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RESEARCH ARTICLE

Isolation of *Flavobacterium psychrophilum* Causing Rainbow Trout Fry Syndrome and Determination of an Effective Antibacterial Treatment in Rainbow Trout *(Oncorhynchus mykiss)* Fry [1]

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Summary

The bacterial pathogen *Flavobacterium psychrophilum (F. psychrophilum)* causes rainbow trout *(Oncorhynchus mykiss)* fry syndorme (RTFS) causes economic losses and widespread in many countries. The aims of the present study are isolation of *F. psychrophilum*, and determination of antibacterial susceptibility and an effective antibacterial treatment in rainbow trout fry. For this purpose, weighted 2-5 g fry were obtained from a private fish farm in west Aegean region of Turkey. It was estimated according to clinical findings that they were naturally infected with *F. psychrophilum* originate from the rearing unit. Following fenotipical, biochemical and enzyme tests aimed the identification of bacteria. Kirby-Bauer disc diffusion method was used for determination of antibacterial susceptibility. According to antibiotic susceptibility tests, *F. psychrophilum* was susceptible to oxytetracycline, enrofloxacin, ciprofloxacin and florfenicol. The antibiotic concentrations in the medicated feeds were to give a dose of 75 mg/kg/day oxytetracycline and 10 mg/kg/day enrofloxacin, ciprofloxacin and florfenicol each antibiotics for 10 days. When considered the clinical signs and death rates among the infected rainbow trout fry, oxytetracycline, enrofloxacin and ciprofloxacin could not displayed enough efficacy. However, florfenicol showed much higher efficacy for controlling the infection and at the end of the treatment, the death rate caused by the infection was decreased significantly.

Keywords: *Flavobacterium psychrophilum, Antimicrobial Susceptibility, Florfenicol, Treatment*

Gökkuşağı Alabalıklarında *(Oncorhynchus mykiss)* RTFS'ye (Rainbow trout Fry Syndrome) Neden Olan *Flavobacterium psychrophilum* Etkeninin Izolasyonu ve Antibakteriyel Sağaltım Seçeneğinin Belirlenmesi

Özet

Gökkuşağı alabalık *(Oncorhynchus mykiss)* yavrularında *Flavobacterium psychrophilum (F. psychrophilum)*'un neden olduğu Yavru Gökkuşağı Alabalığı Sendromu (Rainbow Trout Fry Syndrome, RTFS) birçok ülkede yaygın olarak görülen ve ekonomik kayıplara neden olan bakteriyel bir hastalıktır. Bu çalışmada *F. psychrophilum* suşunun izolasyonu ve identifikasyonu, antibakteriyel duyarlılığı ve etkili antibakteriyel ilaç/ilaçlarla sağaltım seçeneklerinin belirlenmesi amaçlanmıştır. Bu amaçla Ege Bölgesi'nin batısında bulunan özel bir işletmede, doğal yolla *F. psychrophilum* ile enfekte olduğu tahmin edilen yavruhane bölümünde meydana gelen enfeksiyon sonucunda, canlı ağırlıkları 2-5 g arasında hastalıklı gökkuşağı alabalık yavrusu kullanıldı. İdentifikasyon amacıyla bakterilerin fenotipik, biyokimyasal ve enzim testleri gerçekleştirildi. Kirby-Bauer disk difüzyon yöntemine göre yapılan antibiyogram test sonuçlarına göre etkenin oksitetrasiklin, enrofloksasin, siprofloksasin ve florfenikole duyarlı olduğu belirlendi. Sağaltım gruplarına oksitetrasiklin 75 mg/kg/gün dozda, enrofloksasin, siprofloksasin ve florfenikol 10 mg/kg/gün dozda ve her biri 10 gün süreyle uygulandı. Gökkuşağı alabalık yavrularında klinik bulgular ve ölüm oranları dikkate alındığında; oksitetrasiklin, enrofloksasin ve siprofloksasinin sağaltım için yeterli etkinlik gösteremedikleri, florfenikolün ise diğer sağaltım gruplarına göre enfeksiyonu kontrol almada önemli derecede etkin olduğu ve sağaltım sonunda enfeksiyondan kaynaklı ölüm oranlarını azaltığı belirlendi.

Anahtar sözcükler: *Flavobacterium psychrophilum, Antimikrobiyal duyarlilik, Florfenikol, Sağaltım*

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INTRODUCTION

The bacterial pathogen *Flavobacterium psychrophilum* (formerly *Cytopahaga psychrophila* or *Flexibacter psychrophilus*) causes rainbow trout (*Oncorhynchus mykiss*) fry syndorme (RTFS) and also is the agent of bacterial coldwater disease in larger fish [1-6]. RTFS widespread in many countries and causes severe mortalities and economic losses of rainbow trout fry in hatcheries in Turkey as well as in most other European countries. The RTFS is considered to be on of the most serious bacterial diseases of rainbow trout in Turkey, and cumulative mortility rates can be as high as 70% [7-11]. Similar mortality rates are seen in aquaculture in other countries [2-6,12-15].

F. psychrophilum has been found in skin mucus, and connective tissue of the fins, gills and operculum of salmonid fish [1,16]. Lorenzen [17] demonstrated *F. psychrophilum* in the lumen and the mucosa/submucosa of the stomach of naturally infected rainbow trout fry, pointing towards the involvement of the gastro-intestinal tract as a portal of entry. On the other hand, it has been isolated from diseased fish and from fish without any sign of disease [1,14]. Handling and stress can exacerbate the disease [18]. The most manifest sign is anaemia as revealed by pale gills, kidney, intestine and liver. Clinically, the fry appear lethargic, inappetant and hang at the water surface [19]. Primarily observed splenomegali, a white, fragile intestine and a hemorrhagic, protruding anus. In some cases, skin erosions have been reported, and these usually are found behind the dorsal fin or on the flank [18]. Fish infected with *F. psychrophilum* as fry may display ataxia, spiral swimming and eventually spinal column deformities [2,13]. By comparison to other bacterial fish pathogens, there is no commercial vaccine available to prevent *F. psychrophilum* [4,20]. Antimicrobial therapy is still the most effective (and used) way of combating *F. psychrophilum* infections [19].

Proper management of fish culture conditions, monitoring and maintenance of high standards for water quality, and adequate sanitation procedures are other essential requisites that may help to avoid and/or limit the severity of RTFS epizootics [20]. The risk of antibiotic treatments inadvertently selecting for drug resistance in bacterial fish pathogens that may also cause disease in humans remains a grave concern in fish health control practices [21,22]. Antimicrobial agents are released into the surrounding water during medical treatment of bacterial disease [23]. Many studies show that *F. psychrophilum* is resistant to most antibiotics, and antibiotic resistance may be different by geographic region. *F. psychrophilum* is capable of acquiring resistance quite easily as seen with oxytetracycline, oxolinic acid, and amoxicillin in Danish freshwater fish farms [23,24]. Furthermore *F. psychrophilum* strains were resistante to both nalidixic acid and oxolinic acid in Japan and United States [5]. In France, chloramphenicol resistance was more frequent than florfenicol resistance in *F. psychrophilum* isolated from cases of RTFS [22]. *F. psychrophilum spp.* were resistante to cephalexin, cephalothin, chloramphenicol, florfenicol, nalidixic acid, gentamicin, kanamycin, erythromycin and oxytetracycline in Australia [25]. Of the increasing resistance problem, it is imperative to base the antibiotic to be used upon susceptibility testing in the laboratory.

The aim of the current study was to demonstrate the presence of *F. psychrophilum* in the west Aegean region of Turkey, and determination of antibacterial susceptibility and an effective antibacterial treatment in rainbow trout fry. Antibiotic susceptibility of strains were determined using disk diffusion method.

MATERIAL and METHODS

Rainbow Trout Samples

A large fish hatchery located in the west Aegean region of Turkey were sampled. Increased mortality occurred in five concrete raceways of rearing unit. From the different raceways a total of 50 (10 of each raceways) rainbow trout fry (weight = 2-5 g; total length = 3-6 cm) showing signs of infection with *F. psychrophilum* (dark skin, dorsal fin white spots or lysis, spiral swimming, spinal column deformities, and failure to feed) were brought to the laboratory. In the hatchery, mean (±SD) water temperature was 9.2±1.5°C, dissolved oxygen was 9.8±0.4 mg/L, and pH was 6.8±0.3. The living animals were carried in containers filled with freshwater, and dead fish were stored on ice in cold boxes. This study was approved by Animal Ethic Committee of University of Adnan Menderes (Decision date 12.04.2007 and number HEK/2007/0004).

Isolation of F. psychrophilum

Samples were taken from the gill, spleen, kidney, liver, intestine and brain of each fish and from observed caudal peduncle lesions. The samples were streaked onto *Cytophaga* agar (CA) containing 1.5% agar as modified by Bernardet and Kerouault [26] (0.05% tryptone, 0.05% yeast extract, 0.02% beef extract, and 0.02% sodium acetate; pH = 7.2-7.4). All plates were incubated aerobically at 15°C for 48-96 h. After incubation, the yellow-pigmented colonies were stained using the Gram staining technique, and Gram negative isolates were observed by microscopic examination [27].

Identification of F. psychrophilum

Identification was based on colony morphology, Gram staining, and biochemical tests. Biochemical characteristics of the isolates were determined using catalase, oxidase, indol, urease, methyl red, Voges-Proskauer, hydrogen sulfide (H_2S), nitrate reduction, oxidation-fermentation, and motility tests, gelatin, Congo red, Simmons' citrate, flexirubin-like pigment (20% KOH), growth on CA for 48-

96 h at 37°C, and growth on CA and media containing 0.5, 1.5, and 3.0% NaCl (weight/volume). In addition, tests for fermentation (e.g., production of acid from glucose, lactose, and mannitol) were also carried out [27,28]. Activities of 19 enzymes were tested using API ZYM strips (bioMe´rieux, Marcy l'Etoile, France). The API ZYM test was performed according to the manufacturer's instructions, but the strips were incubated at 15°C for 16-20 h and *F. psychrophilum* NCIMB (National Collection of Industrial, Marine, and Food Bacteria) 1947 was used as the control.

Determination of Antimicrobial Susceptibility of F. psychrophilum

Antimicrobial susceptibility was assessed by disk diffusion method. This method is used for more-routine screening, when sensitivity is not as important. The Kirby-Bauer disk diffusion method [29] was performed using multidisks (Oxoid, Ltd., Basingstoke, UK) of ampicillin (AMP; 10 µg), amoxicillin-clavulanic acid (AMC; 30 µg), enrofloxacin (ENR; 5 µg), erythromycin (E; 15 µg), florfenicol (FFC; 30 µg), gentamicin (CN; 10 µg), oxytetracycline (OT; 30 µg), penicilin G (P; 10 U), ciprofloxacin (CIP; 5 µg), and sulfamethoxazole-trimethoprim (SXT; 25 µg). Isolates were plated onto Mueller-Hinton agar (Oxoid) that was modified for testing *F. psychrophilum*, as described by Hawke and Thune [30]. The plated isolates were incubated at 15°C for 48-96 h.

The antimicrobial susceptibility was determined for each isolate by use of the disk diffusion method described by the National Committee for Clinical Laboratory Standards [31]. Endpoint determinations were performed after 96 h. Disk diffusion was evaluated as the lowest zone diameter produced by an antimicrobial agent.

Antibiotic Treatment

Oxytetracycline (Terramycin, Pfizer, Turkey), enrofloxacin (Baytril, Bayer Turk, Turkey), ciprofloxacin (Ciprobiotik, Eczacıbaşı, Turkey) and florfenicol (Florocol, Schering-Plough, UK) were selected according to the *in vitro* study. Commercial rainbow trout fry pellets (1-1.5 mm) (Bayem, Pro-feed, Turkey) were moistened with cornflower oil to ensure attachment of the powdered drugs to the pellets. The drugs were added and carefully mixed with the pellets. The feed for the control fish was moistened with cornflower oil, without a drug [32]. The antibiotic concentrations in the medicated feeds were to give a dose of 75 mg/kg/day oxytetracycline and 10 mg/kg/day enrofloxacin, ciprofloxacin and florfenicol each antibiotics for 10 days [20,33,34]. Five concrete raceways of rearing unit were used for this purpose. Each group consisted approximately 25 000 naturally infected rainbow trout fry at the start of the experiment. Chi-square tests with Yates' correction were used to compare the groups.

RESULTS

Twenty isolates that were phenotypically identified as *F. psychrophilum* were cultured from the gill (14% of 50 fish samples; n = 7 isolates gill), kidney (6%; n=3), liver (6%; n = 3), spleen (4%; n = 2), brain (4%; n = 2), intestine (4%; n = 2) and from observed pathological lesions of the caudal peduncle (2%; n=1). Results obtained from biochemical, cultural, and physiological characteristics and API ZYM profile tests are given in *Table 1*. The biochemical and morphological characteristics of the isolates closely resembled those of the type srtain *F. psychrophilum* NCIMB 1947. Mean zone diameter (mm) for each antimicrobial agent are presented in *Table 2*. The susceptibility of *F. psychrophilum* isolates to the antimicrobial agents, as indicated by the disk diffusion method, is shown in *Table 3*.

Disk diffusion results showed that 100% of the isolates were resistant to AMC, 95% were resistant to AMP and SXT, 80% were resistant to P and, 65% were resistant to CN. However, 100% were susceptible to ENR and CIP, 70% were susceptible to FFC, and 55% were susceptible to OT. Also, 50% of the isolates were intermediate susceptible to E *(Table 3)*. In the study, The number of daily death before and after application of antibiotics, depending on the RTFS are presented in *Table 4*.

The mortality was very higher in the oxytetracycline group compared the other treatment groups. Although oxytetracycline significantly decreased the mortality on the second day (x^2_{yates} = 14.57, Degree of Freedom (DF) = 3, P<0.05). After the third day the mortality were reduced and blocked with florfenicol. However, that was not significantly (P>0.05). After the fourth day of therapy were not significanlty decreased the effects of enrofloxacin and ciprofloxacin (P>0.05). Deaths due to infection in both groups continued after the application of antibiotic. There was no significant difference between groups of enrofloxacin and ciprofloxacin (x^2_{yates} = 0.36, DF = 1, P>0.05) compared the cumulative mortality. Control (x^2 = 11200.55, DF = 4, P<0.001), oxytetracycline (x^2_{yates} = 5534.4, DF = 3, P<0.001) and florfenicol (x^2_{yates} = 296.04, DF = 2, P<0.001) groups were significantly different compared the cumulative mortality.

There was no specific findings about RTFS in FFC group after the treatment. Between 5 to 10 fish died every day in this group. Bacteriological examination yielded no *F. psychrophilum* or other fish pathogens.

Deaths continued to increase in the control group and the other treatment groups (OT, ENR and CIP) after the treatment. For this purpose 10 surviving fish in each raceway were bacteriological examined for 5 days. Eighteen isolates that were phenotypically identified as *F. psychrophilum*. The isolates were cultured from the control group (4 isolates spleen, one each from brain, liver and

Table 1. *Biochemical, cultural, physiological characteristics and API ZYM profile results for 20 F. psychrophilum isolates obtained from rainbow trout fry at a fish hatchery in the west Aegean region of Turkey*

Tablo 1. *Ege Bögesi'nin batısında bulunan Gökkuşağı alabalık işletmesine ait yavruhane bölümünden izole edilen F. psychrophilum (n=20) suşlarının biyokimyasal, kültürel ve fizyolojik karakterleri ile API ZYM profil sonuçları*

Character	Reactions		API ZYM Profile	Reactions	
	Isolates	(r)		Isolates	(r)
Color of coloni	yellow	yellow	Alkaline phosphatase	20/20	+
Gram	0/20	-	Esterase	0/20	-
Catalase	20/20	+	Esterase lipase	20/20	+*
Oxidase (cytochrome oxidase)	20/20	+	Lipase	0/20	-
Hydrogen sulfide	0/20	-	Leucine arylamidase	20/20	+
Glucose	0/20	-	Valine arylamidase	20/20	+*
Lactose	0/20	-	Cystine arylamidase	0/20	-
Mannitol	0/20	-	Tyripsin	0/20	-
Motility	0/20	-	α-chymotrypsin	0/20	-
Nitrate reduction	0/20	-	Acid phosphatase	11/20	+
Urease	0/20	-	Napthol-AS-BI-phosphohydrolase	17/20	+*
Indol	0/20	-	α-galactosidase	0/20	-
Oxidation-Fermentation	5/20	-	β-galactosidase	0/20	-
Gelatine	20/20	+	β-glucuronidase	0/20	-
Methyl red	0/20	-	α-glucosidase	9/20	-
Voges proskauer	0/20	-	β-glucosidase	0/20	-
Flexirubin pigment	20/20	+	N-acetyl-β-glucosaminidase	0/20	-
Congo red	0/20	-	α-mannosidase	0/20	-
Simmon's citrate	0/20	-	α –fucosidase	0/20	-
Growth on CA					
5 °C	20/20	+			
37 °C	0/20	-			
added 0.5 % NaCl	20/20	+			
added 1.5 % NaCl	20/20	+			
added 3 % NaCl	0/20	-			

+ = positive; + = weak positive; - = negative included in results;* ***r*** *= NCIMB 1947 F. psychrophilum referance strain;* ***CA****= Cytophaga agar*

intestine), OT group (2 isolates spleen, 2 isolates brain, one each from kidney and liver), ENR group (one each from spleen, kidney and brain) and CIP group (one each from spleen and kidney).

DISCUSSION

The method of susceptibility testing used most widely in diagnostic laboratories is the agar disk diffusion technique. It is simple to perform, and a single bacterial isolate can easily be tested with several antimicrobial agents [35]. The diluted Mueller-Hinton Agar as described by Hawke and Thune [30] has been recommended by Bruun et al. [24] for susceptibility testing of *F. psychrophilum*.

Data on the antimicrobial susceptibility of *F. psychrophilum* isolated from rainbow trout fry are limited in Turkey. Kum et al.[11] reported that 20 isolates of *F. psychrophilum* were resistance to AMC (90%), SXT (75%), CN (70%), E (65%), and sensitive to ENR (10%), OT (20%) and FFC (25%). Didinen et al.[10] reported the resistance of 13 *F. psychrophilum* isolates to (84.6%), E (61.6%), P (53.9%), ENR (23.1%), AMC (15.4%), and CN (7.7%). Ispir et al.[37] demonstrated sensitivity to AMC, CN, E, and OT and resistant to P for five isolates of *F. psychrophilum*. Using the disk diffusion method, Diler et al.[8] reported that two *F. psychrophilum* isolates were sensitive to AMP, AMC, CN, CIP and OT and were resistant to trimethoprim. Similarly, Korun and Timur [38] reported that 20 isolates of *F. psychrophilum* were sensitive to OT (100% of isolates) but resistant to sulfadimethoxine and trimethoprim (100%), as determined using the disk diffusion method.

In the present study, disk diffusion method indicated that the 20 isolates of *F. psychrophilum* were susceptible to ENR and CIP (100%), FFC (70%), and OT (55%). Although

Table 2. *Mean zone diameter (mm) from the disk diffusion method used to determine the susceptibility of 20 F. psychrophilum isolates to ten antimicrobial agents*

Tablo 2. *İzole edilen F. psychrophilum suşlarının (n=20) disk difüzyon metoduyla on antimikrobiyal ajana karşı oluşan zon çapları (mm)*

Antimicrobial Agent	Mean Zone Diameter (mm)		
	R	I	S
AMP	≤ 13	14-16	≥ 17
AMC	≤ 14	15-19	≥ 20
ENR	≤ 15	16-20	≥ 21
E	≤ 13	14-22	≥ 23
FFC	≤ 14	15-18	≥ 19
CN	≤ 12	13-14	≥ 15
OT	≤ 14	15-18	≥ 19
P	≤ 14	-	≥ 15
CIP	≤ 15	16-20	≥ 21
SXT	≤ 10	11-15	≥ 16

AMP = ampicillin; AMC = amoxicillin-clavulanic acid; ENR = enrofloxacin; E = erythromycin; FFC = florfenicol; CN = gentamicin; OT = oxytetracycline; P=penicilin G; CIP = ciprofloxacin; SXT = sulfamethoxazole-trimethoprim. Results are presented for isolates demonstrating resistance (R), intermediate response (I), and susceptibility (S) to each agent

Table 3. *Number (percentage in parentheses) of F. psychrophilum isolates (n=20) demonstrating resistance, susceptibility, or an intermediate response to antimicrobial agents (codes defined in Table 2), as assessed using the disk diffusion method*

Tablo 3. *İzole edilen F. psychrophilum suşlarının (n=20) disk difüzyon metoduyla antimikrobiyal ajanlara (kısaltmalar Tablo 2.'de belirtilmiştir) karşı belirlenen duyarlılık ve dirençlilik durumları (parantez içindekiler; hesaplanan yüzdeliklerdir)*

Isolate Response	Antimicrobial Agent									
	AMP	AMC	ENR	E	FFC	CN	OT	P	CIP	SXT
Resistant	19 (95)	20 (100)	-	9 (45)	6 (30)	13 (65)	4 (20)	16 (80)	-	19 (95)
Intermediate	1 (5)	-	-	10 (50)	-	1 (5)	(25)	-	-	1 (5)
Susceptible	-	-	20 (100)	1 (5)	14 (70)	6 (30)	11 (55)	4 (20)	20 (100)	-

Disk sizes: AMP = 10 µg, AMC = 30 µg, ENR = 5 µg, E= 15 µg, FFC = 30 µg, CN = 10 µg, OT = 30 µg, P = 10 U, CIP = 5 µg, and SXT = 25 µg

Table 4. *The number (percentage in parentheses; calculated by the number of live fish of that day) of daily death before and after application of antibiotics, depending on the RTFS*

Tablo 4. *Antibiyotik uygulama öncesi ve sonrası RTFS'ye bağlı günlük ölüm sayıları (parantez içindekiler; o günkü canlı alabalık sayısı üzerinden hesaplanan yüzdeliklerdir)*

Time (Day)		Groups					x^2
		Control	OT	ENR	CIP	FFC	
Before application of antibiotics		397 (1.59)	372 (1.49)	373 (1.49)	434 (1.74)	365 (1.46)	8.38 NS
After application of antibiotics	1	112 (0.46)	114 (0.46)	106 (0.43)	130 (0.53)	106 (0.43)	3.42 NS
	2	168 (0.69)*	86 (0.35)**	132 (0.54)	129 (0.53)	142 (0.58)	11.58
	3	312 (1.28)*	135 (0.55)	96 (0.39)	106 (0.44)	121 (0.50)	209.61
	4	286 (1.19)*	243 (1.00)*	126 (0.52)	126 (0.52)	96 (0.40)	160. 68
	5	615 (2.59)*	367 (1.53)*	130 (0.54)	137 (0.57)	81 (0.34)**	765.46
	6	887 (3.84)*	335 (1.41)*	140 (0.58)	136 (0.57)	63 (0.26)*	1471. 66
	7	911 (4.10)*	496 (2.12)*	156 (0.65)	146 (0.61)	41 (0.17)*	1706.23
	8	1167 (5.48)*	963 (4.21)*	133 (0.56)	137 (0.58)	22 (0.09)*	2427.27
	9	1346 (6.68)*	1114 (5.09)*	187 (0.79)	148 (0.63)	8 (0.03)*	2806.93
	10	1217 (6.47)*	988 (4.76)*	195 (0.83)	174 (0.74)	6 (0.03)*	2363.28
Cumulative mortality		7143 (28.57)*	4972 (19.89)*	1524 (6.10)	1494 (5.98)	804 (3.22)*	11200.55

***OT** = oxytetracycline; **ENR** = enrofloxacin; **CIP** = ciprofloxacin; **FFC** = florfenicol, * P<0.01; ** P<0.05, **NS** = Not significant*

the deaths originate from the rearing unit of hatchery, it is noteworthy that the isolates exhibited quite a range in susceptibilities to the different antibiotics. This may suggest that the fish were infected with multiple clones of *F. psychrophilum*, each exhibiting different susceptibilities to the antibiotics. Nevertheless, antibiotics used in treatment are not effective except FFC. This reveals a negative correlation between *in vitro* and *in vivo* effect. In Europe, RTFS has been successfully controlled using OT at 75-300 mg/kg/d for 10-14 day [20]. However, isolates of *F. psychrophilum* with resistance to OT are increasingly being described in England [39] and Denmark [14,23,24,36]. Previous investigations of antimicrobial resistance in *F. psychrophilum* have only dealt with the *in vitro* aspect. For example, MIC values can be useful indicators of the probable clinical efficacy, but these results have not been verified by *in vivo* testing [2,39,40]. Branson [18] reported from the antibiotics tested, enrofloxacin, sarafloxacin and florfenicol showed potential for in *in vivo* trials but no therapeutic efffect has been found in subsequent trials with enrofloxacin.

Therapeutic activity remained at low levels, although on the second day of OT treatment to reduce mortality. After the third day of this activity was decreased, while providing a significant therapeutic efficacy initially of CIP and ENR. The reason for this might be chelation or resistance. Burka et al.[41] reported OT and quinolones may be inactivated by exposure to Calcium^{2+} and Magnesium^{2+} in the water and the bowel of the fish. Resistance mechanisms have been examined in recent studies as potential causes of antimicrobial aquaculture treatment failures. Resistance to tetracyclines and/or oxytetracyclines has been commonly reported. Resistance to oxytetracycline in *F. psychrophilum* is caused by uptake of transposons carrying tetracycline resistance determinants or the resistance is caused by other unspecific changes in membrane permeability [36]. Alvarez et al.[4] described the resistance genes *tetQ* conferred tetracycline resistance. DNA gyrase (GyrA) is an important target for quinolones in *F. psychrophilum* [5] and can, as such, only be transferred vertically in bacterial populations [24].

Most of the *F. psychrophilum* isolates in this study were sensitive to FFC (70%). FFC was found to be highly effective in controlling RTFS. After the second day was reduce mortality. Perhaps FFC resistance has not yet occurred. Resistance of *F. psychrophilum* to FFC has rarely been observed in England [39], and Nematollahi et al.[19] indicated that *F. psychrophilum* in salmonids exhibited no resistance to ENR or FFC. Michel et al.[22] and Akinbowale et al.[22] noted that *F. psychrophilum* resistance to FFC has not become widespread in France or Australia, despite the aquacultural use of FFC in those countries.

However, because of the increasing resistance problem, it is imperative to base the antibiotic to be used upon susceptibility testing in the laboratory. The emergence of resistant bacteria may have resulted from improper use of antimicrobial agents. As a result of increasing incidence of resistant bacteria and recurrent outbreaks of disease shortly after a treatment, investigations on alternative treatments and prophylactic schemes should be given high priority. These results were considered indicative of FFC potential in controlling outbreaks of RTFS. Future studies of effective antibiotic application for the treatment of RTFS, this study can be used as a reference.

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