Molecular characterization of tetracycline- and quinolone-resistant Aeromonas salmonicida isolated in Korea

Ji Hyung Kim†, Sun Young Hwang†, Jee Soo Son, Jee Eun Han, Jin Woo Jun, Sang Phil Shin, Casiano Choresca Jr, Yun Jaie Choi, Yong Ho Park, Se Chang Park

Laboratory of Aquatic Animal Medicine, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul National University,
Seoul 151-742, Korea

Laboratory of Animal Cell Biotechnology, Department of Agricultural Biotechnology, Seoul National University,
Seoul 151-742, Korea

The antibiotic resistance of 16 Aeromonas (A.) salmonicida strains isolated from diseased fish and environmental samples in Korea from 2006 to 2009 were investigated in this study. Tetracycline or quinolone resistance was observed in eight and 16 of the isolates, respectively, based on the measured minimal inhibitory concentrations. Among the tetracycline-resistant strains, seven of the isolates harbored tetA gene and one isolate harbored tetE gene. Additionally, quinolone-resistance determining regions (QRDRs) consisting of the gyrA and parC genes were amplified and sequenced. Among the quinolone-resistant A. salmonicida strains, 15 harbored point mutations in the gyrA codon 83 which were responsible for the corresponding amino acid substitutions of Ser83→Arg83 or Ser83→Asn83. We detected no point mutations in other QRDRs, such as gyrA codons 87 and 92, and parC codons 80 and 84. Genetic similarity was assessed via pulsed-field gel electrophoresis, and the results indicated high clonality among the Korean antibiotic-resistant strains of A. salmonicida.

Keywords: Aeromonas salmonicida, minimal inhibitory concentration, pulsed-field gel electrophoresis, quinolone-resistance determining region, tetracycline-resistance

Introduction

Aeromonas (A.) salmonicida is a pathogen that causes furunculosis and bacterial septicemia in a broad variety of fish, and is thus responsible for significant economic losses in the global aquaculture industry [37]. Recently, antibiotic-resistant A. salmonicida strains have been recognized as a serious concern owing to their potential health risks to humans and animals [32,33]. Among the antibiotics utilized in the treatment of furunculosis, both tetracycline and quinolone resistance have been widely documented [10,31]. Tetracycline-resistant strains of A. salmonicida are suspected to be the source of tetracycline resistance dissemination in the aquatic environment because tet genes, the determinants of tetracycline resistance, are generally encoded on plasmids [1,32,33]. Quinolone resistance is a potential public health threat since quinolones are also utilized for the treatment of Aeromonas infections in humans [15,16]. Quinolone resistance in Gram-negative bacteria is primarily attributable to mutations in the quinolone-resistance determining regions (QRDRs) consisting of the gyrA and parC genes, which are the subunits of the target enzymes of quinolones, DNA gyrase subunit A and topoisomerase IV, respectively [2]. The presence of the qnr gene which is associated with the plasmid-mediated quinolone-resistance, or efflux pumps is also known to be associated with mid- to low-level quinolone resistance [6,30].

Antibiotic resistance has been previously reported in several aquatic bacteria isolated in Korea including Edwardsiella tarda [17], Streptococcus iniae, and Streptococcus parauberis [29]. However, the antibiotic resistance of Aeromonas spp. has not previously been addressed. Therefore, in this study we evaluated the antimicrobial susceptibility and clonal relationship in A. salmonicida isolated from both cultured fish and the environmental water in Korea. In particular, the genetic determinants of tetracycline and quinolone resistance were assessed via (i) the detection of tetA to E, (ii) the detection of plasmid-encoded qnr genes, and (iii) the analysis of point mutations in QRDRs.

Materials and Methods

Bacterial isolation and culture conditions

Between 2006 and 2009, sixteen strains of A. salmonicida
were isolated from a variety of samples from fish and sewage water from two private aquariums and three salmonid farms in Korea (Table 1). Two reference strains were purchased from the American Type Culture Collection (ATCC, USA): *A. salmonicida* subsp. *salmonicida* ATCC 33658 (ASS) and *A. salmonicida* subsp. *masoucida* ATCC 27013 (ASM). *A. salmonicida* isolates were first screened using a Vitek System 2 (bioMérieux, France). All strains of *A. salmonicida* were stored in tryptic soy broth (Difco, USA) with 10% glycerol at −80°C and sub-cultured for 48 h on tryptic soy agar (Difco, USA) at 22°C. To assess strain purity, single colonies were selected and sub-cultured three times, and the resulting bacterial cells were harvested for further experiments.

**Antimicrobial susceptibility test**

Antimicrobial susceptibility tests were conducted via broth micro-dilution methods according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and ASS was utilized as a quality control bacterial strain [7,8]. Since cut-off values have not been determined for all antibiotics, three references [7,8,24] were used for interpretation, as was the case in other previous reports [2,5,29]. Seven antimicrobials were diluted in following ranges: ampicillin (0.06 to 32 μg/mL), florfenicol (0.12 to 64 μg/mL), gentamicin (0.06 to 32 μg/mL), oxolinic acid (0.004 to 8 μg/mL), oxytetracycline (0.03 to 16 μg/mL), and trimethoprim-sulfamethoxazole (0.03/0.6 to 2/38 μg/mL). All antimicrobials were purchased from Sigma-Aldrich (USA). The antimicrobials were serially diluted two-fold in cation-adjusted MHB (CAMHB; Difco, USA) and 100 μL volumes of the dilutions were placed into 96-well micro-titer plates. The inoculations were prepared as follows: 18 strains of *A. salmonicida* were adjusted to a McFarland value of 0.5 and diluted 10-fold with CAMHB. With the addition of 5 μL of inocula into each micro-titer wells, the final cell densities were adjusted to 5 × 10^8 CFU/mL. In all cases, two control wells without antimicrobials or inocula were maintained. After 44 to 48 h of incubation at 22°C, the lowest concentration of antibiotics that visibly inhibited bacterial growth was defined as the minimal inhibitory concentration (MIC). The MIC results of *A. salmonicida* subsp. *salmonicida* were used to classify the strains as resistant or sensitive in accordance with the cut-off values established by Miller et al. [24] and the guidelines of M49-A [7] and M31-A3 [8].

**DNA extraction and polymerase chain reactions (PCR)**

Genomic DNA was extracted by harvesting the cells with sterile water followed by 10 min of boiling. After 3 min of centrifugation at 10,000 ×g, the supernatants were collected and 1 : 100 dilutions in sterile water were utilized as a PCR template. All isolates were confirmed to be *A. salmonicida* using Fer-3 and Fer-4 PCR primers [3].

**Table 1. Aeromonas (A.) salmonicida strains used in this study**

| Name | Source | Isolated year | Bacterial identification | 16S rRNA sequence |
|------|--------|---------------|--------------------------|------------------|
| AS01 | Cherry salmon (*Oncorhynchus masou masou*) | 2006 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS02 | Cherry salmon (*Oncorhynchus masou masou*) | 2006 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS03 | Crucian carp (*Carassius carassius*) | 2006 | *A. salmonicida* + − | subsp. *achromogenes* |
| AS04 | Neon tetra (*Paracheirodon innesi*) | 2007 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS05 | Rainbow trout (*Oncorhynchus mykiss*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS06 | Rainbow trout (*Oncorhynchus mykiss*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS07 | Rainbow trout (*Oncorhynchus mykiss*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS08 | Rainbow trout (*Oncorhynchus mykiss*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS09 | Chum salmon (*Oncorhynchus keta*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS10 | Chum salmon (*Oncorhynchus keta*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS11 | Chum salmon (*Oncorhynchus keta*) | 2008 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS12 | Chum salmon (*Oncorhynchus keta*) | 2009 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS13 | Malma trout (*Salvelinus malma malma*) | 2009 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS14 | Malma trout (*Salvelinus malma malma*) | 2009 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS15 | Cherry salmon (*Oncorhynchus masou masou*) | 2009 | *A. salmonicida* + + | subsp. *salmonicida* |
| AS16 | Sewage water | 2007 | *A. salmonicida* + − | subsp. *flounderacida* |
| AS | *A. salmonicida* subsp. *salmonicida* ATCC 33658 | – | *A. salmonicida* + + | subsp. *salmonicida* |
| ASM | *A. salmonicida* subsp. *masoucida* ATCC 27013 | – | *A. salmonicida* + − | subsp. *masoucida* |
Subspecies were determined by *A. salmonicida* subsp. *salmonicida*-specific PCR with MIY1 and MIY2 primers [4,26] and confirmed by 16S rRNA sequencing at Macrogen (Korea). Two multiplex PCR procedures were conducted as previously described to amplify the five tetracycline resistant genes (tetA to E) [27] and to detect the qnr genes [5]. The QRDRs of the gyrA and parC genes were detected using the following primers: ASGYRA1, ASGYRA2, ASPARC3, and ASPARC4 [10]. The primers used in this study are shown in Table 2.

**Sequence analysis**

Sequencing was conducted by Macrogen (Korea) and the sequences were analyzed with the AlignX tool in the Vector NTI program (Invitrogen, USA). BLAST searches were conducted using both the blastn and blastx algorithms provided by the National Center for Biotechnology Information (NIH, USA).

**Pulsed-field gel electrophoresis (PFGE)**

Harvested bacterial cells were diluted with cell suspension buffer (100 mM Tris-HCl and 100 mM EDTA, pH 8.0) up to an optical density of 1.0 at 600 nm. A cell suspension volume of 100 μL was mixed with an equal volume of 1.6% SeaKem Gold agar (FMC Corporation, USA) and solidified in a 100 μL plug mold. The plugs were then incubated for 2 h with 1 mg/mL of lysozyme (Sigma-Aldrich, USA) at 37°C and treated with 1 mg/mL of proteinase K (Sigma-Aldrich, USA) at 50°C for 8 h. DNA in the plugs was digested for 18 h with 30 U of *SpeI* (New England Biolabs, USA) at 37°C and electrophoresed in 1.0% SeaKem Gold agarose gel with a CHEF-Mapper III PFGE system (Bio-Rad, USA). The running conditions were 6 V/cm at 14°C for 22 h, and the pulse times were 1.5 to 25 sec. The Lambda ladder PFGE marker (New England Biolabs, USA) was included as a size marker. The gels were stained with ethidium bromide and photographed under UV trans-illumination. The genetic relationships among isolates were analyzed with Bionumerics software (Applied Maths, Belgium) and the clusters were determined using the UPGMA algorithm with the 70% Dice-coefficient of similarity (2.0% position tolerance).

**Results**

**Bacterial identification**

The 16 isolates were successfully identified as *A. salmonicida* using Vitek System 2 and species-specific PCR.
(Table 1). Among the various categories of biochemical tests in Vitrek System 2, four (D-glucose, D-mannitol, and sucrose fermentation tests, and H2S production test) which were noted in only one isolate (AS16). A total of 9 multidrug-resistant strains were detected. Enrofloxacin resistance was exhibited in all 16 isolates, eight oxytetracycline-resistant strains and sixteen oxolinic acid-resistant strains were detected. Enrofloxacin resistance was noted in only one isolate (AS16). A total of 9 multidrug-resistant (MDR) strains were observed: 7 strains (AS09 to AS15) that were resistant to oxytetracycline and oxolinic acid, one strain (AS03) that was resistant to ampicillin and oxolinic acid, and one strain (AS16) that was resistant to five antibiotics (ampicillin, gentamicin, oxolinic acid, enrofloxacin, and oxolinic acid). Moreover, strain AS16 exhibited a high level of resistance to both enrofloxacin (≥ 4 μg/mL) and oxolinic acid (≥ 8 μg/mL) although the other 15 quinolone-resistant strains were susceptible to enrofloxacin (< 0.03 μg/mL) and showed low-level oxolinic acid resistance (1 ~ 2 μg/mL).

**tet genes in A. salmonicida isolates**

The tetA gene (211 bp) was detected in seven isolates (AS09 to AS15) while the tetE gene (744 bp) was detected in strain AS16 (Fig. 1). The amplified PCR products were sequenced and aligned with the tet gene sequences from
Antibiotic-resistant *Aeromonas salmonicida* in Korea

Fig. 1. Multiplex PCR assay of tetracycline resistance genes (*tet*A of 211 bp and *tet*E of 744 bp) in two reference strains and 16 isolates of *Aeromonas* (*A.* salmonicida). Lane M: molecular mass marker; lane 1 to 18: strains AS01, AS02, AS03, AS04, AS05, AS06, AS07, AS08, AS09, AS10, AS11, AS12, AS13, AS14, AS15, ASS, ASM, and AS16, respectively. Marker sizes (bp) are indicated.

GenBank. All amplified *tet*A fragments in this study showed 100% homology with the *tet*A gene of pRAS1, a drug resistance plasmid of *A. salmonicida* (GenBank accession No. AJ517790.2). The *tet*E gene, which was detected in AS16, showed 100% homology with the *tet*E gene of *A. salmonicida* subsp. salmonicida A449 plasmid 4 (pAsa4; GenBank accession No. CP000645.1) and *A. salmonicida* plasmid pYA90644 (GenBank accession No. DQ366299.1).

*qnr* genes and codon mutations in the QRDRs of *A. salmonicida* isolates

The *gyr*A (663 bp) and *par*C (418 bp) genes of QRDRs were successfully amplified from all 16 isolates and the ASS reference strain (Table 3). The amplified products were sequenced and their corresponding amino acid sequences were aligned with the sequences of *gyr*A (GenBank accession No. L42453.1) and *par*C (GenBank accession No. AF473701.1) of *A. salmonicida* ATCC 14174. AS03 and ASS strains had no point mutations while the AS16 strain harbored a Ser83 → Asn83 substitution in *gyr*A codon 83 (Table 3). Other 14 isolates showed Ser83 → Arg83 substitutions in the same loci. Additionally, AS16 had a single nucleotide mutation (AAA → AAG) at the *par*C codon 80 without an amino acid substitution. No other substitutions were detected on *gyr*A codon 87 (Asp95) and 92 (Leu97), or *par*C codon 80 (Lys84) and 84 (His96). The *qnr* gene was not detected in any of the *A. salmonicida* strains in this study.

Strain typing by PFGE

All *A. salmonicida* strains utilized in this study were clustered into four types based the PFGE results (Fig. 2). The ASM, AS03, and AS16 were divided into type A, B, and C, respectively. The other 14 *A. salmonicida* subsp. salmonicida isolates and ASS were classified into the same cluster designated as type D.

Discussion

Based on Bergey’s Manual of Determinative Bacteriology [14], at least three subspecies were identified among all 16 isolates using the biochemical results of Vitek System 2. All of these interpretations were in concordance with the subspecies-specific PCR and 16S rRNA sequencing results. Interestingly, the AS16 strain that showed distinctive biochemical characteristics (D-glucose (+), D-mannitol (+), sucrose (+) and H2S production (−)) was identified as the recently-reported *A. salmonicida* subsp. flounderacida (GenBank accession no. AY786177.1). Based on these results, we were able to confirm the presence of three subspecies of *A. salmonicida* (subsp. salmonicida, subsp. achronogenes and subsp. flounderacida) among those Korean isolates.

Considering the widespread use of tetracycline and quinolones in the aquaculture industry [12], the resistance of *A. salmonicida* to the two antibiotic classes was the focus of this study. According to the epidemiological cut-off values for *A. salmonicida* for oxytetracycline and oxolinic acid [24], eight oxytetracycline-resistant strains were detected, and sixteen isolates were oxolinic acid-resistant. Although enrofloxacin is one of the quinolones, like oxolinic acid [8], only one isolate (AS16) noted resistance to it. Interestingly, ampicillin resistance was detected only in three isolates (AS03, AS16, and ASM) although there have been some reports showing that *A. salmonicida* is naturally resistant to narrow-spectrum β-lactams [7]. One isolate showed resistance to gentamicin, and all strains were found to be susceptible to florfenicol and trimethoprim-sulfamethoxazole. Tetracycline resistance in *A. salmonicida* was strictly related to the presence of the *tet*A and *tet*E genes. These genes were also detected in other *A. salmonicida* strains from a variety of fish species in other countries [25,36]. The sequenced *tet*A
and tetE genes in this study showed 100% homology to tetA in pRAS1 and tetE in pAsa4. Since the pRAS1 and pAsa4 plasmids can be transferred into or replicate within certain strains of *Escherichia coli* [31,35], it has been suspected that tetracycline resistance has been disseminated between various bacterial species. The location and transferability of the tetA and tetE genes in *A. salmonicida* clearly warrants further investigation.

Despite the high levels of activity of quinolones against *Aeromonas* species [16,18], the number of quinolone-resistant *Aeromonas* strains has increased [10,34]. In this study, all 16 isolates showed resistance to oxolinic acid, and the AS16 strain was resistant to enrofloxacin. Genetic analysis through sequencing QRDRs revealed that these resistant strains except AS03 harbored point mutations in gyrA codon 83. The AS01 to AS15 (except AS03) strains had Ser→Arg substitutions and the AS16 strain harbored a Ser→Asn substitution. In particular, the AS16 strain having a Ser→Asn substitution exhibited high-level resistance to both oxolinic acid and enrofloxacin. It is well known that quinolone resistance is principally related to mutations of the QRDRs, particularly in gyrA codons 83, 87, and 92, and in parC codons 80 and 84 [10,11]. In addition to previous studies that found Ser→Ile and Ser→Val substitutions on gyrA codon 83 in strains of *Aeromonas* [2,11,28], here we report two more putative substitutions that might contribute to quinolone resistance. Moreover, based on our results, amino acid substitutions on gyrA codon 83 may affect the level and spectrum of quinolone resistance in *A. salmonicida*.

In addition to the point mutations in the QRDRs, acquisition of qnr genes or efflux pumps also contribute to quinolone resistance [6,30]. In this study, the AS03 strain showed low-level resistance to oxolinic acid without mutations in the QRDRs. Since no qnr genes were detected, further investigation should be performed for determining whether this strain contains other quinolone-resistant mechanisms such as the efflux pump. The other possibility that could explain the differences in quinolone resistance between the isolates might be subspecies-specific natural resistance mechanisms that have yet to be elucidated; AS03 (*A. salmonicida* subsp. *achromogenes*) and AS16 (*A. salmonicida* subsp. *flounderacida*) were different subspecies from *A. salmonicida* subsp. *salmonicida* strains.

The PFGE results of this study typed isolates at the subspecies level. The genetic heterogeneity between subspecies was consistent with previous reports that described differences between typical and atypical *A. salmonicida* strains [9,13]. Interestingly, 14 *A. salmonicida* subsp. *salmonicida* strains isolated from Korea were found to be distinct from ASS although they were included in the same cluster; this result suggests geographical differences in the distribution of *A. salmonicida*.

Besides the subspecies classification, most of biochemical results and MDR patterns of the *A. salmonicida* strains also concurred with the PFGE types. For example, AS16 (type B) was identified as *A. salmonicida* subsp. *flounderacida* and resistant to the highest number of antibiotics including ampicillin, gentamicin, oxytetracycline, enrofloxacin, and oxolinic acid. Strains in other PFGE types (type A and D) were identified as *A. salmonicida* subsp. *achromogenes* or subsp. *salmonicida* that were resistant to one or two antibiotics. On the other hand, the similar PFGE pattern of the tetracycline-resistant and susceptible *A. salmonicida* subsp. *salmonicida* strains within type D appears to imply horizontal transfer of *tet* genes among these isolates. These results suggest a potential risk of the spread of MDR strains or dissemination of antimicrobial resistance genes in the Korean aquatic industry.

Thus far, only a few antibiotics are approved for use in animals in the worldwide aquatic industry [19,24]; nevertheless, antibiotic resistance is expected to continue to become more frequent [20-23]. The detection of MDR in Korean strains of *A. salmonicida* suggests that antibiotic resistance in aquaculture can pose a risk to both humans and animals. Thus, stricter guidelines for the use of tetracycline and quinolones will be necessary to prevent the dissemination and acquisition of antibiotic resistance in aquaculture.

**Acknowledgments**

This study was financially supported by a Korean Research Foundation Grant (KRF-2008-331-E00385) and by the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science, and Technology (2009-0074437).

**References**

1. Adams CA, Austin B, Meaden PG, McIntosh D. Molecular characterization of plasmid-mediated oxytetracycline resistance in *Aeromonas salmonicida*. Appl Environ Microbiol 1998, **64**, 4194-4201.
2. Alcaide E, Blasco MD, Esteve C. Mechanisms of quinolone resistance in *Aeromonas* species isolated from humans, water and eels. Res Microbiol 2010, **161**, 40-45.
3. Beaz-Hidalgo R, Magi GE, Balboa S, Barja JL, Romalde JL. Development of a PCR protocol for the detection of *Aeromonas salmonicida* in fish by amplification of the *fotA* (ferric siderophore receptor) gene. Vet Microbiol 2008, **128**, 386-394.
4. Byers HK, Gudkovs N, Crane MS. PCR-based assays for the fish pathogen *Aeromonas salmonicida*. I. Evaluation of three PCR primer sets for detection and identification. Dis Aquat Organ 2002, **49**, 129-138.
5. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing entrobacterial
isolates. J Antimicrob Chemother 2007, 60, 394-397.
6. Cattoir V, Poireil L, Aubert C, Soussy CJ, Nordmann P. Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental Aeromonas spp. Emerg Infect Dis 2008, 14, 231-237.
7. Clinical and Laboratory Standards Institute (CLSI). Methods for Broth Dilution Susceptibility Testing of Bacteria Isolated from Aquatic Animal; Approved Guideline. CLSI document M49-A. CLSI, Wayne, 2006.
8. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals: Approved Standard-3rd Edition. CLSI document M31-A3. CLSI, Wayne, 2008.
9. García JA, Larsen JL, Dalsgaard I, Pedersen K. Pulsed-field gel electrophoresis analysis of Aeromonas salmonicida ssp. salmonicida. FEMS Microbiol Lett 2000, 190, 163-166.
10. Giraud E, Blanc G, Bouju-Albert A, Weill FX, Donnay-Moreno C. Mechanisms of quinolone resistance and clonal relationship among Aeromonas salmonicida strains isolated from reared fish with furunculosis. J Med Microbiol 2004, 53, 895-901.
11. Goïl-Urriza M, Arpin C, Capdepy M, Dubois V, Caumette P, Quinet C. Type II topoisomerase quinolone resistance-determining regions of Aeromonas caviae, A. hydrophila, and A. sobria complexes and mutations associated with quinolone resistance. Antimicrob Agents Chemother 2002, 46, 350-359.
12. Guérin-Faulbée V, Delignette-Muller ML, Vigneulle M, Flandrois JP. Application of a modified disc diffusion technique to antimicrobial susceptibility testing of Vibrio anguillarum and Aeromonas salmonicida clinical isolates. Vet Microbiol 1996, 51, 137-149.
13. Hänninen ML, Hirvelä-Koski V. Pulsed-field gel electrophoresis in the study of mesophilic and psychrophilic Aeromonas spp. J Appl Microbiol 1997, 83, 493-498.
14. Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST. Bergey’s Manual of Determinative Bacteriology. 9th ed. pp. 254-255, Williams & Wilkins, Baltimore, 1994.
15. Janda JM, Abbott SL. Evolving concepts regarding the genus Aeromonas: an expanding Panorama of species, disease presentations, and unanswered questions. Clin Infect Dis 1998, 27, 332-344.
16. Jones BL, Wilcox MH. Aeromonas infections and their treatment. J Antimicrob Chemother 1995, 35, 453-461.
17. Jun LJ, Jeong JB, Huh MD, Chung JK, Choi DI, Lee CH, Jeong HD. Detection of tetracycline-resistance determinants by multiplex polymerase chain reaction in Edwardsiella tarda isolated from fish farms in Korea. Aquaculture 2004, 240, 89-100.
18. Ko WC, Chiang SR, Lee HC, Tang HJ, Wang YY, Chuang YC. In vitro and in vivo activities of fluoroquinolones against Aeromonas hydrophila. Antimicrob Agents Chemother 2003, 47, 2217-2222.
19. Kwon NH, Park KT, Jung WK, Youn HY, Lee Y, Kim SH, Bae W, Lim JY, Kim JY, Kim JM, Hong SK, Park YH. Characteristics of methicillin resistant Staphylococcus aureus isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet Microbiol 2006, 117, 304-312.
20. L’abé-Lund TM, Sørum H. Functional Tn5393-like transposon in the R plasmid pRAS2 from the fish pathogen Aeromonas salmonicida subspecies salmonicida isolated in Norway. Appl Environ Microbiol 2000, 66, 5533-5535.
21. L’abé-Lund TM, Sørum H. Class 1 integrons mediate antibiotic resistance in the fish pathogen Aeromonas salmonicida worldwide. Microb Drug Resist 2001, 7, 263-272.
22. L’abé-Lund TM, Sørum H. A global non-conjugative Tet C plasmid, pRAS3, from Aeromonas salmonicida. Plasmid 2002, 47, 172-181.
23. McIntosh D, Cunningham M, Ji B, Fekete FA, Parry EM, Clark SE, Zalinger ZB, Gilg IC, Danner GR, Johnson KA, Beattie M, Ritchie R. Transferable, multiple antibiotic and mercury resistance in Atlantic Canadian isolates of Aeromonas salmonicida subspecies salmonicida is associated with carriage of an IncA/C plasmid similar to the Salmonella enterica plasmid pSN254. J Antimicrob Chemother 2008, 61, 1211-1228.
24. Miller RA, Reimischauessel R. Epidemiologic cutoff values for antimicrobial agents against Aeromonas salmonicida isolates determined by frequency distributions of minimal inhibitory concentration and diameter of zone of inhibition data. Am J Vet Res 2006, 67, 1837-1843.
25. Miranda CD, Kehrenberg C, Ulep C, Schwarz S, Roberts MC. Diversity of tetracycline resistance genes in bacteria from Chilean salmon farms. Antimicrob Agents Chemother 2003, 47, 883-888.
26. Miyata M, Inglis V, Aoki T. Rapid identification of Aeromonas salmonicida subspecies salmonicida by the polymerase chain reaction. Aquaculture 1996, 141, 13-24.
27. Nawaz M, Sung K, Khan SA, Khan AA, Steele R. Biochemical and molecular characterization of tetracycline-resistant Aeromonas veronii isolates from catfish. Appl Environ Microbiol 2006, 72, 6461-6466.
28. Oppegaard H, Sørum H. gyrA mutations in quinolone-resistant isolates of the fish pathogen Aeromonas salmonicida. Antimicrob Agents Chemother 1994, 38, 2460-2464.
29. Park YK, Nho SW, Shin GW, Park SB, Jang HB, Cha IS, Ha MA, Kim YR, Dalvi RS, Kang BJ, Jung TS. Antibiotic susceptibility and resistance of Streptococcus iniae and Streptococcus parauberis isolated from olive flounder (Paralichthys olivaceus). Vet Microbiol 2009, 136, 76-81.
30. Pool E. Efflux-mediated resistance to fluoroquinolones in gram-negative bacteria. Antimicrob Agents Chemother 2000, 44, 2233-2241.
31. Reith ME, Singh RK, Curtis B, Boyd JM, Bouevitch A, Kimball J, Munholland J, Murphy C, Sarty D, Williams J, Nash JHE, Johnson SC, Brown LL. The genome of Aeromonas salmonicida subspecies salmonicida A449: insights into the evolution of a fish pathogen. BMC Genomics 2008, 9, 427-441.
32. Rhodes G, Huys G, Swings J, Megann P, Hiney M, Smith P, Pickup RW. Distribution of oxytetracycline resistance plasmids between aeromonads in hospital and aquaculture environments: implication of Tn j721 in dissemination of the tetracycline resistance determinant Tet A. Appl Environ Microbiol 2000, 66, 3883-3890.
33. Schmidt AS, Bruun MS, Dalsgaard I, Larsen JL.
Incidence, distribution, and spread of tetracycline resistance determinants and integron-associated antibiotic resistance genes among motile aeromonads from a fish farming environment. Appl Environ Microbiol 2001, 67, 5675-5682.

34. Sinha S, Chattopadhyay S, Bhattacharya SK, Nair GB, Ramamurthy T. An unusually high level of quinolone resistance associated with type II topoisomerase mutations in quinolone resistance-determining regions of Aeromonas caviae isolated from diarrhoeal patients. Res Microbiol 2004, 155, 827-829.

35. Sørum H, L’abée-Lund TM, Solberg A, Wold A. Integron-containing IncU R plasmids pRAS1 and pAr-32 from the fish pathogen Aeromonas salmonicida. Antimicrob Agents Chemother 2003, 47, 1285-1290.

36. Sun K, Wang HL, Zhang M, Xiao ZZ, Sun L. Genetic mechanisms of multi-antimicrobial resistance in a pathogenic Edwardsiella tarda strain. Aquaculture 2009, 289, 134-139.

37. Wiklund T, Dalsgaard I. Occurrence and significance of atypical Aeromonas salmonicida in non-salmonid and salmonid fish species: a review. Dis Aquat Organ 1998, 32, 49-69.