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Fatty Acid Profile and Antimicrobial Susceptibility of *Aeromonas salmonicida* Isolated from Rainbow Trout

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Abstract: *In vitro* susceptibilities of 30 *Aeromonas salmonicida* strains isolated from rainbow trout (*Oncorhynchus mykiss*) were determined against to 23 antimicrobials by using disc diffusion method. According to antimicrobial susceptibility tests results, *A. salmonicida* strains were found susceptible to all antibiotics tested except for the ampicilin and vancomycine. The major fatty acids, used as indicators for identification of the bacteria, were found as 14:0 3OH/16:1 ISO I, 16:1 w7c/15 ISO 2OH, 16:0 and 18:1 w7c, respectively.

Key words: *Aeromonas salmonicida*, rainbow trout (*O. mykiss*) fatty acid profile, antimicrobial susceptibility, ampicilin, vancomycine

INTRODUCTION

Aeromonas salmonicida has a wide range of hosts and survives in both fresh and seawater environments (Enger, 1997). It is associated with a variety of clinical diseases in non-salmonids including Cprinidae, Anoplopomidae and Serramidae. Moreover, it is the causative agent of furunculosis which is one of the most important bacterial diseases in terms of causing economical loss in salmonid cultures (Bernoth *et al*., 1997). The analysis of fatty acid patterns is well introduced for classification and identification of bacteria. Fatty acids are mainly located in the cytoplasmic membrane as constituents of phospholipids and lipopolysaccharides of the outer membrane of Gram-negative bacteria as well as lipoteichoic acids in Gram-positive bacteria. The importance of this method for the identification of bacteria is based on the large structural differences within these molecules. For classification or identification of bacteria the presence of distinct fatty acids and their relative amount is analyzed and compared with the fatty acid profiles of reference strains (Busse *et al*., 1996).

Intensive fish farming has resulted in massive use of antimicrobial agents for prevention and therapeutic treatment of bacterial fish diseases. The extensive use of antibiotics in human and veterinary medicine, aquaculture agriculture is contributing to the selection and dissemination of antibiotic resistant microorganisms (Henriques *et al*., 2006). The development of resistance to antibiotics commonly used in animal production is an increasing concern for agriculture industries. Antimicrobials, in and of themselves do not cure a fish, merely control the population growth of bacteria in a fish long enough for its immune system to eliminate them (Uhland and Higgins, 2006). During the development of salmonid farming, furunculosis has been routinely treated with antibiotics and *Aeromonas salmonicida* developed resistance to the some antimicrobials (Yanong, 2006). However it is also known that using antibiotics against to bacterial diseases is time dependent since resistance is occurring against to certain antibiotics by bacteria. Therefore, it is very important to use the suitable drug for treatment of unhealthy fish at the treatment time.

Antibiotics are used in companion, food, exotic and other types of animals to prevent, control or treat a variety of infections. In general, the antibiotic classes are similar to those used in human medicine (Magnadóttir *et al*., 2002).

Considering this fact the aim of the present study was to determine the antimicrobial susceptibilities of the *A. salmonicida* strains, identificated with MIS (Microbial Identification System) by using disk diffusion method which is most popular for routine testing.

MATERIALS AND METHODS

Fish and fish tank facilities: This study was conducted in Fisheries Department of Agricultural Faculty at Ataturk University (Erzurum/Turkey) in 2004. A total of 30 rainbow

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trout (*Oncorhynchus mykiss*) weighting 50-100 (75±0.255 g) were obtained from a freshwater farm. They were kept in 300 L circular fiberglass tank and held under natural light conditions, a constant water flow of 1.5 L min^{-1} of aerated dechlorinated tap water and a temperature of 9-11°C. Dissolved oxygen, pH and total hardness of the water were 8-9 ppm, 7.8 and 102 mg in $CaCO_3$, respectively. The fish were acclimated in these conditions for two weeks before the start of the experiment. The fish were fed with pelleted dry food at a rate of 1.5% of their body weight per day.

Experimental infection: Lyophilized *A. salmonicida* A-480 strain was originally received from Japon, cultured on BA at 25°C. A suspension of approximately 10^7 cfu (Colony forming units) was diluted 1000-fold with phosphate buffered saline (0.001 M PBS, pH 7.4) and 0.05 of this was injected intramuscularly in each fish. The fish were anaesthetized with MS 222 (Sigma) before injection.

Isolation of the bacteria: Experimentally infected fish were slaughtered in sterile conditions and aseptically. Inoculums were taken from the kidney, liver and spleen of infected fish and used for the isolation of bacteria. The inoculums were streaked on the Blood agar (blood agar base, supplemented 5% sterile sheep blood) at 25°C for 24 h (Gray and Shryock, 2005).

Identification of the bacteria: A commercially available system for microbial identification by fatty acid analysis (Microbial Identification System (MIS), MIDI, Newark, DE, USA) was used for identification of bacteria. Specimens were processed on a MIDI Sherlock® Microbial Identification System with a Hewlett-Packard automatic sampler and integrator. For identification, clinical library was used (Buyer, 2002).

Antimicrobial sensitivity tests: The following antimicrobial susceptibility test discs with their concentrations shown in parentheses were used to determine antimicrobial susceptibility of the bacterial isolates Amikacin (30 μg), Amoxicillin/Clavulamic Acid (30 μg), Amoxicillin (10 μg), Ampicillin (10 μg), Ampicillin Sulbactam (20 μg), Cefaclor (30 μg), Cefazolin (30 μg), Cefepime (30 μg), Cefotaxime (30 μg), Cefoxitin (30 μg), Ceftriaxone (30 μg), Ciproflaxacin (5 μg), Clindamycin (2 μg), Erythromycin (30 μg), İmipenem (10 μg), Meropenem (10 μg), Netilmicin (30 μg), Oxacillin (1 μg), Penicillin (10 μg), Piperacillin (100 μg), Tetracycline (30 μg), Trimethoprim/Sulfamethoxazole (25 μg), Vancomycin (30 μg). All the discs containing antimicrobials were purchased from BBL (Becton Dickinson, USA).

The disc diffusion assay was carried out according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 1987). Basically bacteria were harvested after 48 hour growth on Trypticase soy agar and suspended in sterile 0.85% saline in order to achieve a McFarland 2 turbidity standard, diluted 1:20 and streaked on Mueller-Hinton agar (Difco) using a cotton swab (Bauer *et al*., 1996). Plates were incubated at 25°C for 24 h. Zones of inhibition, formed around the discs were measured and antimicrobial sensitivity was assayed from the length of the diameter of the zones (in mm). Zone diameters were interpreted as susceptible, intermediate and resistant according to the manufacturers instructions (Saha and Pal, 2002).

RESULTS AND DISCUSSION

In the present study overall, 7 fatty acids with aliphatic chain lengths of 12 to 18 carbon atoms were identified in the bacterial lipid extracts. Analysis of the fatty acid patterns demonstrated that relative amounts of unsaturated fatty acids was 54.45% while the amount of saturated fatty acids were 44.48% in *A. salmonicida* strains.

The fatty acid compositions of *A. salmonicida* strains were presented in Table 1.

Diameters of the inhibition zones for the 15 bacterial isolates against 23 antimicrobial agents are shown in Table 2. Based on this inhibition zones isolates categorized as sensitive or resistant.

A. salmonicida strains were found resistant to ampicillin and vancomycin. However they were found susceptible to all other antimicrobials tested.

For bacteriological study, the organs of choice are kidney and spleen, but preferably kidney (Furones, 2001). In present study, bacterial strains were isolated from the kidney, liver and spleen of the fish, presented characteristic symptoms of furunculosis. Kidney found most suitable for the isolation of bacteria in the present study.

In the present study total 7 different fatty acids were identified in the bacterial lipid extracts. Among this, major fatty acids for the identification of *A. salmonicida* determined as 16:1 w7c/15 ISO 2OH (46.12%), 18:1 w7c (8.33%) monounsaturated fatty acids and 14:0 3OH/16:1 iso I (8.83%), 16:0 (23.75%) saturated fatty acids.

Species differing in their motility levels also differed in the quantitative ratios of individual fatty acids in their lipids. For example, the total content of unsaturated fatty acids of motile bacteria (motile aeromonads and vibrios) was higher compared to nonmotile bacteria. It is also known that the fatty acid composition of membrane lipids can be used in determination of a number of their

Table 1: Comparison of the fatty acid composition of *A. salmonicida*

Peak name	Percent
Unknown	1.07
12:0	5.11
11:0 ISO 3 OH	3.51
14:0	3.28
14:0 3OH/16:1 ISO I	8.83
16:1 w7c/15 ISO 2OH	46.12
16:0	23.75
18:1 w7c	8.33

Table 2: Inhibition zone diameters for *A. salmonicida*

Antibiotic (µg/disc)	Inhibition zone (mm)	Sensitivity
Amixacin (30)	34	S
Amoxcicillin/Clavulanic acid (30)	28	S
Amoxicillin (10)	26	S
Ampicillin (10)	24	R
Ampicillin sulbactam (20)	32	S
Cefaclor (30)	42	S
Cefazolin (30)	28	S
Cefepime (30)	30	S
Cefotataxime (30)	34	S
Cefoxitin (30)	36	S
Ceftriaxone (30)	32	S
Ciproflaxacin (5)	32	S
Clindamycin (2)	32	S
Erythromycin (30)	36	S
İmipenem (10)	25	S
Meropenem (10)	30	S
Netilmicin (30)	36	S
Oxacillin (1)	24	S
Penicillin (10)	30	S
Piperacillin (100)	28	S
Tetracycline (30)	43	S
Trimethoprim/Sulfamethoxazole (25)	32	S
Vancomycin (30)	16	R

R: Resistant, S: Susceptible

physicochemical properties (Bogdan *et al*., 2001). The viability of micro-organisms is known to be dependent upon cell membrane integrity and for Gram-negative bacteria, lipids play an important role in the structure and the function of cell membranes, species differing in their motility levels differed also in the quantitative ratios of individual fatty acids in their lipids (Brissonnet *et al*., 2000). Therefore it can be concluded that *A. salmonicida* strains in our study should be considered motile bacteria. A considerable overall increase in drug resistant, fish pathogenic bacteria in parallel with the extensive use of chemotherapeutic agents, which has created a great deal of difficulty in the treatment of bacterial infections in fish culture (Aoki, 1992). Drug resistance in *A. salmonicida* was first reported in the USA with the finding of strains resistant to sulphonamides (Snieszko and Bullock, 1957). Thereafter the occurrence of antimicrobial resistance in *A. salmonicida* has tended to follow drug usage (Aoki *et al*., 1983). Today increased prevalence of antibiotic resistance is still a growing concern and generally accepted that the main risk factor for this increase in resistance in pathogenic bacteria is the increased use of antibiotics against pathogenic bacteria (Levy, 1992). Based on the *in vitro* antimicrobial susceptibility testing, *Aeromonas salmonicida* strains, isolated from rainbow trout were found mainly resistant to ampicillin and vancomycin, however they were found susceptible to all antimicrobials tested in the present study. This finding is also corroborated with observations by other researchers (Dixon and Issvoran, 1993; Radu *et al*., 2003). Gram-negative orgamisms are not sensitive to vancomycin, perhaps because channels in the cell wall of the gram-negative organism do not accommodate the large, bulky vancomycin molecule.

Antibiotics effective in human medicine like, oxytetracycline, sulfamerazine and ormethoprim, are used for treatment of bacterial infections in some commercially raised fish species. The most frequent fish infections treated with antibiotics are skin ulcers, diarrhea and blood sepsis. The microorganisms related with these infections belong to bacteria that also produce infections in humans that is why transference of antibiotic resistance is highly probable (Serrano, 2005).

Present study suggested that usage of ampicillin and vancomycin should be avoided for treating bacterial disease caused by *Aeromonas salmonicida* in trout farms.

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