



Food and Agriculture
Organization of the
United Nations

FMM/RAS/298: Strengthening capacities, policies and national action plans on
prudent and responsible use of antimicrobials in fisheries Final Workshop
in cooperation with AVA Singapore and INFOFISH
12-14 December, Concorde Hotel, Singapore

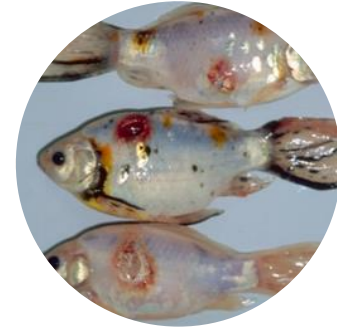
Global ornamental fish trade as a risk of AMR development and transfer

Olga Haenen

Olga.haenen@wur.nl



WBVR National Reference Laboratory for Fish, Shellfish and Crustacean Diseases



Betty van Gelderen
Ineke Roozenburg
Michal Voorbergen
Olga Haenen
Marc Engelsma



Background

- Globally: > 1 billion ornamental fish transported
- 50% from all tropical ornamental fish originates from SE-Asia
- 80% from this is freshwater cultured fish
- The Netherlands is an important import- and transfer port: billions of ornamental fish per year
- In thousands of Dutch households 11 billion ornamental fish are kept in aquaria



Background (2)



- Transport of live ornamental fish ➡ stress ➡ immune suppression ➡ susceptible to bacterial infections...
- Often preventive treatment with antibiotics at transport ➡ development of AMR in commensals and fish pathogens (including potential zoonotic)
- AMR bacteria in ornamental fish and AB residues in transport water ➡ **contact** with professionals in ornamental fish trade (both at export and import sites), and hobbyists, and **spread** to environment



...government
concern...

J Antimicrob Chemother 2014; **69**: 287–291
doi:10.1093/jac/dkt392 Advance Access publication 3 October 2013

Journal of
Antimicrobial
Chemotherapy

Carbapenemase-producing Enterobacteriaceae and
non-Enterobacteriaceae from animals and the environment:
an emerging public health risk of our own making?

Neil Woodford^{1,2*}, David W. Wareham², Beatriz Guerra³ and Christopher Teale⁴

**Action taken:
surveillance**



Rijksinstituut voor Volksgezondheid
en Milieu
Ministerie van Volksgezondheid,
Welzijn en Sport

“Active surveillance and monitoring for carbapenem-resistant bacteria in the food chain and other non-human sources is urgently needed, with an enhanced and rigorous follow-up of all positive results”.
(cit. Woodford et al., 2014)



➔ Schiphol-Project

“Investigation into Extended Spectrum Beta-Lactamase (ESBL)-, en Carbapenemase Producing (CP)-bacteria and potential zoonotic bacteria in ornamental fish imported into the Netherlands, and residues of antibiotics and antiparasitics in the transport water”

This was a project of WBVR, in name of our Dutch Vet Service, NVWA, and in cooperation with RIKILT of WUR



Potential zoonotic bacteria from fish, focus on:

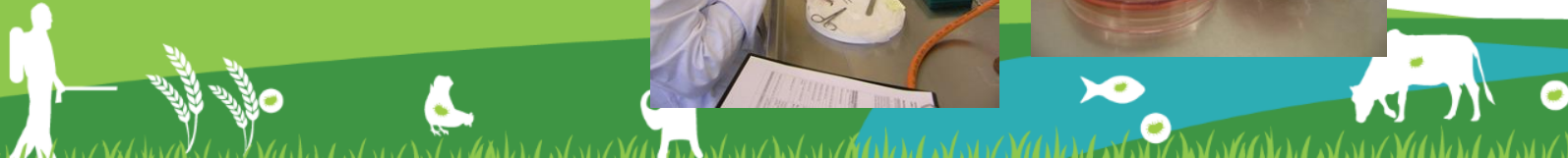
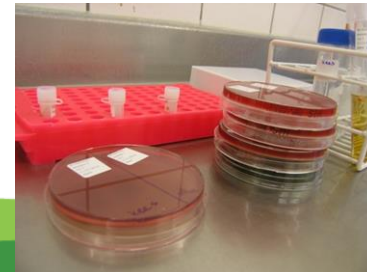
- *Edwardsiella tarda*
- *Streptococcus iniae*
- *Streptococcus agalactiae*
- *Vibrio vulnificus*
- *Mycobacterium marinum* and other *M. spp.*



Materials & Methods: Necropsy, bacteriology, and water sampling at WBVR



PROJECTNAMER 2024: nr. CVT 200000-02 VESSEN - 232285	
<p>Waarachtigheidsverklaring De afgegeven informatie is waarachtig en juist.</p> <p>Water De afgegeven informatie is waarachtig en juist.</p> <p>Miscel De afgegeven informatie is waarachtig en juist.</p>	<p>Bevestiging De afgegeven informatie is waarachtig en juist.</p> <p>Waarheid De afgegeven informatie is waarachtig en juist.</p>
<p>Waarheid en juistheid De afgegeven informatie is waarachtig en juist.</p> <p>Waarheid De afgegeven informatie is waarachtig en juist.</p>	<p>Waarheid De afgegeven informatie is waarachtig en juist.</p> <p>Waarheid De afgegeven informatie is waarachtig en juist.</p>
<p>Waarheid De afgegeven informatie is waarachtig en juist.</p> <p>Waarheid De afgegeven informatie is waarachtig en juist.</p>	<p>Waarheid De afgegeven informatie is waarachtig en juist.</p> <p>Waarheid De afgegeven informatie is waarachtig en juist.</p>



Materials & methods AMR lab

Ornamental Tropical Fish & Transport Water

- 50 batches for importers
- From 13 third countries
- 2-3 fish, 500 + 100ml water (AMR & residues)
- Nov 2014-Feb 2015

In collaboration with NVWA

Additional water sampling: Surface water (NL)

- 24 samples
- 100 ml
- March 2015



Real-Time PCR

Detect in a single assay

OXA-48
IMP
VIM
KPC
NDM

- Sample inoculated in TSB
- DNA extraction using Qiagen blood and tissue kit
- RT-PCR (CarbaCheck MDR RT) to detect bla_{KPC} , bla_{NDM} , bla_{IMP} , bla_{OXA-48} and bla_{VIM} families

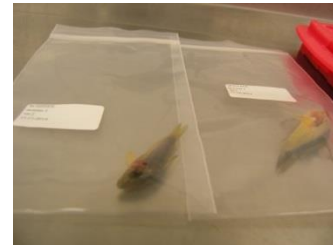


Fish species (36 species/genera from 13 countries)



Fish sampling

- Euthanized with overdose 2-Phenoxy-ethanol, decap
- Skin inoculated on sheep blood- and TCBS agars
- Liver inoculated at sheep blood agar
- Liver smear for Ziehl Neelsen stain, for *Mycobacterium spp.* In case positive: isolation and PCR
- Gut inoculated at MacConkey-agar to isolate *E. coli* ➔ AMR lab
- Piece of gut put into Trypton Soy Broth (TSB) to culture possible resistant bacteria ➔ AMR lab
- Rests stored at -20°C

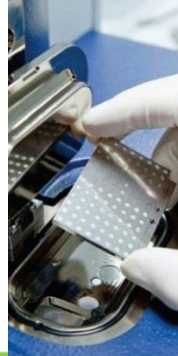
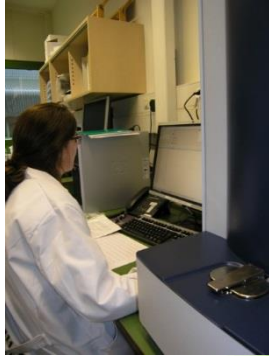


Identification of bacteria

- After 2-7d incubation at 20°C: 1 of 50 batches no bact growth
- 321 pure cultures ➡ MALDI-TOF
 - 49 not identifiable
 - 55 up to genus
 - 217 up to species
 - Of these: 53 x *Aeromonas* spp. and 3x *Vibrio* spp.
- *Aeromonas* en *Vibrio* spp. selected for antibiogram
- *Shewanella*'s sent to AMR lab



MALDI-TOF to identify bacteria



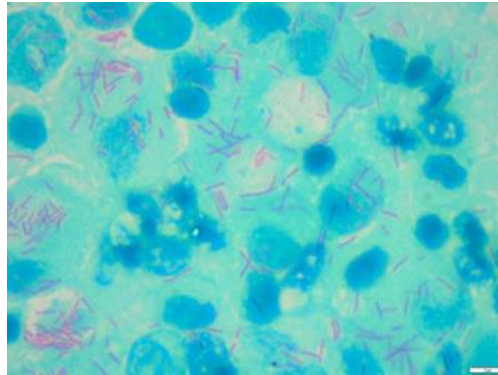
Potential zoonotic bacteria

- Not found: *Vibrio vulnificus*, *Edwardsiella tarda*, *Streptococcus iniae*, *Streptococcus agalactiae*
- Found: other potential zoonotic bacteria: ***Elisabethkingia meningoseptica*** in 9 of 50 batches fish, from:
 - Singapore (5 batches: 2x goldfish, 1x *Corydoras pataetus*, 1x *Corydoras aeneus* longfin, and 1x *Hyphessobrycon bentosii*)
 - Sri Lanka (3 batches: 1x Dalmatian molly, 2x *Poecilia reticulata* (guppy))
 - Brazil (1 batch: *Otocinclus* spp.)



TBC-lab: *Mycobacterium*

- No *M. marinum* (fish TBC) found
- 1x strong positive ZN in *Hemigrammus caudovittatus* from Indonesia
- PCR tests ➔ ***Mycobacterium haemophilum***



- And 1 goldfish from China: ***Mycobacterium* spp.** (no *marinum/ulcerans/haemophilum*)



Risks of these potential zoonotic bacteria

Elisabethkingia meningoseptica:

- Tuon et al. 2007: may cause meningitis, especially in neonatal intensive care. Seldomly noso-comial pneumonia, endocarditis, post-operative bacteremia, and other, especially when immuno-compromized

Mycobacterium haemophilum:

- Lindeboom et al., 2011: skin ulcers and arthritis in humans, seldomly lung inflammation, when immuno-compromized, in healthy children cervical and perihilar lymphadenitis



Antibiogram against 6 “fish”-antibiotics: *Aeromonas* and *Vibrio* spp. (n = 53 and 3)

- 84,7% resistant against **tetracycline**
- **52.5%** against **flumequine**
- **30,5** and **33.9%** against **trimethoprim-sulfa**, and **neomycine** respectively
- **8,5 %** against **florfenicol**
- **17%** against **nitrofurantoin**
- *Aeromonas* species from Singapore and Congo showed relatively much resistance



Transport water (analysed at RIKILT)



- **98%** of 50 water samples contained one or more of the antibiotics, mostly **tetracyclines** and **quinolones**, in concentrations from 0.02 to 10000 µg/L.
- **36%** of 50 water samples contained **chloramphenicol**,
- **68%** **nitrofuranes** and
- **14%** non-licenced **malachite green**
- There was a high correlation between residues in water and resistance of isolated *Aeromonas* spp. to tetracyclines and quinolones.



AMR lab: OXA48 detection

- All samples were negative for carbapenemase families bla_{KPC} , bla_{NDM} , bla_{IMP} and bla_{VIM}
- Variants of bla_{OXA-48} were identified by RT-PCR in 92 samples, independent of the source, chromosome located (**non transferable** = good news)

Ornamental Fish & Transport Water

- 76 positive samples (fish = 37, transport water = 39)
- 41 out of 50 lots (**82%**)
- Water samples more positive (**78%**) than fish (**36%**)

Surface water (NL)

- 9/24 surface water samples (**37,5%**)
- 5 provinces across the Netherlands



Conclusions: OXA48 detection

- *bla*_{OXA48}-like genes widespread
- High prevalence is a result of high sensitivity of the method
- *Shewanella* isolation is not surprising as it is an ubiquitous aquatic organism and was proven to be the environmental reservoir of the *bla*_{OXA-48} family.
- These genes are considered of environmental origin and **not** a public health risk
- MIC values only marginally increased to ertam-, mero- and imipenem
- We need to stay cautious, as other resistance genes may be transferred through ornamental fish trade
- Is there contact with edible fish culture?

Ceccarelli D et al., 2017. Chromosome-Based *bla*OXA-48-Like Variants in *Shewanella* Species Isolates from Food-Producing Animals, Fish, and the Aquatic Environment. *Antimicrob Agents Chemother.* 61(2). pii: e01013-16. doi: [10.1128/AAC.01013-16](https://doi.org/10.1128/AAC.01013-16).

Business card Daniela Ceccarelli is available



Conclusions (2)

- Most transport water samples contained residues of authorized and non-authorized antibiotics, some also malachite green.
- Fish mostly carried resistant opportunistic *Aeromonas* spp. mainly against oxytetracycline.
- Fish bacteria from Singapore, Congo showed relatively high levels of multi-resistance to antibiotics.
- Fish imports may pose a risk to man, at direct contact with fish and transport water. Current EU border inspections for import control do not consider these risks.
- Awareness about these risks for the ornamental fish branch, fish hobbyist, veterinarians, medical practitioners and governmental authorities important. Hygienic measures to be in place.
- Regular screening for potential zoonotic bacteria and antimicrobial resistance of bacteria from imported ornamental fish is important.



Participants, and Acknowledgements

We thank **NVWA** for funding and assistance in sampling

- **RIKILT:** Lina Stolker, Tina Zuidema
- **AMR lab:** Dik Mevius, Daniela Ceccarelli, Kees Veldman, Alieda van Essen, Joop Testerink, Marga Japing, Cindy Dierickx
- **Fish disease lab:** Ineke Roozenburg, Michal Voorbergen, Betty van Gelderen, Marc Engelsma, Olga Haenen
- **TBC-lab:** Karel Riepema, Robin Ruuls, Douwe Bakker, Ad Koets
- Dutch importers of ornamental fish

I thank the organizers of this meeting for inviting me

Thank you for your attention