Pathogenic Gram-negative bacteria isolated from ornamental fish in Uruguay: characterization and antibiotic resistance

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Abstract

Ornamental fish culture and trade is a major global activity. Bacteria are the main pathogenic microorganisms affecting fish, generally in the form of opportunistic pathogens. Gram-negative bacteria found following a surveillance of ornamental fish farms and pet shops in Uruguay are described. A total of 65 fish were examined (31 goldfish and 34 tropical fish). Isolates were phenotypically identified using the Microbact 2000 kit (OXOID) and antibiotic sensitivities were assessed using Oxoid BBL Sensi-Discs. At least 20 bacterial species were identified, with *Aeromonas*, *Pseudomonas* and *Vibrio* the most frequent genera. Mesophilic motile *Aeromonas* spp. were the most frequently identified isolates. Significant differences concerning antibiotics sensitivity were found, and they were divided into two groups: those with high sensitivity (chloramphenicol; tetracycline, oxytetracycline, oxolinic acid and sulphamethoxazole/trimethroprim), and those with low sensitivity (penicillin and ampicilin/sulbactam). Multi-resistance was observed in 45% of the isolates. All these organisms likely pose a limited risk to humans, ornamental fish handlers should be warned of the potential zoonotic risks involved.

Introduction

Ornamental fish culture and trade is a major activity with greater than 1 billion animals per year traded globally (Whittington and Chong, 2007). Ornamental fish are exposed to many stressors, mainly as a result of their complex supply chain, making them susceptible to many diseases. Ectoparasites and bacteria are the most significant ornamental fish pathogens. Gram negative bacteria are the main pathogenic microorganisms affecting fish. Extrinsic stressors, including crowding, shipping and poor water quality can predispose ornamental fish to bacterial diseases (Lewbart, 2001). The use of antibiotics for aquaculture and companion animals is restricted in most countries (FDA, 2012; Costello et al., 2001). However ornamental fish are often subjected to treatments without a veterinary prescription. These treatments include the use of a number of antibiotics that can be applied inappropriately. As a result, bacteria isolated from ornamental fish often show multi-resistance to several antibiotics (Yanong, 2010; del Rio-Rodriguez and Turnbull, 2002). The determination of the resistance levels of bacteria to various antibiotics is a helpful guide to therapy. A further concern is that some fish pathogenic

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bacteria may be zoonotic. Ornamental fish are a potential reservoir of infectious agents for wild and farmed fish and humans. This study is a description of the Gram-negative bacteria found following surveillance of ornamental fish farms and pet shops in Uruguay.

Material and methods

Fish were obtained from pet shops or ornamental fish farms. Only fish with evident signs of disease were sampled. A total of 65 fish were examined (31 goldfish and 34 tropical fishes). The fish species examined were: goldfish, *Carassius auratus*; guppy, *Poecilia reticulatus*; platy, *Xiphophorus maculatus*; swordtail, *Xiphophorus helleri*; Molies, *Poecilia sphenops* and *P. latipinna*; discus, *Symphisodon aequifasciata*; dwarf gourami, *Trichogaster lalius*; and peppered catfish, *Corydoras paleatus*. Microbiological samples were taken from the skin (from fish with skin ulcers) and kidney (in all cases). The samples were plated onto trypticase soy agar (TSA) and incubated at 25 °C for 24 h. Resultant colonies were tested for Gram stain, cytochrome oxidase activity and motility. Microbact 2000 kit (OXOID) was used for identification, with samples incubated at 25 °C for 48 h prior to analysis. To determine the sensitivity to a range of antibiotics Oxoid BBL Sensi-Discs were used. These included penicillin (P; 10 IU), chloramphenicol (C; 30 μg), tetracycline (TE; 30 μg), oxytetracycline (OT; 30 μg), Oxolinic Acid (OA; 2 μg), ampicillin/sulbactam (SAM; 10/10 μg) and sulfametoxazole/trimethroprim (STX; 23.5/1,25 μg). Isolated colonies were suspended in saline and adjusted to a density of 0.5 according to McFarland turbidity standards. TSA plates were inoculated by streaking with swab over the entire agar surface and incubated at 25 °C for 24 h. Four discs per plate were used (NCCLS, 2002). Criteria for determining susceptibility are shown in Table 1. Bacteria falling into the categories of susceptible or intermediately susceptible were both considered as being susceptible, according to del Rio-Rodriguez and Turnbull (2002). Results were statistically evaluated using χ^2 for

Table 1. Criteria for antimicrobial susceptibility determination

Antimicrobial agent	Inhibition zone diameter (mm) Interpretative criteria		Source
	Sensitivity	Resistant	
Penicillin (P)	≥20	≤19	NCCLS, 2002
Chloramphenicol (C)	≥ 13	≤ 12	del Rio-Rodriguez & Turnbull, 2002
Tetracycline (TE)	≥ 15	≤ 14	CLSI, 2013
Oxytetracycline (OT)	≥ 14	≤ 13	del Rio-Rodriguez & Turnbull, 2002
Oxolinic Acid (OA)	≥ 17	≤ 16	del Rio-Rodriguez & Turnbull, 2002
Ampicillin/Sulbactam (SAM)	≥12	≤ 11	CLSI, 2013
Sulfamethoxazole/Trimethroprim (SXT)	≥ 11	≤ 10	CLSI, 2013

the proportions of isolates susceptible to the different antibiotics.

Results

A total of 67 isolates were obtained: 35 from pet shop fishes and 32 from farm fishes. A total of 20 bacterial species were identified (Table 2). The most common bacterial genera were: *Aeromonas*, *Pseudomonas* and *Vibrio*, with representatives from all three genera found from both pet shops and fish farms identified. There was no significant difference between the number of bacterial genera identifications in each place (farm or pet shop). The most frequently identified bacteria was *Aeromonas* spp. (49.2 % isolates).

Antibiotic sensitivities for the different isolates are shown in Table 2. There were significant differences between the sensitivity of the isolates to the different antibiotics ($\chi^2 = 32.39$; $p<0.01$). The results indicated that antibiotics may be broadly divided in two groups: those with the isolates highly sensitive to (C; TE, OT, OA and STX) and those with most of the isolates resistant to (P and SAM). Multi-resistance was observed in 45% of the isolates.

Discussions and conclusions

The identification of isolates indicated at least 20 different bacterial species and three prevalent genera: *Aeromonas*, *Pseudomonas* and *Vibrio*. This is similar to the results obtained by Stoskopf (1993), Aoki (1999) and Lewbart (2001).

The genus *Aeromonas* is widely distributed in nature and found especially in freshwater environments, especially if they are rich in organic matter. *Aeromonas* species are associated with a wide range of infections in poikilotherm animals (amphibians, fish and reptiles), the most frequently isolated species are *A. hydrophila*, *A. caviae* and *A. veronii* (Janda et al, 1995; Lewbart, 2001). Our results showed *A. hydrophila* -like isolates were the most dominant TSA-culturable bacteria recovered (37.3 %). This is similar to previous studies from Uruguay (Chaves et al., 2003; Carnevia et al., 2010) and other countries (Kuo and Chung, 1994; Shome and Shome, 1999; Manfin et al, 2002; Locatelli et al, 2003). *A. hydrophila* can be a primary pathogen, however it is more commonly implicated as a secondary opportunistic pathogen, often associated with poor water quality or a serious errors in production management (Padrós and Furones; 2004). *Pseudomonas stutzeri*, *P. aeruginosa* and *P. fluorescens* have been associated with diseases in farmed fish and crustaceans (Follet and Grischkowsky, 1981; Nabi et al., 2000) as well as ornamental fish (Barker, 2001; Negrete et al., 2003). They can also act as opportunistic pathogens, producing pathologies characterized by bleeding skin and fins and tail ulcers. *Vibrio* spp. are important pathogens of marine organisms, but have also been isolated from freshwater ornamental fish, resulting in septicemia, fin rot, skin ulceration and ophthalmitis (Hacking and Budd, 1971; Hettiarachchi and Cheong, 1994; Kuo and Chung, 1994; Manfin et al., 2002). Other bacteria isolated in this study (*Escherichia coli* – inactive; *Acinetobacter haemolyticus*, *Stenotrophomonas maltophila*, *Burkholderia pseudomallei*) may also be opportunistic pathogens that can affect weakened fish (Kuo and Chung, 1994; Evans et al., 2001; Musa et al., 2008).

Some of the bacterial species which cause disease in fish may also cause disease in humans, including organisms from the genera *Aeromonas*, *Pseudomonas, Vibrio, Acinetobacter* and *Plesiomonas* (Wolf, 1998; Lowry and Smith, 2007; Austin,

Table 2. Bacterial species isolated from ornamental fish in Uruguay and antibiotic sensivity (% susceptible strains).

Bacterial species	N° ident.	P	C	TE	OT	OA	SAM	SXT
Acinetobacter haemolyticus	1	0	100	-	100	0	100	0
Actinobacillus sp.	3	66.6	100	100	100	100	0	100
Aeromonas caviae	3	0	100	0	100	100	0	100
Aeromonas hydrophila	22	9.4	91.2	47.8	80	80	24.2	79.4
Aeromonas veronii bv sobria	6	0	100	50	50	100	20	80
Aeromonas veronii bv veronii	2	0	100	50	-	0	0	50
Brevundimonas vesicularis	1	0	100	-	100	100	100	100
Burkholderia pseudomallei	3	0	100	100	66.6	66.6	100	66.6
Cedecea davisae	1	0	100	100	-	100	-	100
Comamonas acidovorans	2	0	100	-	100	100	0	100
Escherichia coli- inactive	1	0	100	-	100	100	0	100
Pseudomonas aeruginosa	6	0	33.3	66.6	100	33.3	33.3	33.3
Pseudomonas fluorescens	4	0	50	100	100	50	0	50
Pseudomonas stutzeri	3	0	100	100	100	100	66.6	100
Stenotrophomonas maltophilia	2	100	100	100	0	100	50	100
Vibrio alginolyticus	3	100	100	100	66.6	33.3	100	100
Vibrio mimicus	1	0	100	-	100	100	0	100
Vibrio parahaemolyticus	1	100	100	100	100	100	100	100
Ochrobactrum anthropi	1	100	100	-	100	100	100	100

P: penicillin; C: chloramphenicol; TE: tetracycline; OT: oxytetracycline; OA: oxolinic acid; SAM: ampicillin/sulbactam; SXT: sulfamethoxazole/trimethroprim; - not tested.

2010). In humans, these organisms can produce gastroenteritis, cellulitis, and septicemia, but usually have low virulence, typically affecting people with reduced immunocompetence (Stoskopf, 1993). Most of the bacteria isolated from ornamental fish in this study, pose a limited potential zoonotic risk, so people with depressed immunity should not handle these fish.

Contrary to expectations, bacteria in this study showed high sensitivity to most antibiotics tested, with low sensitivity only to penicillin and ampicillin/sulbactam. Although this is expected with Aeromonads, due to intrinsic resistance to beta-lactams. These results differ from those obtained by Musa et al. (2008) who found that 41% of the bacteria isolated from ornamental fish had low sensitivity to antibiotics and 23% intermediate sensitivity, with only 34% showing high sensitivity. Verner-Jeffreys et al (2009) also found low sensitivity to most of the antibiotics tested (tetracycline, quinolones,

fluoroquinolones, chloramphenicol, sulfamethoxazole / trimethoprim). These results are similar to those found by del Rio-Rodriguez and Turnbull (2002), who found low antimicrobial susceptibility in ornamental fish from South America and high in ornamental fish from South East Asia. In countries where ornamental fish culture is highly developed, the use of veterinary antibiotics is a common practice. This sustained selection pressure has likely resulted in reduced bacterial sensitivity to these drugs (Yanong, 2010). In Uruguay, production is based on small-scale artisan producers, who typically use antimicrobials reserved for human use. This explains why the observed sensitivity to the main Veterinary antimicrobial tested, tetracycline, oxytetracycline, oxolinic acid and sulfametoxazole/trimethoprim, was very high. The results of this study in general suggest that oxytetracycline, oxolinic acid and sulfametoxazole/trimethoprim should all be effective for the control of bacteria in Uruguay ornamental fish. Although these bacteria typically are of low virulence for humans, ornamental fish handlers should still be aware of the zoonotic risks involved and exercise appropriate precautions.

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