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

Carla Dias1,2, Vânia Mota1, António Martinez-Murcia1 and Maria José Saavedra2,3,4*

1CECAV-University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal
2CITAB-University of Trás-os-Montes e Alto Douro, 5000-801 Vila Real, Portugal
3Department of Veterinary Sciences, School of Agriculture and Veterinary Science, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal
4Universidad Miguel Hernández, Orihuela E-03500, Alicante, Spain
5CIMAR/CIMAR - Centro Interdisciplinar de Investigação Marinha e Ambiental and ICBAS - Instituto

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, tioflavin, carbamycin, 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 cocobacilli 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 salmonica* 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. salmonica* 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).

*Corresponding author: Maria José Saavedra, Department of Veterinary Sciences, School of Agriculture and Veterinary Science, University of Trás-os-Montes e Alto Douro, Vila Real, Portugal, E-mail: saavedra@utad.pt

Received March 27, 2012; Accepted April 26, 2012; Published May 05, 2012

Citation: Dias C, Mota V, Martinez-Murcia A, SaavedraMJ (2012) Antimicrobial Resistance Patterns of *Aeromonas* spp. Isolated from Ornamental Fish. J Aquacult Res Dev 3:131 doi:10.4172/2155-9546.1000131

Copyright: © 2012 Dias C, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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, 10µg), carbenicillin (CAR100µg), amoxicillin (AM, 30µg), amoxicillin/ clavulanic acid (AMC, 30µg), piperacillin (PRL, 100µg), piperacillin/ tazobactam (TZP, 100µg), ticarcillin (TIC, 75µg), ticarcillin/ clavulanic acid (TIM, 30µg), cefalothin (KF, 30µg), cefotaxime (CTX, 30µg), cefoperazone (CP, 100µg), cefotaxime (CAZ, 30µg), ceftriaxone (CRO, 30µg), ceftazidime (CTZ, 30µg), aztreonam (ATM, 30µg), imipenem (IMP, 10µg), gentamicin (CN, 10µg), kanamycin (K, 30µg), tobramycin (TOB, 10µg), amikacin (AK, 30µg), netilmicin (NET, 30µg), tetracycline (TE, 30µg), ciprofloxacin (CIP, 5µg), norfloxacin (NOR, 5µg), erythromycin (E, 5µg), trimethoprim/sulfamethoxazole (SXT, 5µg) and chloramphenicol (C, 5µg) were used. All disks were obtained from Oxoid. After 24 h incubation at 30°C, organisms were classified as sensitive (S), intermediate resistant (I) or resistant (R) on the basis of the size of the zone of bacterial 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 gyrB and rpoD 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 caviae (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 species [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. caviae, 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. caviae (n = 16) | Total % |
|------------|-----------------------|-----------------------|---------------------|-------------------|---------|
| R          | 100                   | 100                   | 100                 | 100               | 100     |
| I          | 0                     | 0                     | 0                   | 0                 | 0       |
| S          | 0                     | 0                     | 0                   | 0                 | 0       |
| R          | 100                   | 100                   | 100                 | 100               | 100     |
| I          | 0                     | 0                     | 0                   | 0                 | 0       |
| S          | 0                     | 0                     | 0                   | 0                 | 0       |
| AMP        | 100                   | 100                   | 100                 | 100               | 100     |
| CAR        | 100                   | 100                   | 100                 | 100               | 100     |
| AML        | 100                   | 100                   | 100                 | 100               | 100     |
| AMC        | 33                    | 5                     | 62                  | 9                 | 78      |
| TIC        | 42                    | 69                    | 52                  | 81                | 5       |
| TIM        | 2                     | 19                    | 65                  | 15                | 11      |
| PRL        | 7                     | 93                    | 7                   | 93                | 6       |
| TZP        | 5                     | 95                    | 1                   | 99                | 3       |
| LF         | 88                    | 12                    | 37                  | 5                 | 58      |
| FOX        | 98                    | 2                     | 1                   | 99                | 9       |
| CRO        | 0                     | 100                   | 0                   | 100               | 0       |
| CAZ        | 0                     | 100                   | 1                   | 99                | 5       |
| CFP        | 16                    | 83                    | 3                   | 4                 | 93      |
| CTX        | 0                     | 100                   | 0                   | 100               | 0       |
| FEP        | 0                     | 100                   | 0                   | 100               | 0       |
| ATM        | 0                     | 98                    | 0                   | 100               | 0       |
| IMP        | 2                     | 98                    | 0                   | 100               | 0       |
| CIP        | 7                     | 92                    | 43                  | 4                 | 53      |
| NOR        | 7                     | 93                    | 34                  | 0                 | 66      |
| TOB        | 51                    | 48                    | 7                   | 6                 | 87      |
| AK         | 16                    | 84                    | 3                   | 0                 | 97      |
| K          | 40                    | 2                     | 58                  | 34                | 66      |
| CN         | 26                    | 74                    | 31                  | 0                 | 69      |
| NET        | 23                    | 77                    | 6                   | 0                 | 94      |
| TE         | 88                    | 12                    | 69                  | 0                 | 31      |
| C          | 14                    | 86                    | 25                  | 0                 | 75      |
| E          | 93                    | 7                     | 96                  | 0                 | 4       |
| SXT        | 49                    | 51                    | 40                  | 0                 | 60      |

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. cuniculica (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-lactamas production [19]. The combination of amoxicillin and carboxpenicillin with a β-lactamas 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. cuniculica 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 underestimated 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 cefalothin, first and third-generation (namely, cephalothin and cefoxatime). 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.

Aztreaman, 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 species 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 sequencing 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.

Acknowledgment

The authors acknowledge the financial support provided by the Portuguese
Portuguese Foundation for Science and Technology (Carla Dias-SFRH/ BGCT/33354/2008) and strategic research project PEst-OE/AGR/ UI0772/2011).

References

1. Barker G (2001) Bacterial diseases. BSAVA manual of ornamental fish. Wild-goose WH (Ed), 185-194.
2. Musa N, Wei SL, Shasharom 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 Microb 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, Aprailz 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 sM030. Rev Med Microbiol 16: 145-153.
7. Beaz-Hidalgo R, Alperi A, Bujan N, Romaine 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 rain-bow 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 (2001) Characterization of class 1 integrons associated with R-plasmids in clinical Aeromonas spp. isolated from South African aquaculture environments: implication of Tn1721 in dissemination of the tetra-cycline resistance determinant Tet A. Appl Environ Microbiol 66: 3883–3890.
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’Abée-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: 4908–4915.
16. 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.
17. L’Abée-Lund TM, Sorum H (2001) Class 1 integrons mediate antibiotic resistance in the fish pathogen Aeromonas salmonicida worldwide. Microb Drug Resist 7: 263-272.
18. 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.
19. 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: 4908–4915.
20. 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.
21. L’Abée-Lund TM, Sorum H (2001) Class 1 integrons mediate antibiotic resistance in the fish pathogen Aeromonas salmonicida worldwide. Microb Drug Resist 7: 263-272.
22. Imazin B (2001) Occurrence and antibiotic resistance of mesophilic Aeromonas in three riverine freshwater of Marrakech, Morocco. ScientificWorldJournal 1: 796–807.
23. Ottaviani D, Santarelli S, Bacchioddi 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-Escarpuili G, Figueras MJ, Aguiler-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 tetra-cycline 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 Anti-microb 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 fish intended for human consumption in Mexico. Int J Food Microbiol 79: 185–194.
33. Campos AC, Burborow RM, Hemstreet WG, Thune RL, Hawke JP (1998) Aeromonas bacterial infections – motile aeromonad septicemia. Southern Regional Aquaculture Center (SRAC) Publication 478.