

Histopathology and Antibiotic Susceptibility of *Aeromonas hydrophila* Isolated from Diseased Guppy (*Poecilia reticulata*)

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Abstract

This study was carried out to determine the reason of mortality in guppy (*Poecilia reticulata*) kept in an aquarium fish-rearing farm in Istanbul province and confirm damage caused in tissues and antibiotic susceptibility of isolated pathogen bacteria. Externally diseased guppies (n=15) showed sloughing scales, ulcerative skin lesions on the body surface and abdominal distension; internally pale liver and accumulation of a liquid in the abdominal cavity were observed. Bacteriological inoculation from internal organs such as liver, spleen and kidney of guppies (n=15) were made onto Tryptic Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA). After incubation, all isolated bacteria were identified as *Aeromonas hydrophila* according to their psychological, morphological, biochemical characteristic, and API 20E profile. Also, these isolates (n=15) were determined to be sensitive to chloramphenicol and florfenicol. Histopathologically, multifocal liquefactive necrosis in the spleen, liver, kidney and heart, degeneration of tubular epithelium and necrosis in the kidney, desquamation of mucosa epithelium and necrotic gastric glands in the stomach, discharge of the white pulp in the spleen, desquamation of the lamina propria and mucosa epithelium in the intestine, and sloughed off the necrotic gill epithelium cells were observed.

Keywords: *Aeromonas hydrophila*, antibiotic susceptibility, API 20E, guppy, histopathology

Hasta Lepistes Balığından (*Poecilia reticulata*) İzole Edilen *Aeromonas hydrophila*'nın Antibiyotik Duyarlılığı ve Histopatolojisi

Öz

Bu çalışma İstanbul ilinde bulunan bir akvaryum balığı üretim işletmesindeki hasta lepestes balıklarında görülen ölümlerin nedenini ortaya koymak ve izole edilen patojen bakterinin dokularda oluşturduğu hasarı ve antibiyotik duyarlılığını tespit etmek amacı ile yürütülmüştür. Eksternal olarak, ölmek üzere olan lepestes (*Poecilia reticulata*) balıklarında pullarda dökülme, vücut yüzeyinde ülseratif deri lezyonları ve abdominal genişleme görüldü; internal olarak karaciğerin renginde açılma, abdominal boşlukta sıvı birikimi tespit edildi. Karaciğer, böbrek ve dalak gibi iç organlardan Tryptic Soy Agar (TSA) and Brain Heart Infusion Agar (BHIA) besiyerlerine ekimler yapıldı. İnkubasyon sonrasında izole edilen tüm bakteriler fizyolojik, morfolojik ve biyokimyasal özellikleri ve API 20E profiline göre *Aeromonas hydrophila* olarak tanımlandı. Bu izolatların (n=15) kloramfenikol ve florfenikol'e duyarlı olduğu tespit edildi. Histopatolojik olarak dalak, karaciğer, böbrek ve kalp dokusunda multifokal liquefaktif nekroz, tubuler böbrek epitelyumunda dejenerasyon ve nekroz, mide bezlerinde ve mukoza epitelyumunda dökülme, dalağın kırmızı pulpasında boşalma, bağırsağın mukoza epiteline ve lamina propriasında dökülme yanı sıra nekrotik solungaç epitel hücrelerinde dökülme tespit edilmiştir.

Anahtar Kelimeler: *Aeromonas hydrophila*, antibiyotik duyarlılığı, API 20E, lepestes, histopatoloji

1. Introduction

Ornamental fish production is a profitable industry in the world. This industry has 60-year history in Turkey; it is particularly involved in the production of fish such as guppy (*Poecilia reticulata*), angelfish (*Pterophylum scalarae*) and gold fish (*Carassius auratus*) (Savaş, 1996; Türkmen and Alpbaz, 1999). However, it has been reported that bacterial infections in the aquarium fish industry cause severe economic losses at every step of the production (Wildgoose, 2001).

Gram-negative, facultative anaerobic *Aeromonas hydrophila* is normal bacterial flora of fish and responsible for the disease known as Motile Aeromonas Disease (MAD) or Motile Aeromonas Septicemia (MAS) in fish at the time of the stress (Lewbart, 2001, Janda and Abbott, 2010; Austin and Austin, 2016). This bacterium also is a zoonotic pathogen causing infection in human (Li and Saghaian, 2011) so an important pathogen both fish and human. *A. hydrophila* has been recovered as a pathogen from different ornamental fish species (Hettiarachchi and Cheong, 1994). Clinically, MAS is generally characterized by the presence of the body surface lesions and hemorrhages, eroded fins, sloughing scales, exophthalmia and abdominal distension (Hettiarachchi and Cheong, 1994; Pathiratne et al. 1994; Musa et al., 2008; Jagoda et al., 2014). *A. hydrophila*, the agent of MAS causes histologic damage in the liver and kidney of infected fish (El-Barbary, 2010).

The aim of the study is to determine the reason of mortalities in the diseased guppy (*Poecilia reticulata*) kept in an aquarium fish-rearing farm in Istanbul province, characterize the pathogen and determine the sensitivity of the

agent involved to commonly used antibiotics with a view to provide the aquarists with the information on effective treatment and suitable treatment against agent caused mortality.

2. Material and Method

2.1. Fish Samples

Fifteen diseased guppy were obtained from an aquarium fish-rearing farm in Istanbul/Turkey.

2.2. Bacteriology

For microbiological analysis, samples were taken from liver, spleen and kidney from all diseased guppies. They were inoculated onto Brain Heart Infusion Agar (BHIA) and Tryptic Soy Agar (TSA). Petri plates were incubated during 24-48 h at 24-25 C°. The isolates (n=15) recovered from guppies were identified by using the conventional bacteriological method. In addition, fifteen isolates were determined together with their biochemical characteristics using API20E (Buller, 2004; Austin and Austin, 2016).

2.3. Histopathology

Samples of tissues from visceral organs (liver, kidney, spleen, gut, heart) and gill immediately fixed in 10% buffered formalin and processed for paraffin embedding. Histological sections (4-5µm) were stained with haematoxylin and eosin (H&E) and examined by light microscopy (Bullock, 1978).

2.4. Antibiotic Susceptibility

Antibacterial susceptibility of our isolates were determined by using KirbyBauer disc diffusion method (Barry and Thornsberry, 1985). For this reason, 12 commercial

antibiotic disc such as chloramphenicol (30 µg/disc), kanamycin (30 µg/disc), flumequine (30 µg/disc), erythromycin (5 µg/disc), streptomycin (10 µg/disc), ciprofloxacin (1 µg/disc), sulphamethoxazole (25 µg/disc), ampicillin (10 µg/disc), enrofloxacin (5 µg/disc) florfenicol (30 µg/disc), oxytetracycline (30 µg/disc) and furazolidone (50 µg/disc) (Oxoid, England) were used. The antibiotics sensitivity testing was carried out according to instruction of the Clinical and Laboratory Standart Institute (CLSI, 2008).

3. Research Findings

Fifteen diseased guppy exhibited externally large hemorrhagic ulcerative skin lesions on the body surface, sloughing scales, eroding fin and abdominal distension (Figure 1a); internally, pale liver, hemorrhage on the visceral organs and accumulation of a liquid in the abdominal cavity (Figure 1b). After incubation of kidney, spleen and liver swabs, cream colonies were observed on TSA and BHIA. Isolated bacteria (n=15) were motile, Gram-negative basil, oxidase and catalase positive and resistant to O129/10-150. According to all biochemical tests result, isolates were identified as *Aeromonas hydrophila* (Table 1). In addition, of these biochemical results, all isolates showed 324612757 (96.9 %) API 20E profile number and thus biochemical result was confirmed with API-web results.

Histopathologically, multifocal liquefactive necrosis in the spleen, liver (Figure 2a), kidney and heart were observed. Tubular epithelium degeneration and necrosis was present in the kidney tissue (Figure 2b). Discharge of the white pulp in the spleen (Figure 2c), lymphocyte infiltration between the cardiac muscle (Figure 2d), desquamation

of mucosa epithelium and necrotic gastric glands in the stomach, desquamation of the lamina propria and mucosa epithelium (Figure 2e) and sloughed off the necrotic gill epithelium (Figure 2f) were observed.

All isolates were sensitive to florfenicol, chloromphenicol, enrofloxacin, flumequine, ciproflaxacin, sulphamethoxazole and highly resistant to erythromycine, oxytetracycline and ampicillin (Figure 3, Table 2).



Figure 1. Diseased guppy infected with *A. hydrophila*; (a) ulcerative skin lesion (arrowed), eroding caudal fin (b) pale liver (arrowed) and accumulation of a liquid in the abdominal cavity

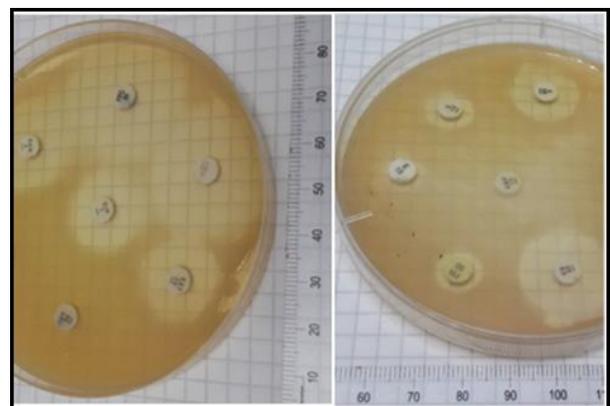


Figure 3. Antibiotic sensitivity test results of *A. hydrophila*

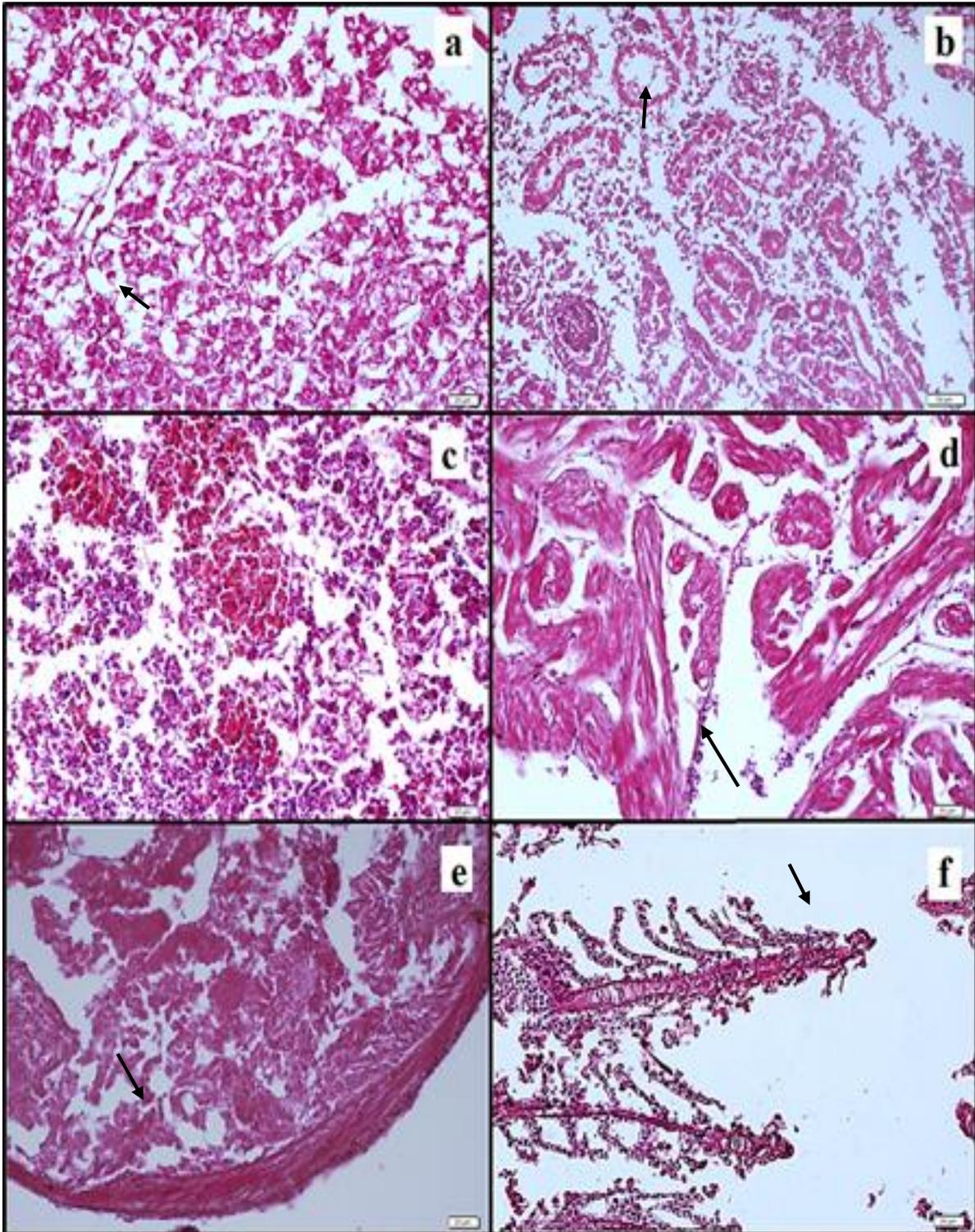


Figure 2. (a) Vacuolation (arrowed) and multifocal liquefactive necrosis; (b) degeneration and necrosis of the tubular epithelium (arrowed); (c) discharge of the white pulp; (d) lymphocyte infiltration between the cardiac muscle (arrowed); (e) desquamation of the lamina propria and mucosa epithelium (arrowed); (f) sloughed of the necrotic gill epithelium (arrowed)

Table 1. Morphological, physiological and biochemical characteristics of the isolates recovered from diseased guppy

Characteristics	Isolates (n=15)	Characteristics	Isolates (n=15)
Gram staining	-	Growth at 1% NaCl	+
Motility	+	Growth at 2% NaCl	+
Oxidase	+	Hemolysis	β
Catalase	+	Arginine dihydrolase	+
O/F	F	Ornithine decarboxylase	-
O/129 (10-150µg/disc)	-/-	Lysine decarboxylase	V
Metil Red	+	Carbohydrate utilization	
Voges Proskauer	-	Lactose	-
Indole	+	Arabinose	+
Citrate	-	Sucrose	+
Nitrate	+	Mannitol	+
Aesculine	+	Maltose	+
Urease	-	Trehalose	+
Growth on TCBS	yellow	Sorbitol	-
Starch hydrolysis	+	Fructose	+
Gelatin hydrolysis	+	Inositol	-
Growth on MCA	+	Galactose	+
Growth on BHIA	+	Xylose	-

+: positive reaction, -: negative reaction, V: variable, O/F: Oxidative/Fermentative MCA: MacConkey Agar

Table 2. Susceptibility of *A. hydrophila* isolates recovered from moribund guppy to 12 types of antibiotics

Antibiotic (µg/disc)	Isolates (n=15)	Antibiotic (µg/disc)	Isolates (n=15)
Flumequine (30 µg)	S	Sulphamethoxazole (25 µg)	S
Erythromycine (15 µg)	R	Streptomycin (10 µg)	I
Kanamycin (30 µg)	I	Chloromphenicol (30 µg)	S
Oxytetracycline (30 µg)	R	Furazolidon (50 µg)	R
Ciproflaxacin (1 µg)	S	Enrofloxacin (5 µg)	S
Ampicillin (10 µg)	R	Florfenicol (30 µg)	S

R: Resistant, I: Intermediate sentivite, S: Sensitive

4. Discussion

The bacterial diseases are the most important diseases in ornamental fishes and a significant cause of high mortality rates (Wildgoose,

2001). MAS is the most common bacterial disease of freshwater fish species. *A. hydrophila*, the agent of MAS, is a zoonotic

pathogen (Li and Saghaian, 2011) and in human septic arthritis, diarrhea, corneal ulcers, skin and wound infections, meningitis and fulminating septicemia may be caused (Von Mensch, 1968).

In the present study, according to Gram staining, presence of cytochrome oxidase and catalase enzyme, fermentation, O/129 resistivity isolated bacteria were identified as *Aeromonas* sp. According to the other morphological and biochemical test result and API profile of our isolates, in present study isolated bacteria were identified as *A. hydrophila* and comparable to that recorded by Buller (2004), Austin and Austin (2016).

The gross pathology observed in our findings bear similarities to MAS in the other ornamental fish species (Hettiarachchi and Cheong, 1994) such as loss of the scales, hemorrhagic ulcerative skin lesions, and abdominal distension with an accumulation of liquid in the abdominal cavity. We think that ulcerative skin lesions observed moribund guppy may be on account of the chronic motile aeromonas infection described by Huizinga et al (1979). Afifi et al. (2000) reported exophthalmia in the eyes of moribund Nile tilapia infected with *A. hydrophila* however; this clinical finding was not observed in the diseased guppy. Internally infected guppy exhibited pale liver because of bacterial toxins liver may become pale color as described by other researchers (Ventura and Grizzle, 1988; Afifi et al., 2000; Hamid et al., 2017).

Vacuolation and necrosis in the liver has been reported in different fish species such as *C. punctatus*, *Oreochromis niloticus*, *Oreochromis aureus* on account of toxins and extracellular products produced by *A. hydrophila* (Afifi et al., 2000; Laith and

Najiah, 2013; Al-Yahya et al., 2015). In this study, degeneration and necrosis were detected in the visceral organs such as liver, kidney and spleen, however hemorrhage and hyperemia reported by other researchers (Pal et al., 2015; Abdelhamed et al., 2017; Hamid et al., 2018) was not detected in the diseased guppy.

The resistance of *A. hydrophila* to commonly used antibiotics is an emerging problem in the ornamental fish. It has been reported that there is a resistance in *A. hydrophila* against a wide range of antibiotic compound such as chloramphenicol, erythromycin, streptomycin, ampicillin, novobiocin, streptomycin (Aoki, 1988). Tetracycline and oxytetracycline are commonly applied for the treatment of MAS (Laith and Najiah, 2013). However, in this study our isolates are resistant to oxytetracycline. This result shows that the use of uncontrolled antibiotics in fish production causes resistance of pathogenic bacteria. In addition, previous studies describe that *Aeromonas* strains are resistant to ampicillin (Radu et al., 2003; Dias et al., 2012). Similarly, in this study our isolates are resistant to ampicillin.

A. hydrophila causes serious health problems both fish in the aquarium and aquarist contact with infected fish. For this reason, it is important to determine the cause of the disease and to apply the appropriate treatment method for zoonotic agents like *A. hydrophila*. In this study, *A. hydrophila* were recovered from moribund guppy as primer disease. Uncontrolled treatment may cause resistance of pathogen bacteria; for this reason, especially treatment of zoonotic agents is important both aquarists through contact with infected fish and fish health.

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