



Antibiotic Susceptibility of Motile *Aeromonas* spp. Isolated from Foods of Animal Origin and Water *

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Summary: In this study, presence of motile *Aeromonas* spp. in different foods of animal origin and water by cultural method and detection to antibiotic susceptibilities of the isolates were investigated. A total of 224 food samples such as fish, chicken, fishery products, milk, meat and water supplied from points of sale like market and bazaar in and around Izmir have been examined. *Aeromonas* selective agar was used for *Aeromonas* spp. isolation. Gram-negative and oxidase positive colonies were assessed in Vitek 2 system (France) for identification. Of 224 food samples (meat, chicken, fish, fishery products, milk and water) 87 have been found to be positive for motile *Aeromonas* spp. According to the study, 51 (22.76%) of them were determined as *A. hydrophila*, 26 (11.6%) of them as *A. caviae* and 10 (4.6%) of them as *A. sobria*. Antibiotic susceptibility of *Aeromonas* isolates was determined according to the Kirby Bauer disk diffusion method. All *Aeromonas* spp. isolates have been found to be susceptible to imipenem and ciprofloxacin.

Key words: Antibacterial susceptibility, food, isolation, motile *Aeromonas* spp.

Hayvansal Gıdalardan ve Sudan İzole Edilen Hareketli *Aeromonas* Türlerinin Antibiyotik Duyarlılığı

Özet: Bu çalışmada hareketli *Aeromonas*'ların farklı hayvansal gıdalar ve sudaki varlığını ortaya koymak ve elde edilen izolatların antibiyotik duyarlılıklarının saptanması amaçlanmıştır. Çalışmada İzmir ve çevresindeki market ve pazar gibi faaliyet gösteren satış noktalarından tedarik edilen toplam 224 adet gıda (balık, balıkçılık ürünü kıyma, tavuk, süt ve su) örneği incelenmiştir. *Aeromonas* spp. izolasyonu için *Aeromonas* selektif agar kullanılmıştır. Gram-negatif, oksidaz pozitif koloniler, identifikasyon için Vitek 2 sistemi (Fransa) ile değerlendirilmiştir. İncelenen 224 gıda örneğinin 87'si hareketli *Aeromonas* spp. yönünden pozitif bulunmuştur. Örneklerin 51'inde (%22.76) *Aeromonas hydrophila*, 26'sında (%11.6) *Aeromonas caviae* ve 10'unda (% 4.46) *Aeromonas sobria* saptanmıştır. *Aeromonas* izolatlarının antibiyotik duyarlılığı Kirby Bauer disk difüzyon metodu ile belirlenmiştir. Tüm izolatlar, imipenem ve siprofloksasine duyarlı bulunmuştur.

Anahtar kelimeler: Antibakteriyel duyarlılık, gıda, izolasyon, hareketli *Aeromonas* spp.

Introduction

Motile *Aeromonas* spp. exists in the environment especially in marine water, drinking water, fresh and brackish water (10). The reason for defining the motile *Aeromonas* spp. as food pathogens is that they exist widely in the environment (7). Since they have psychrophilic structures, they can reproduce in the foods kept at 4°C, and pose a potential danger for human health (3,5,13). *Aeromonas* spp. have a number of different putative virulence factors such as enterotoxins, haemolysin or cytotoxins and antibiotic resistance to some antibiotics (3,11,13), and are responsible for intestinal and extraintes-

tinal infections in humans (13).

Aquatic environment plays an important role in the dissemination of *Aeromonas* spp., while fish and seafood, raw and cooked meat, chicken, vegetables, and milk can be potential carriers for human and animal infections (13).

This study was conducted both to determine the existence of motile *Aeromonas* spp. in foods of animal origin and in water by a cultural method and to reveal the antibiotic susceptibilities of the isolates.

Material and Methods

Standard strain

Standard *Aeromonas hydrophila* ATCC 7512 strain was obtained from the Bornova Veterinary Control Institute, Culture Collection.

Isolation of *Aeromonas* spp. from the samples

Each sample was subjected to bacteriological

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examination under aseptic conditions. Twenty grams were taken from the samples and placed in sterile sample bags, 225 ml alkaline peptone water was added, and homogenized in stomacher for 2 minutes, and then were left for incubation at 28°C for 18-24 hours. A loopful of the enrichment culture was streaked onto *Aeromonas* Agar (Oxoid, CM0833A, England) used with Ampicillin Selective Supplement (Oxoid, SR0136, England) and the inoculated plates incubated at 28°C for 18-24 hours. After the incubation, the colonies that reproduced in the plates in the form of green, and the button-green colonies in their middle parts were accepted as suspected *Aeromonas* spp. The colonies were plated on the nutrient agar with needle-point loop in the form of single colony, and incubated at 30°C for 24 hours. The Gram-negative and oxidase positive colonies were assessed in the Vitek 2 device for identification (Vitek 2 system-France) (2).

cin (5 mcg), vancomycin (30 mcg), cefuroxime (30 mcg), imipenem (10 mcg), chloramphenicol (30 mcg), tobramycin (10 mcg), ciprofloxacin (10 mcg) antibiotic disks (Oxoid, England) were placed.

After the disks were placed, the agars were taken to an incubator and incubated at 37°C for 18-24 hours. Then, the zone diameters were measured.

Results

In this study, 224 different food samples supplied from the points of sale like market in and around Izmir have been investigated.

Eighty-seven (87%) of the 224 food samples including 46 fish, 25 shrimps, 30 mussels, 43 chickens, 35 minced meat samples, 25 milk and 20 water samples have been found to be positive in terms of motile *Aeromonas* spp. Fifty one (22.76%) of these were observed to have *Aeromonas hydrophila*, 26 (11.6%) to have *Aeromonas caviae*, 10 (4.46%) to have *Aeromonas sobria* (Table 1).

Table 1. The prevalence and distribution of motile *Aeromonas* spp. in various food samples

	Examined Food Samples (n)	<i>A. hydrophila</i>	<i>A. caviae</i>	<i>A. sobria</i>
Fish	46	15 (32.6%)	9 (19.56%)	3 (6.5%)
Mussel	30	9 (30%)	5 (16.6%)	2 (6.6%)
Shrimp	25	8 (32%)	3 (12%)	1 (4%)
Meat	35	5 (14.2%)	3 (8.5%)	1 (2.85%)
Chicken	43	6 (13.9%)	3 (6.9%)	2 (4.6%)
Milk	25	5 (20%)	3 (12%)	1 (4%)
Water	20	3 (15%)	-	-

n=number of samples

Antibiotic Susceptibility Testing

The antibiotic susceptibility of *Aeromonas* isolates was determined according to the Disk Diffusion Method. They were taken from the liquid culture with a 0.1 ml sterile pipette, and was spread on Muller Hinton Agar (Oxoid, CM0337, England) with a glass rod, In order to determine the antibiotic susceptibility of the isolates, erythromycin (15 mcg), streptomycin (10 mcg), gentamicin (30 mcg), amikacin (30 mcg), tetracycline (10 mcg), ampicillin (25 mcg), cephalothin (30 mcg), amoxicillin 20 mcg/clavulanic acid 10 mcg (30 mcg), trimethoprim 1.25 mcg/sulfamethoxazole 23.75 mcg (25 mcg), ofloxa-

The antibiotic susceptibility of 87 *Aeromonas* spp. isolates obtained in the study has been determined according to the disk diffusion method. The zone diameters of the inhibitions have been measured with caliper cage in mm. In this context, it has been observed that 51 *A. hydrophila* species were susceptible to amoxicillin clavulanic acid, ciprofloxacin and imipenem at a rate of 100%, and 81% of the strains were determined to be resistant to erythromycin, 85.4% of the strains were determined to be resistant to vancomycin, trimethoprim/sulfamethoxazole at a rate of 64.1%, respectively. Moreover, the 26 *Aeromonas caviae* isolates were susceptible to

imipenem, amikacin and ciprofloxacin at a rate of 100%; 88.6% of the isolates were determined to be resistant to erythromycin; 76.5% were determined to be resistant to vancomycin; 41% were detected to be resistant to trimethoprim/sulfamethoxazole; 21.5% were detected to be resistant to amoxicillin clavulanic acid and chloramphenicol; 10.9% were detected to be resistant to gentamicin and tetracycline. It was determined that the 10 *A. sobria* isolates had susceptibility to ciprofloxacin and amikacin at a rate of 100%, ampicillin and trimethoprim/sulfamethoxazole at a rate of 66.5%, and 90% were seen as resistant to streptomycin. The antibacterial susceptibility testing results of *Aeromonas* spp. isolates against antibiotics are given in Table 2.

the risk of infection in humans. Especially the fish, mussels, shrimps, living in sea and consumed as food by humans are contaminated with the pathogen bacteria that blend in the sea water together with waste water and pose an important threat for public health.

These examinations were conducted to detect the existence of motile *Aeromonas* spp. which contaminates the products especially food products in a cultural manner, and to reveal the antibiotic susceptibilities of the *Aeromonas* spp.

The frequency of existence and the antibiotic resistance of *Aeromonas* spp. in fish in Malaysia were investigated in 2003 (8). The researchers studied a total of 87 fish samples, and *Aeromonas* spp. were found in 69% of the samples. In the study, a total of 87 fish were used and

Table 2. Antibacterial susceptibility testing results of *Aeromonas* spp. isolated from food samples

Antibiotics	<i>A. hydrophila</i> (n=51)			<i>A. caviae</i> (n= 26)			<i>A. sobria</i> (n=10)		
	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Amikacin	2.4	18.5	79.1	-	-	100	-	-	100
Amoxicillin/ clavulanic acid	-	-	100	21.5	-	78.5	7.1	-	92.9
Ampicilin	28.6	7.1	21.5	12.4	12.4	75.2	66.5	-	33.5
Cefuroxime	14.3	21.4	64.3	-	-	100	11.6	-	88.4
Ciprofloxacin	-	-	100	-	-	100	-	-	100
Erythromycin	81.9	-	18.1	81.00	-	19.00	82.7	-	17.3
Gentamycin	34.6	7.8	57.6	10.9	11	78.1	32.5	-	67.5
Imipenem	-	-	100	-	-	100	4.4	-	95.6
Chloramphenicol	9.9	-	90.1	21.5	-	78.5	13.3	-	84.7
Cephalotine	85.8	7.1	7.1	66.7	-	33.3	83.3	-	16.7
Streptomycine	9	10.5	80.5	-	-	100	90	-	10
Tetracyline	8.3	11.4	80.3	10.9	18.9	70.2	-	16.7	83.3
Tobramycin	35.7	14.3	50	22.2	-	77.8	14.6	-	85.4
Trimethoprim/ sulfamethoxazole	64.1	14.4	21.5	41.00	-	59.00	66.5	-	33.5
Vancomycin	85.4	7.3	7.3	76.5	-	23.5	85.4	-	14.6
Ofloxacin	92.9	7.1	-	88.4	11.6	-	88.2	11.8	-

n=number of isolates

Discussion and Conclusion

The existence of motile *Aeromonas* spp. in healthy animals and in drinking water increases

sixty nine percent of the samples have been found positive for *Aeromonas* spp. *Aeromonas* isolates were defined as *A. sobria*, *A. hydrophila*,

and *A.caviae* at the rates of 55%, 11.5% and 2.3% respectively. All the isolates were found to be resistant to ampicillin, and susceptible to ceftazidime (8). Eighty-two *Aeromonas* spp. isolates were defined in 250 frozen fish samples in biochemical and genetic terms in Mexico (4). In a previous study, antibiotic susceptibility of 57 *Aeromonas* spp. isolates, which were isolated from different foods and water samples, were examined, and the majority of the isolates were found to be resistant to erythromycin, and susceptible to tetracycline, nitrofurantoin, chloramphenicol, quinolone and aminoglycosides (amikacin, gentamicin, tobramycin) (1). Sratev et al. (12) conducted different studies on *A.hydrophila* isolated from milk, dairy products, meat, meat products and vegetables, and reported that it was an opportunistic pathogen, and was resistant to commercial antibiotics. The existence of *A.hydrophila* in foods and its antibiotic resistance pose a threat for public health and for the health of living organisms in aquatic environments (12).

The reason of motile *Aeromonas* spp. for becoming more important in recent years is that their various virulence factors have been defined, and it has been understood better that these virulent factors lead to enteropathogenic. These factors are hemolysins, enterotoxins and cytotoxins (11).

In a study conducted in Ankara in 2006, it was detected that the *A.hydrophila* strains that were obtained from chicken and meat doner kebab samples were susceptible to gentamicin, chloramphenicol, tetracycline, cephalothin and chloramphenicol at a rate of 100%, and resistant to ampicillin and vancomycin at a rate of 100% (14).

In the present study, the *Aeromonas* spp. Isolates have been found to be susceptible to imipenem and ciprofloxacin at a rate of 100%. It has been reported that *Aeromonas* spp. may sustain their lives even in refrigerator temperatures because they are psychotropic. The selection of proper antibiotics is important for the treatment of the diseases caused by the bacteria. The antibiotic resistance of motile *Aeromonas* spp. must be handled with care because of these characteristics. One of the most important infection sources is the water contaminated with motile *Aeromonas* species. In order to prevent this, hygienic rules must be strictly followed, and some precautions must be taken

in the stages from food production to consumption.

With this study, the antibiotic susceptibility of motile *Aeromonas* spp. was investigated. The motile *Aeromonas* spp. are considered as opportunistic pathogens for humans and are isolated especially from fish, sea products, and various foods that are used for human consumption. The selection of proper antibiotic is important in terms of gastrointestinal infections stemming from food, contaminated by the bacteria.

References

1. Awan BM, Maqbool A, Bari A, Krovacecek K. Antibiotic susceptibility profile of *Aeromonas* spp. isolates from food in Abu Dhabi, United Arab Emirates. *New Microbiol* 2009; 32(1): 17-23.
2. Biomerieux, Vitek 2 Technology. Online software user manual, 510773-3EN1, Marcy l'etoile, France, 05/2006.
3. Daskalov H. The importance of *Aeromonas hydrophila* in food safety. *Food Control* 2006; 17(6): 474-83.
4. Castro-Escarpulli G, Figueras MJ, Aguilera-Arreola G, Soler L, Fernandez-Rendon E, Aparicio GO, Guarro J, Chacon MR. Characterisation of *Aeromonas* sp. isolated from frozen fish intended for human consumption in Mexico. *Int J Food Microbiol* 2003; 84(1): 41-9.
5. Gobat PT, Jemmi T. Distribution of mesophilic *Aeromonas* in raw and ready-to-eat fish and meat products in Switzerland. *Int J Food Microbiol* 1993; 20(2):117-20.
6. Gonzalez-Rodrigues MN, Santos JA, Otero A, Garcia-Lopez ML. PCR detection of potentially pathogenic *Aeromonad* in raw an cold-smoked freshwater fish. *J Appl Microbiol* 2002; 93(4): 675-80.
7. Neyts K, Huys G, Uytendaele M, Swings J, Debevere J. Incidence and identification of mesophilic *Aeromonas* spp from retail foods. *Lett Appl Microbiol* 2000; 31(5): 359-63.
8. Radu S, Ahmad N, Ling FH, Reezal A. Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malasia. *Int J Food Microbiol* 2003; 81(3): 261-6.
9. Santos Y, Toranzo AE, Barja JL, Nieto TP, Villa TG. Virulence properties and enterotoxin production of *Aeromonas* strains isolated from fish. *Infect Immun* 1988; 56(12):

- 3285-93.
10. Sechi LA, Deriu A, Falchi MP, Fadda G, Zanetti S. Distribution of virulence genes in *Aeromonas* sp. isolated from Sardinian waters and from patients with diarrhoea. J Appl Microbiol 2002; 92(2): 221-7.
 11. Seethalakshmi I, Subashkumar R, Saminathan P. Distribution of putative virulence genes in *Aeromonas hydrophila* and *Aeromonas salmonicida* isolated from marketed fish samples. J Fish Aquat Sci 2008; 3(2) 145-51.
 12. Stratev D, Odeyemi OA. Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review. J Infect Public Health 2016; 9(5): 535-44.
 13. Praveen K, Debnath H, Shekhar S, Dalai N, Ganguly S. Incidence of *Aeromonas* spp. infection in fish and chicken meat and its related public health hazard: A review. Vet World 2016; 9(1): 6-11.
 14. Topçu S. Ankara'da satışı sunulan döner kebab çeşitlerinden *L. monocytogenes* *S.aureus* *A. hydrophila* izolasyonu ve çeşitli antibiyotik dirençlilikleri Yüksek Lisans Tezi, Gazi Ü. Fen Bilimleri Enstitüsü, Ankara 2003; p. 1-40.

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