

Antimicrobial Susceptibility of *Pseudomonas Putida* Isolated from Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract

Susceptibilities of eight *Pseudomonas putida* strains, isolated from the livers of 10 freshwater rainbow trout (*Oncorhynchus mykiss*), were determined against to 23 antimicrobials by using a disc diffusion method.

The antimicrobial susceptibility tests results showed that *Pseudomonas putida* strains were susceptible to all antimicrobials except for trimethoprim/sulfamethoxazole, cefaclor, ceftriaxone and tetracycline.

Key words: experimental infection, rainbow trout (*Oncorhynchus mykiss*), antibiotic resistance

INTRODUCTION

As with all animal production systems, disease is a considerable constraint on production, development and expansion in the aquaculture industry. The control of disease is particularly difficult in that the fish are often farmed in systems where production is dependent on natural environmental conditions, in contrast to all other intensive animal production where environmental parameters can be very closely controlled [1]. Almost all fish bacterial pathogens are capable of independent existence outside the fish. There are only a very small number of obligatory pathogens. Even these, however, are capable of living for a considerable period within the tissues of their host without any deleterious effect. For the present, it is uncertain whether Pseudomonads represent an emerging problem or a secondary (opportunistic) invader of already diseased hosts [2].

Pseudomonas infections usually occur in conditions of poor water quality, crowding, physical damage or other stresses and results in a hemorrhagic septicemia identical to that seen with *Aeromonas* septicemias. The bacterium has a worldwide distribution in fresh and salt water and it is likely that all fish species are susceptible, although it is most frequently seen in pond culture when fish are stressed by poor environmental conditions. The disease can have an acute or chronic course with large, hemorrhagic skin lesions and hemorrhages of internal tissues being the most frequently encountered clinical signs [1]. Since *Pseudomonas* species are so widespread and numerous, they may be involved in disease processes

and act as secondary invaders of fish compromised by pathogens or other factors Inglis *et al.* [3].

Although *P. anguilliseptica*, *P. fluorescens* and *P. chlororaphis* species are well known aetiological agents of diseases in fish among the genus *Pseudomonas* [2]. *P. putida* is not a common pathogen in fisheries. To date there has been only one report of *P. putida* as a fish pathogen which caused a heavy mortality among rainbow trout (*Oncorhynchus mykiss*) in Turkey [4].

In intensive fish farm, where fish are subjected to stressful conditions, bacterial diseases often occur and result in serious economic losses in such cases, antibacterial drugs are usually used to treat the bacterial diseases of fish. The impact of the intensive use of antimicrobial agents worldwide for prophylactic and therapeutic purposes has been associated with the increase of bacterial resistance in the exposed microbial environment. Pathogenic bacteria often develop drug resistance if exposed to antibacterial drugs long term and this makes infections more difficult to treat [5,6].

Antimicrobial susceptibility testing of bacteria has two components. The first is the generation of laboratory data by the application of a specific test protocol and the second is the interpretation of those data by the application of a break points [7].

For a treatment to be effective, antimicrobial susceptibility experiments should be carried out to evaluate the susceptibility and resistance development to antimicrobial agents [8].

The aim of the present study was to determine the antimicrobial susceptibilities of the *P. putida* strains

isolated from rainbow trout and to provide useful information on the efficacy of antimicrobial treatments in rainbow trout.

MATERIALS AND METHODS

Isolation and identification of isolates

A total of 10 rainbow trout (*Oncorhynchus mykiss*), weighting 40-100 g which were exhibiting characteristic symptoms of disease were taken from a commercial fish farm and transferred to the Fish Disease Laboratory of Atatürk University in cold boxes containing ice [9]. Materials obtained from internal organs and muscle lesions were inoculated onto tryptic soy agar (TSA) and incubated at 25 °C for 48 hours. After incubation, isolates were identified by fatty acid methyl-ester (FAME) gas chromatography analysis using Microbial Identification Systems Software (MIS Delaware, USA) as described by Buyer [10]. *Pseudomonas putida* could isolated for only eight of the suspected fish.

Antimicrobial susceptibility testing

The following antimicrobial susceptibility test discs with their concentrations shown in parentheses were used

to determine antimicrobial susceptibility of the bacterial isolates; Amikacin (30µg), Amoxicillin/Clavulanic Acid (30µg), Ampicillin (10µg), Ampicillin Sulbactam (20µg), Cefazolin (30 µg), Cefepime (30µg), Cefotaxime (30µg), Cefoxitin (30µg), Ciproflaxacin (5µg), Clindamycin (2µg), Erythromycin (30 µg), İmipenem (10µg), Meropenem (10µg), Piperacillin (100µg), Tetracycline (30µg), Trimethoprim/Sulfamethoxazole (25µg), 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) [11]. Basically bacteria were harvested after 48 hours growth on Trypticase soy agar at 25 °C and suspended in sterile 0.85% saline and streaked on Mueller-Hinton agar (Difco) using a cotton swab [12]. Plates were incubated at 25 °C for 48 hours. 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). The zone radius was actually scaled from the centre of the antibiotic disc to the end of the clear zone

Table 1. Inhibition zone diameters for *P.putida*

Antibiotic (µg/disc)	Inhibition zone (mm)	Sensitivity
Amixacin (30)	26	S
Amoxicillin/Clavulanic Acid (30)	26	S
Amoxicillin(10)	19	S
Ampicillin(10)	20	S
Ampicillin Sulbactam (20)	29	S
Cefaclor(30)	15	R
Cefazolin(30)	40	S
Cefepime(30)	23	S
Cefotataxime(30)	30	S
Cefoxitin(30)	22	S
Ceftriaxone(30)	21	R
Ciproflaxacin(5)	22	S
Clindamycin(2)	21	S
Erythromycin(30)	25	S
İmipenem(10)	28	S
Meropenem(10)	30	S
Netilmicin(30)	32	S
Oxacillin(1)	28	S
Penicillin(10)	30	S
Piperacillin(100)	28	S
Tetracycline(30)	10	R
Trimethoprim/Sulfamethoxazole(25)	0	R
Vancomycin(30)	19	S

R: Resistant S: Susceptible

where bacteria could be seen growing. Zone diameters were interpreted as susceptible, intermediate and resistant according to the manufacturer's instructions [13].

RESULTS

In the present study overall, 15 fatty acids with aliphatic chain lengths of 10 to 18 carbon atoms were identified in the bacterial lipid extracts. Among these, 16:1 w7c/15 iso 2OH (33.44%), 16:0 (30.60%) and 18:1w7c (14.21%) were detected predominant fatty acids for *P.putida*. Comparison of the fatty acid composition of *P.putida* presented in Table 1.

P. putida strains were found susceptible to all of the antimicrobials tested, except for the trimethoprim/sulfamethoxazole, cefaclor, ceftriaxone and tetracycline. The antimicrobial susceptibility results of *P. putida* are presented in Table 2.

Table 2. Comparison of the fatty acid composition of *P.putida*

Peak name	Percent
10:0 3OH	3.52
12:0	2.18
11:0 ISO 3OH	0.55
12:0 2OH	4.13
12: 3OH	3.81
14:0	0.45
15:0 ISO	0.57
15:0 ANTEISO	1.38
16:1 w7C/15 iso 2OH	33.44
16:0	30.60
17:0 ISO	0,50
17: ANTEISO	0.34
17:0 CYCLO	3.37
18:1w7c	14.21
18:0	0.95

DISCUSSION

Bacterial identification is a growing field of interest within microbiology. In term of a bacterial disease for a treatment to be effective it is important to know which bacteria is the agent of disease. The earlier you identify the bacteria the earlier you can start the treatment process.

Biochemical, nutritional and physiological characterization tests are time consuming and laborious tests. The analyses of fatty acid patterns used in the present study, is well introduced for classification and identification of bacteria. The importance of this method for the identification of bacteria is based on the large structural differences within phospholipids and lipopolysaccharides molecules of the outer membrane

of Gram-negative bacteria as well as lipoteichoic acids in Gram-positive bacteria [14].

Major mono-unsaturated and saturated fatty acids were found as 16:1 w7c/15 iso 2OH (33.48%), 18:1 w7c (14.56%), and 16:0 (31.91%) respectively in present study. Similarly, predominance of palmitic acid (16:0), palmitoleic acid (16:1) and oleic acid (18:1) in *Pseudomonas* strains have also been reported by several researchers [15,16]. These results show that, these fatty acids may be used as markers for *P.putida*. Both our results and the relevant published data also demonstrate that high variabilities of bacterial lipids and fatty acid compositions allow their usage as taxonomic characteristics only in combination with other, more stable features.

According to disc diffusion assay in Mueller-Hinton agar *P. putida* strains were found resistant to tetracycline, trimethoprim/sulfamethoxazole, cefaclor, and ceftriaxone. *P.putida* is an uncommon opportunistic pathogen, usually susceptible to antimicrobial agents. Data concerning resistance to antimicrobial agents in clinical *P.putida* isolates are limited [17]. Similar results have also been reported by several researchers [18,19]. Finding two antibacterial resistant strains is also confirmed the statement of Aoki [20], saying that 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.

Effective and successful treatment of bacterial diseases depends on susceptibility testing of pathogen bacteria isolates prior to antibiotic treatment. Large commercial ponds for fish generally are not drained after harvest, so high levels of drugs remain, affecting newly introduced fish. This fish are then exposed to the antibiotic residues and any active resistant bacteria. Therefore the findings of the present study suggest that antimicrobials only should be used if absolutely necessary therapeutically in fish farms.

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