

Siderophore, hemolytic, protease, and pyrazinamidase activities and antibiotic resistance in motile *Aeromonas* isolated from fish

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Received: 29.01.2009

Abstract: A total of 120 fresh water fish samples were evaluated for the presence of *Aeromonas* spp. *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria* were isolated from the gills, intestines, livers, and skins of fish, and 78 isolated *Aeromonas* spp. strains were further examined for siderophore, hemolytic, protease, and pyrazinamidase activities and antibiotic resistance. Siderophore production was demonstrated in almost all of the *A. hydrophila* and *A. caviae* strains except for 2 strains of *A. veronii* bv. *sobria*. The strains identified as *A. hydrophila* and *A. veronii* bv. *sobria* were the stronger producers of hemolysin, whereas the *A. caviae* strains were nonhemolytic. It was found that 92% of *A. hydrophila* and 91% of *A. caviae* strains hydrolyzed pyrazinamide in under 48 h, whereas 5% of *A. veronii* bv. *sobria* strains hydrolyzed pyrazinamide. Protease activity was found in 100% of isolates of *A. hydrophila* and *A. veronii* bv. *sobria*, but in 81% of *A. caviae* isolates. In addition, all *Aeromonas* species were examined for antibiotic resistance patterns and were found to be resistant to ampicillin and tetracycline.

Key words: *Aeromonas*, siderophore, hemolysin, pyrazinamidase, antibiotic resistance, fish

Balıklardan izole edilen hareketli *Aeromonas*'larda siderofor, hemolitik, proteaz, pirazinamidaz aktiviteleri ve antibiyotik direnç

Özet: Toplam 120 tatlı su balığı örneklerinin, solungaç, bağırsak, karaciğer ve deri numunelerinden izole edilen *Aeromonas* cinsine ait *A. hydrophila*, *A. caviae* ve *A. veronii* bv. *sobria* varlığı değerlendirilmiştir. İzole edilen 78 *Aeromonas* suşunun siderofor, hemolitik, pirazinamidaz, proteaz aktiviteleri ve antibiyotik direnç durumları incelenmiştir. Siderofor üretimi *A. hydrophila*, *A. caviae* suşlarının neredeyse tamamında görülürken *A. veronii* bv. *sobria* suşlarının yalnızca ikisinde gözlenmiştir. *A. hydrophila* and *A. veronii* bv. *sobria* suşları hemolizin üretirken *A. caviae* suşları nonhemolitikdir. *A. hydrophila* suşlarının % 92'si, *A. caviae* suşlarının % 91'i pirazinamid'i 48 saatten daha az bir zamanda hidroliz etmiş oysa *A. veronii* bv. *sobria* suşları yüzde beş oranında aktivite göstermiştir. Proteaz aktivitesi, *A. hydrophila*, ve *A. veronii* bv. *sobria* izolatlarının tamamında (% 100) bulunurken *A. caviae* izolatlarının % 81,8'de saptanmıştır. Bunlara ilaveten, tüm *Aeromonas* suşları ampisilin ve tetrasikline karşı dirençli bulunmuştur.

Anahtar sözcükler: *Aeromonas*, siderofor, hemolizin, pirazinamidaz, antibiyotik direnç, balık

Introduction

The motile mesophilic *Aeromonas* species are autochthonous inhabitants of aquatic environments (1). The fact that they have been recently isolated from the acute intestinal infections identified in people has added to the importance of these microorganisms and led researchers to study this topic (2). They are increasingly being noted as significant pathogens for humans and lower vertebrates, including amphibians, reptiles, and fish (3). Some *Aeromonas* species are responsible for causing a variety of human infections, including septicemia, wound infections, meningitis, pneumonia, and gastroenteritis (4,5).

That motile *Aeromonas* are found in both chlorinated and nonchlorinated water, are transmitted from these environments to food, and can reproduce at refrigerator temperatures due to the psychrophilic characteristics of some of its types suggest that they may be pathological agents critical for human health (6-10). Several investigators reported that members of the genus *Aeromonas* are common contaminants of food, and they have been isolated from a wide range of food products such as fish, chicken, meat, and vegetables (2-4). Characteristically freshwater bacteria, motile types of *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria*, which are dominant elements in the natural microbial flora of the aquatic animals, can cause various diseases in freshwater fish, like carp (11,12).

Siderophores are bacterially secreted molecules with a high affinity for iron that scavenge iron from the bacterial cells' environment for growth. Production of siderophores has been reported in different species of *Aeromonas* and might be related to virulence properties. However, the role of siderophores, whether enterobactins or the recently characterized and named amonobactins, in *A. hydrophila* infections is not clearly determined (13,14). Various putative virulence factors (aerolysin/hemolysin, proteases, lipases, DNases, enterotoxins, cytotoxins, and siderophores) that may play an important role in the development of diseases, either in humans or in fish, have been described in several species of the genus (15-18). Some authors reported that the production of cytotoxins and hemolysin is related to *A. hydrophila* and *A. veronii*

bv. *sobria* (19), and hemolytic molecules seem to be related to enterotoxigenicity (16). McMahon (20) indicated a correlation between the pathogenic potential and the hemolytic and proteolytic activity of *Aeromonas* species isolated from different sources. There are several reports dealing with pyrazinamidase activity, which has been extensively studied in *Mycobacterium* spp. and is considered a differentiating feature in the classification of mycolic acid-containing actinomycetes and a virulence-associated marker in the genus *Yersinia* (21,22). Some isolates of *Aeromonas* have pyrazinamidase activity, a valuable phenotypic marker to assist in the differentiation of *A. veronii* bv. *sobria* from *A. hydrophila* and *A. caviae*. Regarding the resistance to antimicrobial agents, several authors have stated that *Aeromonas* species are rapidly adapting to new drugs commonly used in medicine, becoming a potential risk to public health (23,24).

In view of increasing evidence supporting the role of aeromonads in human and fish diseases, we isolated *Aeromonas* spp. from freshwater fish obtained from the local bazaar and characterized them with respect to their siderophore, hemolytic, protease, and pyrazinamidase activities and antibiotic resistance in *in vitro* conditions.

Materials and methods

Isolation and identification of motile *Aeromonas*

Fish samples were randomly purchased from the local bazaar in Kırşehir, Turkey. A total of 120 samples of fish were screened for the presence of *Aeromonas* spp. Samples were immediately transferred to the laboratory in ice chests at 4-7 °C. Gill, intestine, liver, and skin contents of fish samples (5 g) were aseptically swabbed using sterile cotton buds and inoculated into 45 mL of alkaline peptone water (APW, pH 8.4) containing 30 µg/mL of ampicillin (A-9393, Sigma Chemical Co., St. Louis, MO, USA) and homogenized for 2 min in sterile Stomacher bags. After 18 h of incubation at 28 °C, 0.1 mL of the APW was streaked on the glutamate starch phenol red agar (GSP, Merck, Darmstadt, Germany). After incubation at 28 °C for 24 h, yellow colonies surrounded by a yellow zone were picked and grown on fresh GSP agar plates for

confirmation (25). The presumptive *Aeromonas* colonies were identified at the genus level by testing their Gram reaction, oxidase, catalase, motility, and growth conditions in 0% and 6% NaCl, oxidation/fermentation (glucose), and resistance to vibriostatic agent O/129 (2,4-diamino-6,7-diisopropylpteridine) (150 µg/mL) (1,26). All *Aeromonas* spp. were reidentified biochemically at the species level by using tests chosen from those described by Joseph and Carnahan (26), that is, esculin hydrolysis, production of H₂S from cysteine, production of gas from glucose, growth in KCN broth, salicin and arabinose fermentation, Voges-Proskauer reaction, and lysine decarboxylase. As reference strains, *A. hydrophila* (ATCC 7966), *A. veronii* bv. *sobria* (ATCC 43979), and *A. caviae* (ATCC 15468) were used. The strains were stored in trypticase soy broth (TSB) with 15% (v/v) sterile glycerol at -70 °C until assayed.

Determination of siderophore production

The chrome azurol siderophore detection agar (CAS agar) (27), prepared as described by Barghouthi et al. (14), was used to detect siderophore production. Single colonies of each isolate were transferred to this medium, and the plates were incubated at 28 °C for 48 h. The presence of an orange halo around the colonies was recorded as positive for siderophore production.

Determination of hemolytic activity

The strains were tested for hemolytic activity by streaking them onto trypticase soy agar (TSA) plates containing 7% sheep blood for 48 h at 37 °C. Beta hemolytic zones of 2 mm or more around the colonies were regarded as the sign of positive hemolytic activity (28). Hemolytic activity for the *Aeromonas* spp. strains was categorized as alpha, beta, or gamma (29).

Determination of proteolytic activity

Protease activity was determined on the surface of skim milk agar, in which skim milk was added just before pouring the medium into the petri plates. The plates were incubated at 28 °C for 4 days. After the incubation period, the clear zones of hydrolysis were measured and recorded. The presence of a transparent zone around the colonies indicated protease activity (30).

Pyrazinamidase activity assays

The following tests were additionally performed on all *Aeromonas* strains. Pyrazinamidase activity was assayed by using a supplied medium that contained trypticase soy agar (15 g; Difco), yeast extract (1.5 g; Sigma Chemical Co.), and Tris-maleate buffer (0.2 M, pH 6, 500 mL) and pyrazinamide (0.5 g; Sigma), and was dispensed in 5 mL aliquots into screw-cap tubes (16 × 150 mm), autoclaved at 121 °C for 15 min. These media were inoculated with 24-48 h of bacterial growth taken from tryptic soy agar (Difco) and incubated for 48 h at 37 °C. One milliliter of 1% (wt/vol) freshly prepared ferrous ammonium sulfate aqueous solution was flooded over each slant, and a positive (pinkish rust color) or negative (colorless) reaction was recorded after 15 min. Positive pyrazinamidase activity indicated the presence of pyrazinoic acid resulting from the action of the enzyme pyrazinamidase (21).

Antimicrobial resistance

The resistance of all strains to different antimicrobial agents was determined by the disk diffusion method (31). Bacteria were cultured in TSB and spread onto the surface of Mueller-Hinton agar. The isolates were individually tested against 9 antimicrobial agents. The antibiotics and concentration ranges tested were as follows: ampicillin, 10 µg; amoxicillin, 10 µg; streptomycin, 10 µg; ceftazidime, 30 µg; ciprofloxacin, 5 µg; kanamycin, 30 µg; tetracycline, 30 µg; gentamicin, 10 µg; and trimethoprim, 5 µg.

All antibiotics were obtained from Oxoid Limited (Hampshire, England). Plates with bacteria inoculum were incubated at 28 °C for 4 days. Inhibition zones that formed in the culture media at the end of this period were evaluated in millimeters. The resistance breakpoints were those defined by the National Committee for Clinical Laboratory Standards (NCCLS, 2004) for gram-negative bacteria. *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *A. hydrophila* (ATCC 7966), and *A. caviae* (ATCC 15468) were used as controls.

Results and discussion

Aeromonas spp. were isolated from 78 (65%) of the 120 samples tested. As seen in Table 1, presumptive *Aeromonas* spp. were identified and confirmed as *A. hydrophila*, *A. veronii* bv. *sobria*, and *A. caviae* morphologically, physiologically, and biochemically.

In general, 65% (78/120) of the fish samples examined were positive for *Aeromonas* spp.: 46.1% (36/78) were positive for *A. hydrophila*, the predominant species; 28.2% (22/78) for *A. caviae*; and 25.6% (20/78) for *A. veronii* bv. *sobria* (Table 2). The incidence of *Aeromonas* spp. varied depending on the samples (gills, intestines, skins, or livers) examined. The results indicate that the highest *Aeromonas* incidence is observed on the skin (80%) of fish samples.

As shown in Table 2, *A. hydrophila* was the dominant species (46.1%), followed by *A. caviae* (28.2%) and *A. veronii* bv. *sobria* (25.6%). *A. hydrophila* and *A. veronii* bv. *sobria*, which have been linked with human infections, were isolated from fish examined in this study. More recent investigations on the prevalence of *Aeromonas* species in sources such as environmental, clinical, food, and veterinary origins have focused on 3 mesophilic species, namely *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria*, respectively. Several studies showed pronounced variations in their occurrence (2,7,8,25,32-37).

When the isolates were grown on a CAS agar, on which siderophore production can be detected, all isolates produced a clear zone (18). In our study, siderophore production was found in 54 (69.2%) of

Table 1. Biochemical properties of isolates.

Biochemical test ^a	Phenotypic expression (%)		
	<i>A. hydrophila</i>	<i>A. veronii</i> bv. <i>sobria</i>	<i>A. caviae</i>
Esculin hydrolysis	100	0	100
Growth in KCN broth	100	0	100
Gas from glucose	100	100	0
H ₂ S from cysteine	100	100	0
Fermentation of salicin	100	0	100
Fermentation of arabinose	100	0	100
Voges-Proskauer	98	100	0
Lysine decarboxylase	100	100	0

a: Joseph and Carnahan (1994)

Table 2. The distributions of *Aeromonas* species in different body parts of fish samples examined.

Organs	Positive samples	<i>A. hydrophila</i>	<i>A. veronii</i> bv. <i>sobria</i>	<i>A. caviae</i>
Intestine	23/30 (76.6%)	13 (56.5%)	nd	10 (43.7%)
Skin	24/30 (80.0%)	9 (37.5%)	7 (29.1%)	8 (33.3%)
Gills	21/40 (52.5%)	9 (42.8%)	11 (52.3%)	1 (4.7%)
Liver	10/20 (65.0%)	5 (50.0%)	2 (20%)	3 (30%)
Overall total	78/120 (65%)	36 (46.1%)	20 (25.6%)	22 (28.2%)

nd: none detected

78 isolates. As shown in Table 3, production of siderophores was detected in all aeromonad species. The highest producer of siderophores was *A. caviae*, at a rate of 95.4% (21/22), followed by 86% of the *A. hydrophila* (31/36) and 10% of the *A. veronii* bv. *sobria* (2/20).

Another virulence factor is the ability to scavenge required nutrients such as iron. Many pathogenic bacteria utilize iron uptake pathways, such as the production of siderophores, to access iron for growth. These uptake pathways are considered to be one of the virulence factors, since they facilitate the growth of pathogenic bacteria and the subsequent production of other virulence factors (18). Earlier studies have reported that the ability to produce siderophores correlated with higher virulence in *Aeromonas* species (38).

This study of siderophore synthesis by 78 isolates classified as phenospecies *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria* showed that more than 86% of the *A. hydrophila* isolates synthesized siderophores, while about 21% of *A. caviae* and 10% of *A. veronii* bv. *sobria* may have produced siderophores. This indicates that *A. hydrophila* and *A. caviae* were higher producers of siderophores than *A. veronii* bv. *sobria*, a rare siderophore producer. Some investigators (13,16) indicated that *A. hydrophila*, *A. caviae*, and *A. veronii* bv. *sobria* isolated from fish produced siderophores. Gierer et al. (39) showed that a total of 355 strains of fish-pathogenic bacteria, including *A. hydrophila*, *A. caviae*, *Pseudomonas fluorescens*, and *P. putida* isolates, were determined as siderophore producers. However, a previous investigation mentioned above indicated that the pathogenicity of *A. hydrophila* was not always accompanied by the presence of

siderophores. Whether or not siderophore production is an important virulence factor is not clear (13). As defined by Alavandi and Ananthan (40), there was no significant difference between the clinical and environmental aeromonads with respect to their enterotoxigenicity, cytotoxicity, and ability to produce siderophores. Although it is known that bacteria can produce some siderophore compounds simply for survival, the cross-feeding assays demonstrated that at least aerobactin and enterobactin biosynthesis are not essential virulence determinants in motile *Aeromonas* strains (12). As can be seen in Table 3, our results show no significant relation between siderophore and hemolytic activities. We could propose a minor relation between the production of siderophores and hemolysin. Furthermore, it was shown that *Aeromonas* strains play an important role in iron acquisition. Molecular studies are needed to actually reveal the correlation between these 2 factors.

The hemolysin production by *Aeromonas* strains is shown in Table 3. Among the *Aeromonas* isolates, 90% of *A. veronii* bv. *sobria*, followed by 89% of *A. hydrophila*, from fish samples were beta-hemolytic on sheep blood agar plates, while none of the *A. caviae* strains were hemolytic. This indicates that *A. hydrophila* and *A. veronii* bv. *sobria* were higher producers of hemolysin than *A. caviae*. No type of hemolysis, except for beta-hemolysis, was found in the isolates.

The production of hemolytic toxins has been regarded as strong evidence of pathogenic potential in aeromonads (16,19). Beta hemolysin has been reported as a virulence factor in motile aeromonads (41). In this study, *A. hydrophila* and *A. veronii* bv. *sobria* strains exhibited beta-hemolytic activity to

Table 3. Incidence of some virulences in motile *Aeromonas* spp. isolated from fish samples.

Species	No. of isolates	Siderophore	Hemolysin	Protease	Pyrazinamidase
<i>A. hydrophila</i>	36	31 (86%)	32 (89%)	36 (100%)	33 (92%)
<i>A. veronii</i> bv. <i>sobria</i>	20	2 (10%)	18 (90%)	20 (100%)	1 (5%)
<i>A. caviae</i>	22	21 (95.4%)	nd	18 (81.8%)	20 (91%)
Overall total	78 (65%)	54 (69.1%)	50 (64.1%)	74 (94.8%)	54 (69.2%)

nd: none detected

different extents, but not *A. caviae* strains. Our findings agree with the results recorded by Janda et al. (42), who showed similar percentages among 121 *Aeromonas* strains: 93% of *A. veronii* bv. *sobria* and 95% of *A. hydrophila* were hemolytic. These results are substantiated by the findings of Farag (43) and Yucel and Çitak (44), who showed a significant difference between the hemolysin production of *A. hydrophila* and *A. veronii* bv. *sobria* as compared with that of *A. caviae*. This represents that hemolysin production may be related to *A. veronii* bv. *sobria* and *A. hydrophila*, but not *A. caviae*. Therefore, hemolysin can be one of several factors determining pathogenicity, but are not required for virulence in all *Aeromonas* species.

Table 3 presents protease activity, which was found in 74 (94.8%) of 78 *Aeromonas* isolates from fish. Specifically, 100% of *A. hydrophila* and *A. veronii* bv. *sobria* were producers of protease, followed by 81.8% of *A. caviae*. The majority of the isolates obtained in the present study produced protease.

Proteases are important factors in the spoilage of foods, and the presence of proteases and hemolysins are used as an indicator of potential pathogenicity (20). Both the quantitative and qualitative production of protease is important in establishing the virulence of a particular strain (45). The proteolytic activity of *A. hydrophila* has been correlated with its ability to induce pathology in fish (46). Our findings revealed that proteases, more than hemolysin, may be important virulence factors in *Aeromonas* infections. Proteases are thought to contribute to the virulence of aeromonads for fish and other hosts; however, their contribution to human pathogenicity still needs to be determined.

The summarized results in Table 3 show 78 well-characterized *Aeromonas* strains representing 3 species from fish for pyrazinamidase activity. This activity was detected in 92% of *A. hydrophila*, 91% of *A. caviae*, and 5% of *A. veronii* bv. *sobria* in under 48 h (Table 3).

The presence of pyrazinamidase in *A. hydrophila* and *A. caviae*, but its absence in representatives of other species of *Aeromonas* (*A. veronii* bv. *sobria*, *A. schubertii*, and *A. veronii*), was described 19 years ago (47). Because the taxonomy of aeromonads has been

in a state of constant flux, we considered it imperative to start with strains that were well characterized at the species level. The present study has demonstrated that all (36 of 36) strains of *A. hydrophila*, 21 of 22 *A. caviae*, and 1 of 20 *A. veronii* bv. *sobria* hydrolyzed pyrazinamide. These findings are consistent with the observations by Carnahan et al. (47) and Wakabongo et al. (48). The given results in Table 3 show that pyrazinamidase activity could be a valuable phenotypic marker to aid in the identification of *A. veronii* bv. *sobria* from *A. hydrophila* and *A. caviae*. Several virulence factors have been stated as indicators of pathogenicity in *Aeromonas* spp. However, pyrazinamidase activity by *Aeromonas* is not clearly determined. It has been reported that negative pyrazinamidase might also be a new virulence-associated marker for *A. veronii* bv. *sobria*, *A. schubertii*, and *A. veronii* (47). Further studies are necessary on the determination of the nature of this association.

The antibiotic resistance patterns of the *Aeromonas* species isolated from the fish samples are shown in Table 4. All strains of *A. hydrophila*, *A. veronii* bv. *sobria*, and *A. caviae* were resistant to ampicillin and tetracycline. Furthermore, the highest resistances encountered were 67.9% to trimethoprim, 44.8% to amoxicillin, and 35.8% to ceftazidime. The lowest resistance was detected for streptomycin (8.9%). None of the *Aeromonas* strains encountered in the present study were resistant to ciprofloxacin or kanamycin.

The frequent occurrence of multiple antimicrobial resistances among *Aeromonas* spp. isolates that are both fish pathogens and emerging opportunistic human pathogens has been monitored over the past decades (25,37,44,45,49-57). In this study, all strains were 100% resistant to ampicillin/beta-lactam and tetracycline. *Aeromonas* spp. have been reported to be intrinsically resistant to ampicillin (49). As defined in our experiment, 44.8% of the strains were resistant to amoxicillin/beta-lactam, which is similar to the findings of Hatta et al. (50), who reported that 33.3%-52.7% of the *Aeromonas* strains from freshwater fish were resistant to amoxicillin. Obtained results indicate that beta-lactam agents should be avoided in the treatment of *Aeromonas* spp. infections. As mentioned, 100% of the *Aeromonas* strains exhibited

Table 4. Prevalence of antibiotic resistance among motile aeromonads isolated from fish.

Antibiotics (µg/disk)	Percentage of resistant strains			
	<i>A. hydrophila</i> (n = 36)	<i>A. veronii</i> bv. <i>sobria</i> (n = 20)	<i>A. caviae</i> (n = 22)	Resistant strains (%)
Trimethoprim	50	85	81.8	67.9
Gentamicin	16.6	0.0	0.0	16.6
Tetracycline	100	100	100	100
Kanamycin	0.0	0.0	0.0	0.0
Ceftazidime	27.7	40	45.4	35.8
Ciprofloxacin	0.0	0.0	0.0	0.0
Streptomycin	13.8	10	0.0	8.9
Ampicillin	100	100	100	100

n: number of isolates

resistance against tetracycline in our study. In a previous paper, Ko et al. (51) found as many as 49% tetracycline-resistant *Aeromonas* spp., compared with 14% in the data of Goni-Urizza et al. (52). Additionally, the resistance to tetracycline has been reported to be acquired and encoded by plasmids or transposons (53,54). The resistance level to trimethoprim was comparable to the findings of Yucel and Çitak (44), who reported 63%-100% of trimethoprim resistant strains, and these results are supported by Vivekanandhan et al. (55) and Ansary et al. (56). However, resistance of *Aeromonas* spp. to ceftazidime was 35.8% in this study, compared to the findings Radu et al. (25) and Ceylan et al. (57), who observed no resistance at all (0%) in retail fish and livestock, respectively. We found that around 8.9% of the strains were resistant to streptomycin, but none of the *A. caviae* demonstrated resistance to streptomycin. This result is in contrast to the findings of Hatha et al. (50), who reported that none of the *Aeromonas* strains from different freshwater fish were resistant to this antibiotic. Strains of *Aeromonas* isolated from frozen fish (36) showed 58.4% resistance against gentamicin, while we encountered 16.6% resistance to gentamicin. However, Yucel et al. (37) reported that 10%-54% of the *Aeromonas* strains from retail fish were resistant to this antibiotic. These results are in contrast to the findings of Goni-Urizza

et al. (52), who reported that 1% of *Aeromonas* strains from rivers were resistant to gentamicin.

Results obtained in this study indicate that multiple resistance, particularly to ampicillin and tetracycline, is often seen in *Aeromonas* spp. isolated from fish, since geographic locations and local selective pressure influence the antibiotic resistance levels.

There has been no study about the presence of motile *Aeromonas* types in carp sold in public outdoor bazaars of Kırşehir, Turkey. To our knowledge, this is the first report on the existence of putative virulence factors and antimicrobial resistance of foodborne *Aeromonas*. The present study isolated motile *Aeromonas* spp., which have been recently identified as an important food pathogen, from carp supplied for public consumption in Kırşehir. In order to determine whether they posed a risk to public health, they were tested for putative virulence properties like siderophore, hemolysin, protease, and pyrazinamidase activity, and their antibiotic resistance was examined.

In conclusion, the present work has clearly emphasized an important incidence of *Aeromonas* spp. with virulence and antibiotic resistance in fish intended for human consumption. Consuming raw or undercooked fish increases the risk of developing

human infections because of the high occurrence of pathogenic *Aeromonas* strains. Pyrazinamidase activity, which is more commonly considered a phenotypic marker in the differentiation of some types of *Mycobacterium*, was used in the characterization of *Aeromonas*. This activity has almost never been employed in such studies in Turkey. An increasing presence of multiple drug-resistance among *Aeromonas* species may become a potential human health hazard. Therefore, the results of the present investigation indicate that additional studies in this field are warranted to elucidate the public health significance of aeromonads in the aquatic environment.

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Acknowledgment

This work at AEU was supported by grants from the Gazi University Scientific Research Projects Department.

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