*Cyprinus carpio*)

Alois Čížek [a,*], Monika Dolejská [b], Radana Sochorová [a], Kateřina Strachotová [a], Veronika Piačková [c], Tomáš Veselý [d]

[a] *Institute of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého 1/3, 612 42 Brno, Czech Republic*
[b] *Institute of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic*
[c] *Research Institute of Fish Culture and Hydrobiology, University of South Bohemia, Vodňany, Czech Republic*
[d] *Veterinary Research Institute, Brno, Czech Republic*

ARTICLE INFO

*Article history:*
Received 5 March 2009
Received in revised form 7 October 2009
Accepted 9 October 2009

*Keywords:*
Aquaculture
*Aeromonas* spp.
Antimicrobial resistance
*tet* resistance genes
Integrons

ABSTRACT

The aim of this study was to evaluate antimicrobial susceptibility of *Aeromonas* spp. isolates from common carp and koi carp coming from randomly chosen farms. The isolates were tested for susceptibility to 8 antimicrobial agents using the standard agar dilution susceptibility test. In all isolates, PCR was used to detect the presence of *tet*(A-E) genes, integrase genes, and gene cassettes. From the total 72 isolates of motile aeromonads sampled from koi carp, 36 isolates (50%) were resistant to oxytetracycline, 18 (25%) to ciprofloxacin, 5 (7%) to chloramphenicol, 5 (7%) to florfenicol, and 11 (15%) to trimethoprim. Among 49 isolates of motile aeromonads collected from common carp, 20 (41%) were resistant to oxytetracycline, 3 (6%) to chloramphenicol, and 3 (6%) to florfenicol. The resistance of aeromonads isolated from koi carp was significantly higher to ciprofloxacin ($P = 0.00024$). The presence of class 1 integrons was detected in these isolates only ($P = 0.00024$). *Tet* genes were detected in 40% (48/121) of isolates, with *tet*(E) being the most dominant. Our results demonstrated a significant difference in the incidence of resistant isolates collected from koi carp and common carp ($P = 0.00042$). This difference can be ascribed to a distinct antibiotic policy established on consumer fish farms versus ornamental fish farms. The potential risk for resistant bacteria to spread and transmit infection to humans should be considered in cases of technological crossover between the two types of fish farms.



## 1. Introduction

The Czech Republic is a traditional producer of common carp (*Cyprinus carpio*). Its annual production is approximately 18,000 tons, half of which is exported. Recently, the development of ornamental (koi) carp (*Cyprinus carpio koi*) farming has appeared in the country, both on small-scale farms and large production farms. Intensive carp farming is associated with risk for the incidence and spread of infectious diseases commonly associated with therapeutic and prophylactic use of antibiotics. In general, two or three agents have been granted marketing authorisations in EU countries (Smith, 2008). In the Czech Republic, for example, oxytetracycline and flumequine are permitted, with the former used extensively on common carp farms. On the other hand, the regulation of antibiotic use is not enforced on such ornamental fish farms as koi carp operations. Therefore, the ornamental fish producers tend to administer

* Corresponding author. Tel.: +420 41562274; fax: +420 549248841.
*E-mail address:* cizeka@vfu.cz (A. Čížek).

0378-1135/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2009.10.001

antibiotics in a non-systematic and uncontrolled manner, thus making the selection and spread of antibiotic-resistant bacteria possible. The results of existing studies show that administering antibiotics in "aquacultures" leads to higher antibiotic resistance across the entire microbial water ecosystem (Schmidt et al., 2000; Sørum, 2006) and thus also to higher risk for transmitting genetic resistance determinants to bacteria that are pathogenic for fish. Indicator bacteria suitable for studying the incidence and development of antibiotic resistance on fish farms include motile aeromonads. These bacteria are interconnected with the water ecosystem, colonize fish, and can cause various infectious processes in them (Austin and Austin, 2007). Sporadically, aeromonads have been proven to cause human infections (Janda et al., 1994; Ko et al., 2000).

To date, very little has been published on the susceptibility of bacteria isolated from common and koi carp worldwide (Guz and Kozinska, 2004; Taylor, 2003), while no data are available at all about bacterial susceptibility in the Czech Republic. The aims of this study were to identify the levels of antimicrobial resistance and to define resistance determinants in motile aeromonads isolated from koi and common carp coming from randomly chosen farms in the Czech Republic.

## 2. Materials and methods

### 2.1. Bacterial cultures

A total of 138 koi and common carp were tested in 2005 (7 localities) and 2006 (7 localities). Fish were subjected to bacteriological examination for differential diagnosis of KHV (koi herpes virus). No increase of morbidity or mortality was observed. Gills and skin swabs from euthanized fish were cultured on Columbia agar (CM331, Oxoid Ltd., Basinstoke, UK), supplemented with 5% sheep blood, in order to isolate *Aeromonas* spp. If the fish had ulcerous changes on skin or gills, samples were taken from these ulcers preferentially. Inoculated agars were incubated at $28 \pm 2\,^{\circ}C$ for 24–48 h. Primary bacterial cultures thus obtained were preidentified according to morphology of colonies, Gram reaction, motility, catalase and oxidase production, and resistance to vibriostatic agent O/129 (Rahman et al., 2002). Presumptive cultures of *Aeromonas* spp. were examined using API 20E test (bioMérieux, France). Esculin hydrolysis and growth on triple sugar iron agar slant were used as supplementary tests. Phenotypic identification was done using Aerokey II (Carnahan et al., 1991). Identified cultures were stored in cryoprotective medium at $-80\,^{\circ}C$ and tested for susceptibility to antimicrobials and the presence of resistance determinants. For these tests, no more than 1 *Aeromonas* spp. isolate from each fish was used. Quality control was done with reference strains *Escherichia coli* ATCC 25922 and *Aeromonas salmonicida* subsp. *samonicida* ATCC 33658.

### 2.2. Determination of susceptibility to antimicrobials

Susceptibility tests were performed by agar dilution method in accordance with Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guidelines (CLSI, 2006) and using the following antibacterial substances: chloramphenicol, ciprofloxacin, florfenicol, oxolinic acid, oxytetracycline, trimethoprim, spectinomycin, and streptomycin (Sigma–Aldrich, Prague, Czech Republic). Antimicrobial agents were incorporated into Mueller-Hinton agar (Oxoid Ltd., Basingstoke, UK), with each plate containing the agent in log 2 doubling dilutions of the following ranges: chloramphenicol, florfenicol (2–64 μg/ml); ciprofloxacin, oxolinic acid and trimethoprim (0.25–16 μg/ml); oxytetracycline (1–64 μg/ml); streptomycin (0.5–64 μg/ml); and spectinomycin (2–1024 μg/ml). Frozen *Aeromonas* spp. isolates and control strains were inoculated on nutrient agar (CM3, Oxoid Ltd., Basingstoke, UK) and incubated at $28 \pm 2\,^{\circ}C$ for 24 h. Growth was resuspended in 2 ml sterile PBS using a sterile cotton-tipped swab at a concentration of $1.5 \times 10^8$ CFU/ml, in accordance with 0.5 McFarland standard. The turbidity was adjusted with a photometer (Densi-La-Meter, LIAP, Latvia). Plates were inoculated using a multipoint inoculator (Trios, Czech Republic), which delivered $10^5$ organisms per spot. Inoculated plates were incubated at $28 \pm 2\,^{\circ}C$ for 24–28 h.

The cultures were compared with a growth control plate containing no antibiotic, and minimum inhibitory concentration (MIC) for each agent was determined as the lowest concentration of an antimicrobial that inhibited the growth of a given culture. Each isolate was examined three times and each batch of media was checked using the aforementioned control strains. The values obtained were used to calculate $MIC_{50}$, $MIC_{90}$ and the MIC range. Criteria published in *Standards for susceptibility tests for bacteria isolated from animals* (CLSI, 2008) were used to evaluate susceptibility of isolates to chloramphenicol, ciprofloxacin, florfenicol, oxytetracycline, and trimethoprim. Cut-off values for interpreting MICs for oxolinic acid, streptomycin, and spectinomycin were not determined.

### 2.3. Detection of resistance genes, integrons and integron-associated genes

All isolates were tested by PCR for *tet*(A-E), the class 1 and 2 integrase genes *intI1* and *intI2*, respectively, the variable region of class 1 and class 2 integrons (designed by primers 5CS-3CS and Hep74-Hep51), gene cassettes inside the integron structure, and the *sul1* gene which is an essential part of the class 1 integron. This test technique has been previously described by Dolejská et al. (2007) and Wilkerson et al. (2004).

### 2.4. Data analysis

The results obtained were statistically evaluated by chi-square test using MS Excel® software with the *Analyse-it* module. Differences with $P \leq 0.05$ were considered as significant.

## 3. Results

### 3.1. Susceptibility to antimicrobials

In total, 121 presumptive isolates were identified as *A. sobria* (60 isolates), *A. hydrophila* (55 isolates) and *A.*