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Antimicrobial Resistance Patterns of *Aeromonas* spp. Isolated from Ornamental Fish

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Abstract

The potential risk of occurrence of new diseases associated with the trade of live animals is well known. However, little importance is still given to the problematic of the dissemination of resistance genes that pass along with the animal trade. In this study we aimed to isolate *Aeromonas* spp. strains from water and skin of ornamental fish and test their resistance to antibiotics. The samples were collected from a national ornamental fish importer, with the intent of obtaining a collection of *Aeromonas* strains. The identification of the strains was made by *gyrB and rpoD* gene sequencing. A total of 288 strains grouped in seven different species - *Aeromonas veronii, Aeromonas media, Aeromonas jandaei, Aeromonas hydrophila, Aeromonas caviae, Aeromonas culicicola, Aeromonas aquariorum*, were isolated. The susceptibility profile was determined for 28 antibiotics commonly used. All the strains presented multi-resistance to the tested antibiotics. The antibiotic susceptibility profile to tetracycline, ticarcillin, carbenicillin, ampicillin and erythromycin revealed resistance levels of more than 80%. Few strains resistant to aztreonam and imipenem were identified. On the other hand, all were sensitive to cefotaxime and cefepime. The results show that these *Aeromonas* spp. strains are potentially reservoirs of antibiotic resistance genes.

Keywords: *Aeromonas* spp.; Antibiotic resistance; Ornamental fish

Introduction

Bacterial disease is one of the most important diseases in ornamental fishes and a significant cause of high fish morbidity and mortality rates [1]. Many stress factors could contribute to bacterial infection in ornamental fish, namely, poor water quality, crowding, transportation and inadequate nutrition [2].

The genus *Aeromonas* belongs to the family *Aeromonadaceae* within the *Gammaproteobacteria* and comprises Gram-negative, nonspore-forming, motile bacilli or coccobacilli rods with rounded ends which measure 1-3,5 μm across [3]. They are facultative anaerobic, oxidase, catalase and indol-positive, able to reduce nitrate to nitrite and are, glucose-fermenting, generally resistant to the vibriostatic agent O/129 [4,5].

Members of the genus *Aeromonas* are found in a wide variety of ecological niches. They are able to inhabit surface water (rivers, lakes), sewage, drinking water (tap and bottled mineral), thermal water and sea water [6,7]. Some species, mainly the psychrophilic *Aeromonas salmonicida* and the mesophilic *Aeromonas hydrophila* and *Aeromonas veronii* are recognized causative agents of fish disease [3,8].

Infections caused by motile aeromonads are probably the most common bacterial disease of freshwater fish [9]. Resistance of *Aeromonas* spp. to commonly used antibiotics is an emerging problem in the ornamental fish. An increase in resistance levels of the genus *Aeromonas*, particularly to β-lactam antibiotics has been observed previously [10,11]. Antimicrobial resistance genes, including cassette-borne resistance genes in class I integrons, have been described as occurring in *A. salmonicida* and in motile aeromonads [12-14].

The objective of the present study is to isolate and identify *Aeromonas* spp. from the water of aquarium and the skin of imported ornamental fish and to evaluate their susceptibility to some antimicrobial agents.

Materials and Methods

Bacteria strains isolation and identification

This evaluation was conducted with samples of skin and water (30 and 14 according to the fish and tanks available, respectively) from imported ornamental fish. Water samples filtered onto nitrocellulose membranes and from fish skin were collected aseptically and incubated at 30°C for 24 h on GSP media (Oxoid, Basingstoke, UK). This media was used to isolate (typical colonies, i.e. yellow on GSP medium) and purify the strains. Bacteria strains were identified, following standard procedures, to identify *Aeromonas* at the genus level, and further standard biochemical classification was performed by using API 50 CH (bioMérieux) at 30°C for 48 h, following the manufacturer's instructions. Procedures and characteristics of oligonucleotide primers for amplification and PCR-based sequencing house-keeping genes (*gyrB and rpoD*) are as described previously [15]. PCR products were purified with QIAquick PCR purification kit (QIAGEN, Germany), following the manufacturer`s instructions and prepared for sequencing by using the Big Dye Terminator V.3.1 cycle sequencing kit and amplified genes were sequenced with an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, USA).

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Antibiotic susceptibility testing

Aeromonas spp. strains isolated in the present study were subjected to susceptibility testing against 28 antimicrobials commonly used. Susceptibility was determined by the disk-diffusion technique of Kirby-Bauer on Mueller-Hinton agar plates (Oxoid Basingstoke, UK) with inocula adjusted to an optical density of 0.5 McFarland standard units [16]. Disks containing ampicillin ($AMP_{10\mu g}$), carbenicillin (CAR100µg), amoxicillin ($AML_{10\mu g}$), amoxicillin/ clavulanic acid ($AMC_{30\mu g}$), piperacillin ($PRL_{100\mu g}$), piperacillin/ tazobactam ($TZP_{110\mu g}$), ticarcillin ($TIC_{75\mu g}$), ticarcillin/ clavulanic acid ($TIM_{85\mu g}$), cephalothin ($KF_{30\mu g}$), cefoxitin ($FOX_{30\mu g}$), cefotaxime ($CTX_{30\mu g}$), cefoperazone ($CFP_{30\mu g}$), ceftazidime ($CAZ_{30\mu g}$), ceftriaxone ($CRO_{30\mu g}$), cefepime ($FEP_{30\mu g}$), aztreonam ($ATM_{30\mu g}$), imipenem ($IMP_{10\mu g}$), gentamicin ($CN_{10\mu g}$), kanamycin ($K_{30\mu g}$), tobramycin ($TOB_{10\mu g}$), amikacin ($AK_{30\mu g}$), netilmicin ($NET_{30\mu g}$), tetracycline ($TE_{30\mu g}$), ciprofloxacin ($CIP_{5\mu g}$), norfloxacin ($NOR_{10\mu g}$), erythromycin ($E_{15\mu g}$), trimethoprim/sulfamethoxazole ($SXT_{25\mu g}$) and chloramphenicol ($C_{30\mu g}$) were used. All disks were obtained from Oxoid. After 24 h incubation at 30°C, organisms were classified as sensitive (S), intermediately resistant (I) or resistant (R) on the basis of the size of the zone of bacteria growth inhibition according to the guidelines of the CLSI (2010).

Results and Discussion

The genus *Aeromonas* has been the subject of various antimicrobial susceptibility studies over the last years. Although *Aeromonas* species are distributed throughout the world, there are geographic differences in the frequency of diseases caused by these bacteria [3].

A total number of 299 isolates were obtained from aquaria of ornamental fish shops (221 from skin and 77 from water). Using *gyr*B and *rpo*D sequencing several species of *Aeromonas* were identified, namely 110 *Aeromonas veronii* (36, 8%), 106 *Aeromonas hydrophila* (35, 5%), 43 *Aeromonas aquariorum* (14, 4%), 24 *Aeromonas* culicicola (8, 0%), 3 *Aeromonas media* (1, 0%), one *Aeromonas* caviae (0, 4%), and one *Aeromonas jandaei* (0, 4%). *A. hydrophila* has been the most common bacteria associated with aquatic animal disease. In a Malaysian aquarium shop, 60% of *A. hydrophila* were isolated from sick freshwater ornamental fish [2]. Other reports also refer to the antimicrobial susceptibility of clinical isolates of this specie [17] and in a prevalence study of fish and prawn from south India market, 33.5% and 17.6% of *A. hydrophila* were isolated, respectively [18].

Strains of *Aeromonas* spp. (n = 225) characterized genetically (43 *Aeromonas aquariorum*, 67 *A. hydrophila*, 94 A. veronii, 16 *A. culicicola*, 3 *A. media*, 1 *A. caviae* and 1 *A. jandaei*) were tested for susceptibility to a panel of 28 antibiotics. The results are presented in Table 1 (in percentage); however, the values regarding *A. media, A. caviae* and *A. jandaei* are not included due to the small number of isolates found. Our results show the existence of differences in some of the antibiotics tested according to the species and a high incidence of resistance of *Aeromonas* isolates to β-lactams antibiotics, as 95% were resistant to amoxicillin, 96% to carbenicillin and 94% to ampicillin (Table 1). It is noteworthy, that the main differences were in the isolates of *Aeromonas aquario-*

Antibiotic	*A.aquariorum* (n = 43)			*A. hydrophila* (n = 67)			*A. veronii* (n = 94)			*A. culicicola* (n = 16)			Total %
	R	I	S	R	I	S	R	I	S	R	I	S	R
AMP	100	0	0	93	0	7	96	0	4	100	0	0	94
CAR	100	0	0	99	0	1	96	0	4	100	0	0	96
AML	100	0	0	99	0	1	96	0	4	94	0	6	95
AMC	33	5	62	9	13	78	11	31	42	25	1	74	15
TIC	42	6	52	81	5	14	82	8	10	94	0	6	73
TIM	2	2	96	15	11	74	33	17	50	50	0	50	22
PRL	7	0	93	7	0	93	6	0	94	6	0	94	7
TZP	5	0	95	1	0	99	3	0	97	0	0	100	3
KF	88	0	12	37	5	58	10	0	90	6	0	94	32
FOX	98	0	2	1	0	99	9	0	91	19	0	81	24
CRO	0	0	100	0	0	100	7	0	93	31	0	69	5
CAZ	0	0	100	1	0	99	5	0	95	19	0	81	4
CFP	16	1	83	3	4	93	1	0	99	0	0	100	4
CTX	0	0	100	0	0	100	0	0	100	0	0	100	0
FEP	0	0	100	0	0	100	0	0	100	0	0	100	0
ATM	0	0	98	0	0	100	0	0	100	0	0	100	0
IMP	2	0	98	0	0	100	1	0	99	0	0	100	1
CIP	7	1	92	43	4	53	7	7	86	6	0	94	18
NOR	7	0	93	34	0	66	7	0	93	13	0	87	16
TOB	51	1	48	7	6	87	14	0	86	13	0	87	19
AK	16	0	84	3	0	97	4	6	90	6	0	94	6
K	40	2	58	34	0	66	27	24	49	31	0	69	31
CN	26	0	74	31	0	69	26	10	64	38	0	62	28
NET	23	0	77	6	0	94	9	0	91	13	0	87	11
TE	88	0	12	69	0	31	86	0	14	88	0	12	80
C	14	0	86	25	0	75	5	0	95	6	0	94	13
E	93	0	7	96	0	4	85	7	8	81	0	19	88
SXT	49	0	51	40	0	60	29	0	71	38	0	62	36

Table 1: Susceptibility profile (%) to antibiotics of *Aeromonas* spp. (n=220) isolates.

rum. For this specie, regarding the β-lactams antibiotics, ampicillin, carbenicillin, amoxicillin, cephalothin and cefoxitin were less effective and of the aminoglycosides antibiotics the most effective was amikacin (84%). Moreover, *Aeromonas hydrophila* showed values to quinolones (ciprofloxacin and norfloxacin) about 40% and on the other hand no significant difference in the values of resistance found in the remain species studied.

Identical susceptibility patterns to β-lactams antibiotics were found for the species of *A. hydrophila*, *A. veronii, A. culicicola* (Table 1), *A. media, A. caviae* and *A. jandaei*, with exception of cephalothin and cefoxitin that for these strains were more effective. *Aeromonas* isolates from different sources have been reported to have a relatively high resistance to β-lactams antibiotics, usually correlated with naturally occurring phenotypes of β-lactamases production [19]. The combination of aminopenicillin and carboxipenicillin with a β-lactamases inhibitor was effective in reducing resistance, as shown by the decrease in the proportion of resistant strains: 95% (amoxicillin) versus 15% (amoxicillin/clavulanic acid); 73% (ticarcillin) versus 22% (ticarcillin/clavulanic acid), that was more pronounced with amoxicillin. Nevertheless, these results are in agreement with the statement above, described in others studies [8], indicating that the penicillins resistance is probably due to the action of the inducible penicillinases susceptible to clavulanic acid.

The isolates found in this work from the species of *A. hydrophila*, *A. veronii, A. culicicola* were observed strains with sensitivity to aminopenicillins. The isolates from *A. aquariorum, A. media, A. caviae* and *A. jandaei* did not reveal sensitivity to any of these antibiotics. The results show that by using a culture media with ampicillin for the isolation of the genus *Aeromonas*, we may be underestimating the presence of these microorganisms from the different environments where they are found. Previous studies related to that *Aeromonas* strains are 100% resistant to ampicillin, which is generally included in culture media for the isolation of aeromonads [20]; but this observation was based on studies using clinical isolates and it is possible that in a natural environment the selective constraints are different.

High resistance to first and second-generation cephalosporins (cephalothin and cefoxitin, respectively) has been detected in motile aeromonad isolates [21,22] and are in accordance with our results for the strains of *A. aquariorum* measured in 88% and 98% of the isolates. Decreased susceptibility to third generation cephalosporins were previously reported [19]. A previous work [23] studied the presence of *Aeromonas* strains in mussels from the Adriatic Sea, reported isolates of *A. hydrophila, A. caviae* and *A. bestiarium*. These authors tested the activity of cephalosporins, first and third-generation (namely, cephalothin and cefotaxime). For cephalothin, we were obtained 100% of resistance in all species, which was in accordance with the results obtained in the present study in relation to the isolates of *A. aquariorum*, however, the values found for *A. hydrophila* were lower (37% of resistance). Regarding the results for cefotaxime, the same authors report 4% of resistance to this antibiotic from isolates that belong to *A. hydrophila.*

Aztreonam, a monobactam antibiotic was effective against all species (two isolates resistant). Remarkably in this work, imipenem resistance was observed in three isolates of *Aeromonas* (1 *A. aquariorum, 1 A. veronii and 1 A. jandaei*). Other studies also reported the incidence of strains resistant to this antibiotic [24,25]. Resistance to imipenem in non clinical strains supposed not subjected to selective pressure by use of such drug is a worrying trait as this is a last-resort antimicrobial agent used in the clinical environment. Chloramphenicol showed the highest efficacy against the bacterial strains tested (87% sensitive and 13% resistant). Tetracycline resistance was 80% for *Aeromonas* spp. isolated, with no differences observed in these studied species. The resistance to tetracycline has been reported to be acquired and encoded by plasmids or transposons [26-28]. Ciprofloxacin and norfloxacin resistance was more prevalent among *A. hydrophila* isolates (43% and 34%, respectively) than the other species. Commonly, quinolones are synthetic antibiotics used as first therapeutic options for *Aeromonas* infections in humans [29,30], also used in the treatment of bacterial fish diseases [31]. These drugs can persist for a long time in the environment, which could favor the emergence of resistant strains in environmental samples. The relatively high rates of resistance towards tetracycline and quinolones antibiotic might be due to extensive use of such compounds in hospital environments [30].

The results found for the aminoglycosides (gentamicin, kanamycin, tobramycin, amikacin and netilmicin) were observed, the differences between the susceptibility profiles of the *A. aquariorum* and of the others species. The antimicrobial agent with the most effective activity to *Aeromonas* spp. was amikacin (6% of resistance). The susceptibility tests with gentamicin and kanamycin revealed the highest percentages of resistance (28% and 31%, respectively). Notably, 50% of the isolates of *A. aquariorum* showed resistance to tobramycin.

The trimethoprim/sulfamethoxazole susceptibility tests revealed a percentage of resistance 29% and 49% for the isolates in the present study (Table 1), with the lowest values found for *A. veronii* and the highest for A. aquariorum. The 3 isolates of *A. media* and one of *A. jandaei* revealed sensitivity to this antibiotic, while the isolate from *A. caviae* showed the resistance to trimethoprim/sulfamethoxazole. A previous work [25] on the characterization of *Aeromonas* spp. in samples of frozen fish reported a resistance for this antibiotic of 49%, and the isolates from *A. veronii* presented 25% of resistance, that are similar to the values found in the present work.

A.salmonicida which is a known as fish pathogenic agent was not found in this study. This fact might suggest that this specie is not frequent in ornamental fish infections, as previously reported on South African ornamental fish [32]. Mesophilic aeromonads are considered to be opportunistic pathogens, capable of producing infections in weakened fish or as secondary invaders in fish populations suffering from others diseases [15,33].

The present study revealed *Aeromonas* species are common inhabitants of aquatic ecosystems. Through genetic sequentiation were found 288 isolates that belong to 7 different species of this genus. There is a frequent occurrence and a considerable diversity of *Aeromonas* spp. in ornamental fish. All the isolates tested presented multi resistance to the used antibiotics. Some strains were resistant to all aminoglycosides tested. This was verified in 3% (2 out of 67) of the isolates of *A. hydrophila* and 16% (7 out of 43) of *A. aquariorum*, collected from the water and skin. Also, there was a crossed multi resistance between aminoglycosides, quinolones, tetracycline, chloramphenicol, erythromycin and trimethoprim/sulfamethoxazole. The patterns of antibiotic resistance displayed by these organisms increase their potential health hazard and their broad distribution on different habitats is a problematic question. Therefore, these *Aeromonas* spp. strains showed to be potential reservoirs of antibiotic resistance genes, being of high importance to perform monitoring studies in order to evaluate and control its dissemination in aquatic environments. Thus ornamental fish can be considered a possible transmission route for aeromonads, however, further studies should be performed.

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References

1. Barker G (2001) Bacterial diseases. BSAVA manual of ornamental fish. Wildgoose WH (Ed), 185-194.
2. Musa N, Wei SL, Shaharom F, Wee W (2008) Surveillance of bacteria species in diseased freshwater ornamental fish from aquarium shop. World Appl Sci J 3: 903-905.
3. Janda JM, Abbott SL (2010) The genus *Aeromonas*: taxonomy, pathogenicity, and infection. Clin Microbiol Rev 23: 35-73.
4. Martin-Carnahan A, Joseph S (2005) Genus I. *Aeromonas*. Bergey`s manual of systematic bacteriology, 556-578.
5. Yanez MA, Catalan V, Apraiz D, Figueras MJ, Martinez-Murcia AJ (2003) Phylogenetic analysis of members of the genus *Aeromonas* based on gyrB gene sequences. Int J Syst Evol Microbiol 53: 875-883.
6. Figueras MJ (2005) Clinical relevance of *Aeromonas* sM503. Rev Med Microbiol 16: 145-153.
7. Beaz-Hidalgo R, Alperi A, Bujan N, Romalde JL, Figueras MJ (2010) Comparison of phenotypical and genetic identification of *Aeromonas* strains isolated from diseased fish. Syst Appl Microbiol 33: 149-153.
8. Saavedra MJ, Guedes-Novais S, Alves A, Rema P, Tacao M, et al. (2004) Resistance to beta-lactam antibiotics in *Aeromonas hydrophila* isolated from rainbow trout (*Oncorhynchus mykiss*). Int Microbiol 7: 207-211.
9. Kadlec K, von Czapiewski E, Kaspar H, Wallmann J, Michael GB, et al. (2011) Molecular basis of sulfonamide and trimethoprim resistance in fish-pathogenic *Aeromonas* isolates. Appl Environ Microbiol 77: 7147-7150.
10. Rowe-Magnus DA, Guerout AM, Mazel D (2002) Bacterial resistance evolution by recruitment of super-integron gene cassettes. Mol Microbiol 43: 1657–1669.
11. Schmidt AS, Bruun MS, Dalsgaard I, Pedersen K, Larsen JL (2000) Occurrence of antimicrobial resistance in fish-pathogenic and environmental bacteria associated with four Danish rainbow trout farms. Appl Environ Microbiol 66: 4908–4915.
12. Jacobs L, Chenia HY (2007) Characterization of integrons and tetracycline resistance determinants in *Aeromonas* spp. isolated from South African aquaculture systems. Int J Food Microbiol 114: 295-306.
13. L'Abee-Lund TM, Sorum H (2001) Class 1 integrons mediate antibiotic resistance in the fish pathogen *Aeromonas salmonicida* worldwide. Microb Drug Resist 7: 263-272.
14. Schmidt AS, Bruun MS, Larsen JL, Dalsgaard I (2001) Characterization of class 1 integrons associated with R-plasmids in clinical *Aeromonas* salmonicida isolates from various geographical areas. J Antimicrob Chemother 47: 735-743.
15. Martinez-Murcia AJ, Saavedra MJ, Mota VR, Maier T, Stackebrandt E, et al. (2008) *Aeromonas aquariorum* sp. nov., isolated from aquaria of ornamental fish. Int J Syst Evol Microbiol 58: 1169-1175.
16. CLSI (2010) Performance standards for antimicrobial susceptibility testing. In: Information Supplement M100-S17. Clinical Laboratory and Standards Institute, Wayne, PA, USA, 76–79.
17. Vila J, Ruiz J, Gallardo F, Vargas M, Soler L, et al. (2003) *Aeromonas* spp. and traveler's diarrhea: clinical features and antimicrobial resistance. Emerg Infect Dis 9: 552–555.
18. Vivekanandhan G, Savithamani K, Hatha AA, Lakshmanaperumalsamy P (2002) Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. Int J Food Microbiol 76: 165-168.
19. Fosse T, Giraud-Morin C, Madinier I (2003) Phenotypes of beta-lactam resistance in the genus *Aeromonas*. Pathol Biol(Paris) 51: 290-296.
20. Radu S, Ahmad N, Ling FH, Reezal A (2003) Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malaysia. Int J Food Microbiol 81: 261-266.
21. Rall VLM, Iaria ST, Heidtmann S, Pimenta FC, Gamba RC, et al. (1998) *Aeromonas* species isolated from Pintado fish (*Pseudoplatystoma* sp): Virulence factors and drug susceptibility. Rev Microbiol 29.
22. Imziln B (2001) Occurrence and antibiotic resistance of mesophilic *Aeromonas* in three riverine freshwaters of Marrakech, Morocco. ScientificWorldJournal 1: 796–807.
23. Ottaviani D, Santarelli S, Bacchiocchi S, Masini L, Ghittino C, et al. (2006) Occurrence and characterization of *Aeromonas* spp. in mussels from the Adriatic Sea. Food Microbiol 23: 418-422.
24. Morita K, Watanabe N, Kurata S, Kanamori M (1994) β-Lactam resistance of motile Aeromonas isolates from clinical and environmental sources. Antimicrob Agents Chemother 38: 353-355.
25. Castro-Escarpulli G, Figueras MJ, Aguilera-Arreola G, Soler L, Fernandez-Rendon E, et al. (2003) Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. Int J Food Microbiol 84: 41-49.
26. Rhodes G, Huys G, Swings J, Mcgann P, Hiney M, et al. (2000) Distribution of oxytetracycline resistance plasmids between Aeromonads in hospital and aquaculture environments: implication of Tn1721 in dissemination of the tetracycline resistance determinant Tet A. Appl Environ Microbiol 66: 3883–3890.
27. Miranda CD, Zemelman R (2002) Antimicrobial multiresistance in bacteria isolated from freshwater Chilean salmon farms. Sci Total Environ 293: 207–218.
28. Casas C, Anderson EC, Ojo KK, Keith I, Whelan D, et al. (2005) Characterization of pRAS1-like plasmids from atypical North American psychrophilic *Aeromonas salmonicida*. FEMS Microbiol Lett 242: 59–63.
29. Jones BL, Wilcox MH (1995) *Aeromonas* infections and their treatment. J Antimicrob Chemother 35: 453-461.
30. Alcaide E, Blasco MD, Esteve C (2010) Mechanisms of quinolone resistance in *Aeromonas* species isolated from humans, water and eels. Res Microbiol 161: 40-45.
31. Giraud E, Blanc G, Bouju-Albert A, Weill FX, Donnay-Moreno C (2004) Mechanisms of quinolone resistance and clonal relationship among *Aeromonas salmonicida* strains isolated from reared fish with furunculosis. J Med Microbiol 53: 895-901.
32. Mouton A, Basson L, Impson D (2001) Health status of ornamental freshwater fishes imported to South Africa: A pilot study. Aquarium Sciences and conservation 3: 313-319.
33. Camus AC, Durborow RM, Hemstreet WG, Thune RL, Hawke JP (1998) *Aeromonas* bacterial infections – motile aeromonad septicemia. Southern Regional Aquaculture Center (SRAC) Publication 478.