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RESEARCH ARTICLE

Occurrence and antibiotic susceptibility of *Vibrio* spp., *Aeromonas* spp. and *Listeria* spp. in seafoods

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Deniz ürünlerinde *Vibrio* spp., *Aeromonas* spp. ve *Listeria* spp.'nin varlığı ve antibiyotik duyarlılıkları

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Öz

Amaç: Bu çalışmada, Türkiye'de tüketilen yüzgeçli balıklarda ve karideslerde *Vibrio* spp., *Aeromonas* spp. ve *Listeria* spp. varlığının ve elde edilen izolatların antibiyotik direnç profilinin disk difüzyon yöntemi kullanılarak belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: Araştırmada, farklı zamanlarda toplanan 300 adet deniz ürünü materyal olarak kullanılmıştır. Klasik kültürel yöntemle izolasyonun ardından, izolatları cins düzeyinde doğrulamak ve patojen türleri tanımlamak için klasik PCR yapılmıştır.

Bulgular: Yüzgeçli balık ve karides örnekleri *Vibrio* spp., *Aeromonas* spp. ve *Listeria* spp. ile sırasıyla %19,4 (33/170), %14,7 (25/170), %4,1 (7/170) and %13,8 (18/130), %13,1 (17/130), %6,2 (8/130) oranında kontamine bulunmuştur. *Vibrio* spp. izolatlarının 29 (%9,7)'u ve 9 (%3,0)'u sırasıyla *V. parahaemolyticus* ve *V. cholerae* olarak tanımlanmıştır. *Aeromonas* spp. izolatlarının 30 (%10)'u *A. hydrophila* olarak tespit edilmiştir. Örneklerin hiçbirinde *L. monocytogenes* ve *V. vulnificus* saptanmamıştır. *V. parahaemolyticus, V. cholerae*, *A. hydrophila* ve *Listeria* spp. izolatlarının antibiyotik direnç profili sırası ile streptomisin (%71,4), teikoplanin (%71,4), ampisilin (%87,5), teikoplanin (%75); streptomisin (%80), ampisilin (%75), sefiksim (%75), penisilin (%75), sulfametoksazol/trimetoprim (%100), tetrasiklin (%75); eritromisin (%93,8), vankomisin (%81,2), amoksasilin/klavulanik asit (%78,6), ampisilin (%85,7), sefalotin (%92,9), penisilin G (%92,9); ve sefalotin (%37,5-%42,9), eritromisin (%37,5-%42,9), penisilin G (%37,5-%42,9), tetrasiklin (%37,5-%42,9) olarak tespit edilmiştir.

Öneri: Sonuç olarak, Türkiye'de tüketilen balık ve karideslerin patojen *Vibrio* ve Aeromonas türleri ile kontamine olabilecekleri ve antibiyotik dirençli izolatların halk sağlığı açısından risk oluşturabileceği düşünülmektedir.

Anahtar kelimeler: *Aeromonas* spp., antibiyotik direnç, *Listeria* spp., deniz ürünleri, *Vibrio* spp.

Abstract

Aim: In this study, it was aimed to determine the presence of *Vibrio* spp., *Aeromonas* spp., and *Listeria* spp. in finned fish and shrimps consumed in Turkey and the antibiotic resistance profile of the isolates using disc diffusion method.

Materials and Methods: In the research, 300 seafoods obtained at different times were used as material. Following isolation by classical cultural method, classical PCR was performed to confirm the isolates at genus level and to identify at species level for pathogenic species.

Results: Finfish and shrimp samples were contaminated with *Vibrio* spp., *Aeromonas* spp. and *Listeria* spp. with the rate of 19.4% (33/170), 14.7% (25/170), 4.1% (7/170) and 13.8% (18/130), 13.1% (17/130), 6.2% (8/130), respectively. Twenty-nine (9.7%) and 9 (3.0%) of the *Vibrio* spp. isolates were identified as *V. parahaemolyticus* and *V. cholerae*, respectively. Thirty (10%) of the *Aeromonas* spp. isolates were detected as *A. hydrophila*. *L. monocytogenes* and *V. vulnificus* was not detected in any of samples. Antibiotic resistance profile of the *V. parahaemolyticus, V. cholerae*, *A. hydrophila* and *Listeria* spp. isolates was streptomycin (71.4%), teicoplanin (71.4%), ampicillin (87.5%), teicoplanin (75%); streptomycin (80%), ampicillin (75%), cefixime (75%), penicillin (75%), sulfamethoxazole/trimethoprim (100%), tetracycline (75%); erythromycin (93.8%), vancomycin (81.2%), amoxacillin/clavulanic acid (78.6%), ampicillin (85.7%), cephalothin (92.9%), penicillin G (92.9%); and cephalothin (37.5%-42.9%), erythromycin (37.5%-42.9%), penicillin G (37.5%-42.9%), tetracycline (37.5%-42.9%), respectively.

Conclusion: As a result, it is thought that fish and shrimp consumed in Turkey may be contaminated with pathogenic *Vibrio* and *Aeromonas* species, and antibiotic-resistant isolates may pose a risk to public health.

Keywords: *Aeromonas* spp., antibiotic resistance, *Listeria* spp., seafoods, *Vibrio* spp.

Introduction

There are many factors related to the seafood is to be exposed the bacterial contamination such as the microbiological condition of the sea, water temperature, salt ratio, time and distance between catch and retail and postharvest handling conditions. Seafood-borne infections and intoxications can occur in humans through consumption of seafood contaminated with pathogenic agents or toxins (Dutta et al 2016).

Vibrio species, especially those classified as high risk (*V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*) according to a risk characterization of Food and Agriculture Organization (FAO) are identified as foodborne bacterial agents that threaten public health worldwide and are often isolated from seafood. Diseases caused by pathogenic *Vibrio* species are described as gastroenteritis, soft tissue infections and systemic infections as a result of bacteremia (Baker-Austin et al 2018, Deng et al 2020).

Aeromonas spp. is one of the enteric pathogens commonly found in less developed countries and mostly classified as an emerging pathogen, contaminating food via the sources such as infected water, feces of animals or porter people (Hoel et al 2019, Zhu et al 2020). Although it can cause localized and systemic disease, including soft tissue and wound infections, tonsillitis, pneumonia, bacteraemia or septicemia the most typical symptoms are observed in the gastrointestinal tract. Gastroenteritis may include dysentery-like symptoms including bloody/mucus diarrhea and abdominal cramps. The disease spectrum of infectious diarrhoea is mainly associated with the level of immune system of the individuals. Although *A. hydrophila* can be recovered from a wide variety of foods, the major source of contamination is seafood such as fish, shrimp, oysters, crabs and scallops, and red meat and poultry meat as well (Stratev and Odeyemi 2016).

Listeria spp. is frequently isolated from fresh drinking water and marine waters in coastal areas. However, the ubiquitous nature of the microorganism increases the possibility of contamination of foods. The main cause of seafood-borne listeriosis is *L. monocytogenes* (Baker-Austin et al 2018). Despite the incidence of microorganism is relatively low, high mortality rates are considered as noteworthy. There are several clinical signs may occur following consumption of contaminated foods, ranging the mild gastroenteritis to systemic infections with high mortality especially in immunocompromised individuals (Yamaki and Yamazaki 2018).

Contamination of the aquatic environment with antibiotic resistant seafood-borne pathogens is a public health concern worldwide. The sublethal use of antimicrobial agents in farm animals and aquaculture for growth promotion, feed efficiency, as well as therapeutic use lead to the contamination of the environment and carriage of these bacteria via food or water sources. There are several studies (Jamali et al 2015, Kumar et al 2017, Osman et al 2020) that have reported the isolation of antibiotic resistant and/or multidrug resistant seafood-borne pathogens such as *Salmonella* spp, *A. hydrophila*, *E. coli*, *Vibrio* spp., *Listeria* spp., and *Klebsiella* spp. from fresh, frozen and ready to eat seafood.

In this study, it was aimed to determine the incidence of *Vibrio* spp., *Aeromonas* spp. and *Listeria* spp. in retail seafoods and the antibiotic resistance profile of the isolates obtained against selected antibiotics.

Material and Methods

Sample collection

Between January 2017 and April 2018, 8 independent visits were made bimonthly, to fish markets and national chain supermarkets. A total of 300 seafoods representing the Mediterranean, Black and Aegean sea were collected from supermarkets and fish markets of Konya/Turkey. Of which 170 [*Mullus barbatus* (n=20), *Atherina boyeri* (n=10), *Engraulis encrasicolus* (n=15), *Trachurus trachurus* (n=40), *Dicentrarchus labrax* (n=20), *Merlangius euxmus* (n=10), *Lithognathus mormyrus* (n=10), *Sardina pilchardus* (n=35), *Scomber scombrus* (n=5), and *Mugil cephalus* (n=5)] were 10 different finfish species and 130 [*Parapenaeus longirostris* (n=70), *Penaeus semisulcatus* (n=30), *Melicertus kerathurus* (n=15) and *Squilla mantis* (n=15)] were 4 different shrimp species. The samples were collected randomly and placed individually into the sterile stomacher bags (VWR, 432-3123) then were brought to the laboratory within 2 hours in the boxes containing ice cubes.

Isolation and identification of Vibrio spp., Aeromonas spp. and Listeria spp.

The skin and gill of all the finfish were aseptically removed by using sterile scalpel and rinsed with running potable water to remove the adhering internal organ particles and shrimps were dissected according to a method described previously (Andrews and Hammack 2001) for microbiological analyses. *Vibrio* spp. isolation was carried out according to the horizontal method for the detection of potentially enteropathogenic *Vibrio* spp. published by International Organization for Standardization (ISO/TS 21872-2 2007) with slight modification. In brief, 25 g of sample was weighed in a sterile stomacher bag as described above (VWR, 432-3123) and 225 ml of Alkaline Saline Peptone Water (ASPW, Liofilchem, 610377) was added and homogenized in a stomacher (Interscience France). Following pre-incubation at 41.5°C for 6 h, 1 ml of medium was transferred into the tubes containing 9 ml of fresh ASPW tubes and incubated

at 41.5˚C for 18 h. After incubation, one loopful from the upper level of the enrichment tube was streaked on to the Thiosulfate Citrate Bile Sucrose agar (TCBS, Merck 1.10263) and incubated at 37˚C for 18–24 h. Presumptive yellow and green colonies grown on TCBS Agar were transferred to Nutrient Agar (NA, Merck, 1.05450) supplemented with 3% NaCl and incubated at 37˚C for 24 h for further identification. For this purpose, catalase, oxidase, Gram staining, mobility, growth test on NA supplemented with 6% NaCl were performed. The selected colonies were then suspended in 200 µl of Tris-EDTA (TE) buffer for DNA isolation. The suspension was placed on the heating block at 95˚C for 10 min. and transferred into an icebox for 3 min. Following the tubes were centrifugated at 10000 g for 10 min, the supernatant was transferred into nuclease free tubes and stored at -20˚C for further PCR applications.

The isolation of *Aeromonas* species was performed with the methods described by Popoff (1984) and Palumbo et al (1992) with slight modification. For this purpose, 10 g of the finfish or shrimp samples were weighed and suspended in 90 ml Alkaline Peptone Water (Liofilchem 610098). Following the pre-enrichment at 30˚C for 24 ± 2 hours, a loopful of the pre-enriched suspension was streaked on to *Aeromonas* Agar (LabM, LAB167) and incubated at 30˚C for 24 ± 2 hours. Gram-staining, oxidase, catalase, motility, growth in NB supplemented with 6% NaCl and DNase test were performed with the typical light green colonies. DNA isolation from the colonies was performed as described in *Vibrio* spp.

The isolation and identification of *L. monocytogenes* was performed using the method recommended by ISO 11290-1 (2017) with slight modification. Briefly, 25 g of each samples the samples were incubated for 24-48 hours at 30˚C in Half Fraser Broth (Oxoid, M1053, UK) supplemented with Half Fraser Supplement (Oxoid, SR0166, UK). Then, 10 ml of the suspension was transferred to Fraser Broth (Oxoid, CM0895, UK) supplemented with Fraser Broth Supplement (Oxoid, SR0156, UK) and selective enrichment was performed at 37˚C for 24-48 hours. A loopful of the selective-enriched broth was streaked on to Oxford Listeria Selective Agar (Merck 1.07004) supplemented with Oxford Listeria Selective Supplement (Merck 1.07006). After incubation at 37˚C for 48 h, suspected grayish or black colonies 1-2 mm in diameter, with a black halo were picked and subcultured on to Tyriptic Soy Agar (TSA, Merck 1.05458) supplemented with Yeast Extract (YE, Merck 103753). Biochemical identification was carried out using API *Listeria* kit (Biomeriux, 10300). DNA extraction from the suspected colonies was performed using a commercial DNA extraction kit (Qiagen Blood and Tissue Extraction Kit, Cat No./ID: 69506, USA).

Primers

The primer pairs used to confirm *Vibrio* spp., *Aeromonas* spp. and *Listeria* spp. and to determine the pathogenic strains are shown in Table 1. PCR reaction mix and thermal cycler conditions were followed according to the indicated references.

Reference strains

V. parahaemolyticus ATCC 17802, *V. cholerae* ATCC 14035, *V. vulnificus* ATCC 29307, *A. hydrophila* ATCC 7966, *L.*

Table 1. The primers list

	Primer pairs	Product length	Reference
Vibrio spp.	F: (5'- AGCCAAACNAAAGAYAARYT-3') R:(5'-CGYARYTTRTCYGGRTTRTRYTC-3')	493 bp	(Teh et al 2010)
V. cholerae	F:(5'-CAAGCTCCGCATGTCCAGAAGC-3') R:(5'- GGGGCGTGACGCGAATGATT-3')	154 bp	(Kim et al 2015)
V. parahaemolyticus	F:(5'-TTGGATTCCACGCGTTAT-3') R:(5'- CGTTCAATGCACTGCTCA -3')	183 bp	(Chen and Ge 2010)
V. vulnificus	F:(5'-TGGTTCGGTTAACGGCTG-3') R:(5'-GCCATCAACATAGCGGCTAA-3')	208 bp	(Ren et al 2009)
Aeromonas spp. (16srRNA)	F: (5'-CTACTTTTGCCGGCGAGCGG-3') R: (5'-TGATTCCCGAAGGCACTCCC-3')	953 bp	(Lee et al 2002)
A. hydrophila (*gyrB*)	F: (5'-AGTCTGCCGCCAGTGGC-3') R: (5'- CRCCCATCGCCTGTTCG-3')	144 bp	(Persson et al 2015)
Listeria spp. (*iap*)	F:(5'-ATGTCATGGAATAA-3') R:(5'-GCTTTTCCAAGGTGTTTTT-3')	400-600 bp	(Cocolin et al 2002)
L. monocytogenes (*hlyA*)	F:(5'-CATTAGTGGAAAGATGGAATG-3') R: (5'-GTATCCTCCAGAGTGATCGA-3')	234 bp	(Furrer et al 1991)

monocytogenes ATCC 13932 were used for positive control of PCR amplifications and Escherichia coli ATCC 25922 was used for antibiotic susceptibility testing.

Antibiotic susceptibility testing

The disk diffusion method was used to determine antibiotic resistance profiles of the isolates. Briefly, the reference strain (Escherichia coli ATCC 25922) and the isolates were cultured in Mueller Hinton Broth (Oxoid, CM0405). The optical density was adjusted to 0.5 McFarland with Mc Farland Optic Densitometer (DEN-1B McFarland Densitometer). The broth culture was streaked on to the Mueller Hinton Agar (Oxoid, CM0337) using sterile cotton swabs. The antimicrobial susceptibility test discs were placed onto the surface of the plates (120 mm) with a sterile forceps. The tested antibiotics were as follows; amikacin (Oxoid-CT 0107B-AK 30 µg), amoxicillin / clavulanic acid (Oxoid-CT 0223B-AMC 10 µg), ampicillin (Oxoid-CT 0003B-AMP 10 µg), cefixime (Oxoid-CT 0653B-CFM 5 µg), cephalothin (Oxoid-CT 0010B-KF 30 µg), cefazolin (Oxoid-CT 0011B-KZ 30 µg), chloramphenicol (Oxoid-CT 0013B-C 30 µg), ciprofloxacin (Oxoid-CT 0425B-CIP 5 µg), clindamycin (Oxoid-CT 0064B-DA 2 µg), erythromycin (Oxoid-CT 0020B-E 15 µg), gentamicin (Oxoid-CT 0024B-CN 10 µg), kanamycin (Oxoid-CT 0026B-K 30 µg), nalidixic acid (Oxoid-CT 0031B-NA 30 µg), oxacillin (Oxoid-CT 0159B-OX 1 µg), penicillin G (Oxoid-CT 0043B-P 10 IU), streptomycin (Oxoid-CT 0047B-S 10 µg), sulfamethoxazole / trimethoprim (Oxoid-CT 0052B-SXT 25 µg), teicoplanin (Oxoid-CT 0647B-TEC 30 µg), tetracycline (Oxoid-CT 0054B-TE 30 µg) and vancomycin (Oxoid-CT 0058B-VA 30 µg). Incubation was carried out at 37˚C for 18-24 h. Inhibition zones were measured and the isolates were classified as susceptible, resistant and intermediate according to the Clinical and Laboratory Standards Institute (CLSI 2016).

Results

The distribution of isolates in sample groups is shown in Table 2. The overall incidence of *Vibrio* spp., *Aeromonas* spp. and *Listeria* spp. in seafoods was 17%, 14% and 5%, respectively. At the rate of 38.2% (65/170) of finfish and 33.1% (43/130) of shrimps were contaminated with at least one of these genera.

The highest contamination rate was in *Vibrio* spp. *Vibrio* species were higher in finfish samples (19.4%) than the shrimp samples (13.8%). In the same way, *Aeromonas* spp. was higher in finfish samples (14.7%) than the shrimp samples (13.1%). However, *Listeria* spp. contamination rate in finfish and shrimp samples was 4.1% and 6.2%, respectively.

According to PCR analysis, 29 (56.9%) and 9 (17.6%) of the 51 *Vibrio* spp. isolates were determined as *V. parahaemolyticus* and *V. cholerae*, respectively (Fig. 1). *V. vulnificus* was not detected in any of the samples. 30 (71.4%) of the *Aeromonas*

Table 2. Distribution of isolates tested in sample groups

Sample speciES	Number of Isolates (%)				
	N	VP	VC	AH	LSPP
Finfish	170	21; (12.35%)	5; (2.94%)	16; (9.41%)	7; (4.11%)
Mullus barbatus	20	ND	ND	1; (5%)	ND
Atherina boyeri	10	ND	ND	1; (10%)	ND
Engraulis encrasicolus	15	4; (26.66%)	ND	2; (13.33%)	2; (13.33%)
Trachurus trachurus	40	6; (15%)	1; (2.5%)	4; (10%)	ND
Dicentrarchus labrax	20	2; (10%)	1; (5%)	3; (15%)	2; (10%)
Merlangius euxmus	10	1; (10%)	ND	1; (10%)	ND
Lithognathus mormyrus	10	1; (10%)	ND	ND	ND
Sardina pilchardus	35	4; (11.42%)	2; (5.71%)	4; (11.42%)	2; (5.71%)
Scomber scombrus	5	1; (20%)	1; (20%)	ND	ND
Mugil cephalus	5	2; (40%)	ND	ND	1; (20%)
Shrimps	130	8; (6.15%)	4; (3.07%)	14; (10.76%)	8; (6.15%)
Parapenaeus longirostris	70	4; (5.71%)	2; (2.85%)	7; (10%)	3; (4.28%)
Penaeus semisulcatus	30	1; (3.33%)	1; (3.33%)	3; (10%)	3; (10%)
Melicertus kerathurus	15	ND	ND	2; (13.33%)	1; (6.66%)
Squilla mantis	15	3; (20%)	1; (6.66%)	2; (13.33%)	1; (6.66%)
Total	300	29; (9.66%)	9; (3%)	30; (10%)	15; (5%)

*VP: *V. parahaemolyticus*, VC: *V. cholerae*, AH: *A. hydrophila*, LSPP: *Listeria* spp, ND: Not Detected.

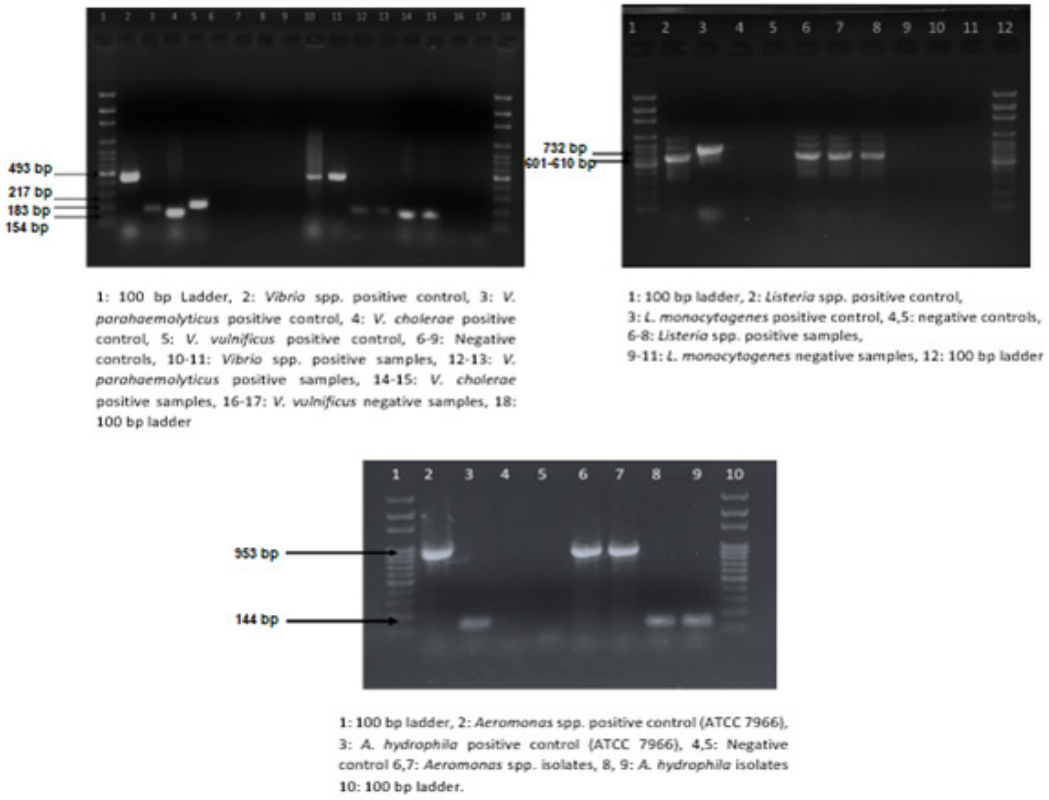

Figure 1. PCR images of isolates

spp. isolates (n = 42) were positive for *A. hydrophila* (Fig. 1). *L. monocytogenes* was not detected in any of the isolates identified as *Listeria* spp. using hlyA gene based PCR (Fig. 1). *V. parahaemolyticus*, *V. cholerae* and *A. hydrophila* contamination rates in finfish and shrimp samples were 12.4%, 2.9% and 9.4%; 6.2%, 3.1% and 10.8%, respectively. The resistance profiles of the isolates from finfish and shrimp sample groups to the tested antibiotics are shown in Table 3. *V. parahaemolyticus* isolates (n=29) showed a high rate of resistance to ampicillin (87.5%) and teicoplanin (75%); oxacillin (66.7%), streptomycin (71.4%) and teicoplanin (71.4%) isolated from shrimp and finfish samples, respectively. More than 70% of the isolates in shrimp and finfish samples were resistant to teicoplanin while they were sensitive to amoxicillin / clavulanic acid and chloramphenicol. *V. cholerae* (n = 9) isolates were resistant to sulfamethoxazole / trimethoprim (100%), ampicillin (75%), cefixime (75%), penicillin G (75%) and tetracycline (75%); erythromycin (60%), oxacillin (60%) and streptomycin (80%) in shrimp and fish samples, respectively.

A. hydrophila (n = 16) isolated from finfish displayed a high percentage of resistance against erythromycin (93.8%), vancomycin (81.3%) but they were suscebtiple to amikacin (100%), sulfamethoxazole / trimethoprim (100%), cefixime (87.5%) and kanamycin (87.5%). Isolates from shrimp showed 92.9% resistance to cephalothin, oxacillin and penicillin G, however, they were found susceptible to chloramphenicol (92.9%), kanamycin (92.9%), ciprofloxacin (85.7%) and gentamicin (85.7%).

The resistance profile of *Listeria* spp. isoletes from finfish and shrimp samples were almost in the same line except for the higher clindamycin resistance in shrimp isolates and ranged between 37.5% to 42.9% against cephalothin, erythromycin, penicillin G, and tetracycline. The clindamycin resistance in shrimp samples (37.5%) were higher than the finfish isolates (28.6%).

Discussion

Vibrio spp. has been reported in seafood worldwide with lower or higher results than our study (17%) in several comparable studies (Messelhäusser et al 2010, Khamesipour et al 2014, Scarano et al 2014, Avşar et al 2016, Azwai et al 2016, Vu et al 2018). In Bavaria-Germany (Messelhäusser et al 2010) and in different locations of Italy (Scarano et al 2014) displayed a lower contamination level with 1.6% and 9.6% in seafish samples, respectively. The relatively higher

Table 3. Antibiotic resistance profiles of isolates

Antibiotic	*V. parahaemolyticus* (n=29)							*V. cholera* (n=9)						*A. hydrophila* (n=30)						*Listeria spp.* (n=15)					
	Fish (n=21)			Shrimp (n=8)				Fish (n=5)		Shrimp (n=4)			Fish (n=16)			Shrimp (n=14)			Fish (n=7)			Shrimp (n=8)		
	R *	I*	S*	R	I	S	R %	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
	%	%	%	%	%	%		%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
AK*	38.09	23.80	38.09	0	12.50	87.50	20.00	20.00	60.00	0	25.00	75.00	0	0	100.00	14.28	7.14	78.57	14.28	28.57	57.14	0	25.00	75.00
AMC*	9.52	0	90.41	0	0	100.00	20.00	0	80.00	25.00	0	75.00	31.25	25.00	43.75	78.57	7.14	14.28	0	14.28	85.71	0	12.50	87.50
AMP*	47.61	9.52	42.85	87.50	0	12.50	20.00	0	80.00	75.00	0	25.00	37.50	6.25	56.25	85.71	0	14.28	14.28	0	85.71	12.50	12.50	75.00
CFM*	9.52	9.52	80.95	0	0	100.00	20.00	0	80.00	75.00	0	25.00	12.50	0	87.50	35.71	0	64.28	0	28.57	71.42	12.50	12.50	75.00
KF*	57.14	14.28	28.57	25.00	0	75.00	20.00	40.00	40.00	0	25.00	75.00	68.75	0	31.25	92.85	0	7.14	42.85	0	57.14	37.50	0	62.50
KZ*	42.85	4.76	52.38	12.50	0	87.50	20.00	0	80.00	25.00	25.00	50.00	56.25	0	43.75	28.57	0	71.42	14.28	14.28	71.42	0	12.50	87.50
C*	0	9.52	90.41	0	0	100.00	20.00	0	80.00	25.00	0	75.00	6.25	12.50	81.25	7.14	0	92.85	0	14.28	85.71	12.50	12.50	75.00
CIP*	9.52	9.52	80.95	12.50	0	87.50	20.00	0	80.00	0	25.00	75.00	6.25	0	93.75	7.14	7.14	85.71	0	28.57	71.42	12.50	25.00	62.50
DA*	19.04	19.04	61.90	0	0	100.00	40.00	20.00	40.00	50.00	0	50.00	25.00	12.50	62.50	57.14	7.14	35.71	28.57	0	71.42	37.50	0	62.50
E*	38.09	0	61.90	12.50	0	87.50	60.00	0	40.00	25.00	0	75.00	93.75	0	6.25	78.57	0	21.42	42.85	0	57.14	37.50	0	62.50
CN*	33.33	4.76	61.90	12.50	12.50	75.00	40.00	0	60.00	25.00	0	75.00	50.00	0	50.00	7.14	7.14	85.71	0	0	100.00	0	0	100.00
K*	4.76	14.78	80.95	0	0	100.00	20.00	40	40.00	0	25.00	75.00	12.50	0	87.50	7.14	0	92.85	0	0	100.00	0	12.50	87.50
NA*	19.04	0	80.95	25.00	0	75.00	20.00	0	80.00	0	0	100.00	18.75	0	81.25	42.85	0	57.14	28.57	14.28	57.14	12.50	25.00	62.50
OX*	66.66	0	33.33	37.50	0	62.50	60.00	0	40.00	25.00	0	75.00	68.75	0	31.25	92.85	0	7.14	14.28	0	85.71	12.50	0	87.50
P*	47.61	0	52.38	50	0	50.00	20.00	0	80.00	75.00	0	25.00	68.75	0	31.25	92.85	0	7.14	42.85	0	57.14	37.50	0	62.50
S*	71.42	0	28.57	62.50	0	37.50	80.00	0	20.00	50.00	0	50.00	12.50	37.50	50.00	57.14	0	42.85	28.57	0	71.42	12.50	0	87.50
SXT*	19.04	0	80.95	25.00	0	75.00	40.00	0	60.00	100.00	0	0	0	0	100.00	7.14	14.28	78.57	14.28	0	85.71	0	12.50	87.50
TEC*	71.42	0	28.57	75.00	0	25.00	40.00	0	60.00	25.00	0	75.00	68.75	0	31.25	42.85	0	57.14	14.28	0	85.71	12.50	0	87.50
TE*	42.85	19.04	38.09	62.50	12.50	25.00	0	60.00	40.00	75.00	0	25.00	25.00	25.00	50.00	64.28	7.14	28.57	42.85	28.57	28.57	37.50	25.00	37.50
VA*	42.85	0	57.14	62.50	0	37.50	40.00	0	60.00	25.00	0	75.00	81.25	0	18.75	64.28	0	35.71	14.28	0	85.71	0	0	100.00

* R: Resistance, I: Intermediate, S: Sensitive, AK: Amikacin, AMC: Amoxicillin / Clavulanic Acid, AMP: Ampicillin, CFM: Cefixime, KF: Cephalothin, KZ: Cefazolin, C: Chloramphenicol, CIP: Ciprofloxacin, DA: Clindamycin, E: Erythromycin, CN: Gentamicin, K: Kanamycin, NA: Nalidixic Acid, OX: Oxacillin, P: Penicillin G, S: Streptomycin, SXT: Sulfamethoxazole / Trimethoprim, TEC: Teicoplanin, TE: Tetracycline, VA: Vancomycine

rates; (19.4%) in shrimp samples in Iran (Khamesipour et al 2014), anchovy and garfish samples (27.3%) in Turkey (Avşar et al 2016) and a markedly higher rate (40%) in finfish samples in Libya were reported (Azwai et al 2016).

Although the presence of *V. parahaemolyticus* (9.6%) (Table 2) was lower compared to the studies of Malcolm et al (2015) (98.7%) and Xie et al (2017) (38%), there was also a study with relatively similar results in finfish (13.8%) and in prawn (15%) (Kshirsagar et al 2013). The detection rate of *V. cholerae* was lower than those of studies of Jones et al (2013) with 19% in finfish samples and Rosec et al (2012) with 10% in shrimp samples in France. Contrary to our findings, it was stated by Irkin et al (2007) in sardine and shrimp samples and Rosec et al (2012) in finfish samples were not contaminated with *V. cholerae*. Considering these results, the re-emergence of *Vibrio* species and especially *V. parahaemolyticus* in regions such as Chile (Gonzalez-Escalona et al 2005) and Alaska (McLaughlin et al 2005), where these agents are extremely rare, may indicate a similar risk may also arise for Turkey. Besides the above-mentioned factors for seafood contamination, the weak competitive interactions of *Vibrio* species in a microbiome may result in a decrease in the *Vibrio* population during the supply chain through the point of sale. Furthermore, different psychrotrophic bacterial species, such as *Pseudomonas* spp. and *Shewanella* spp. may become dominant, especially under the cold storage. In addition, *Vibrio* species, may lose their culturability and enter the viable but not culturable (VBNC) state under the extreme environmental conditions such as cold or frozen storage and competition-related nutrient restrictions (Malcolm et al 2015, Telli and Doğruer 2019).

Aeromonas spp. and *A. hydrophila* contamination rates (Table 2) in finfish (14.7%, 9.4%) and shrimp (13.1%, 10.8%) samples were lower than earlier studies (Khamesipour et al 2014, Lijon et al 2015, Thenmozhi et al 2015), however, our results were relatively higher than other authors (Bulduklu and Özer 2007, Doğruer and Koç 2017). Khamesipour et al (2014), Lijon et al (2015) and Thenmozhi et al (2015) detected *A. hydrophila* in 5 (13.9%) of 36 shrimp, 13 (17.3%) of 25 freshwater shrimp and 8 of 50 carp samples, respectively. A similar study in Turkey conducted by Doğruer and Koç (2017) showed the presence of *A. hydrophila* in 6 (12%) of 50 squid samples and 11 (11%) of 100 shrimp samples. In contrast, Bulduklu and Özer (2007) reported that none of the 120 rainbow trout samples were contaminated with *Aeromonas* species in Turkey. It is thought that the differences between the findings of the studies may be caused by factors such as season, sampling techniques, detection methods, different hygiene and sanitation practices, sample supply times and storage conditions.

In our study, the contamination rates of *Listeria* spp. in finfish and shrimp samples was 4.1% and 6.2%, respectively (Table 2). *L. monocytogenes* was not detected in any of the isolates. A similar study by Abdollahzadeh et al (2016) in Iran, 9 of 63 (14.3%) fresh fish samples were contaminated with *Listeria* spp. and 5 (7.9%) of the isolates were identified as *L. monocytogenes*. The researchers also isolated *Listeria* spp. in 1 (1.7%) of 59 shrimp samples whereas *L. monocytogenes* was not identified in any of the isolates. In a similar study, *L. monocytogenes* was not detected in 85 samples of fish and shrimp by Jalali and Abedi (2008). Another study in Poland, Wieczorek and Osek (2017) detected a markedly higher rate of *L. monocytogenes* in 57 (18.9%) of 301 fresh and smoked fish samples. There are potential sources of *L. monocytogenes* contamination on seafood including water and ice, soiled surfaces, or human and avian sources. Hence, *L. monocytogenes* may commonly found in coastal or fresh waters, seafood captured in these waters may possibly be contaminated by the microorganism.

The highest antibiotic resistance of *V. parahaemolyticus* (n = 29) isolates was against; cephalothin, oxacillin, streptomycin, teicoplanin in fish samples; ampicillin, streptomycin, teicoplanin in shrimp samples. More than 70% of the isolates in finfish and shrimp samples were resistant to teicoplanin and sensitive to amoxicillin / clavulanic acid and chloramphenicol. A relatively similar resistance and sensitivity pattern was reported by Letchumanan et al (2015) in Malaysia and Sudha et al (2012) in India. In *V. parahaemolyticus* isolates, the most predominant antibiotic resistance profiles were declared by Elmahdi et al (2016) as ampicillin, penicillin and tetracycline regardless of the countries. In this context, our study was also in accordance with the mentioned report. Overall sensitivity of the *V. parahaemolyticus* isolates regardless of sample type, was in accordance with the Centre for Disease Control and Prevention (CDC) recommended antibiotic classes for treatment of *Vibrio* spp. infections including fluoroquinolones, cephalosporins, aminoglycosides, and folate pathway inhibitors.

V. cholerae (n = 9) isolates were resistant to sulfamethoxazole / trimethoprim (100%), ampicillin (75%), cefixime (75%), penicillin G (75%) and tetracycline (75%); erythromycin (60%), oxacillin (60%) and streptomycin (80%) in shrimp and fish samples, respectively. The ampicillin-penicillin resistance and tetracycline-ampicillin-trimethoprim / sulfamethoxazole resistance pattern in shrimp samples were similar to the resistance pattern of Raissy et al (2012) from seafood in Iran and Ahmed et al (2018) from crustaceans in Egypt, respectively. Besides, erythromycin resistance in *V. cholerae* isolates from fish samples were similar with Noorlis et al (2011) in Malaysia. *Vibrio* spp. is previously reported (Stevens et al 2014, Elmahdi et al 2016) as susceptible to a wide range of antibiotics whereas improper and extensive use of antibiotics in human medicine and agriculture, antibiotic resistant microoganisms has emerged and evolved

in many bacteria including *Vibrio* spp.

In our study, *A. hydrophila* isolates displayed a high percentage of resistance against erythromycin, cephalotin, oxacillin, penicillin G, ampicillin, vancomycin and amoxicillin / clavulanic acid. In the literature review, there is a similar study (Vivekanandhan et al 2002) that the resistance rate to erythromycin and oxacillin (Methicillin) in fish and prawn samples in India. The high amoxicillin / clavulanic acid resistance in shrimp samples (78.6%) was similar to a recent study by Ahmed et al (2018) in Egypt (80%). High percentage of antimicrobial sensitivity to chloramphenicol, ciprofloxacin, kanamycin was in concurrence with the reports of Kaskhedikar and Chhabra (2010).

Listeria spp. (n = 15) isolates had a lower rate of resistance against the antimicrobial agents tested. Among these, the resistance rate of cephalotin, erythromycin, penicillin G, tetracycline and clindamycin had a rate between 37.5% - 42.8%. Referring to the existing scientific literature there are a few studies on antibiotic resistance of *Listeria* spp. isolated from seafood. Penicillin resistance in *Listeria* spp. isolates (Table 3) were also reported by Rodas-Suarez et al (2006) in Mexico and Fallah et al (2013) in Iran. A relatively similar antibiotic resistance pattern was observed in a more recent study by Jamali et al (2015) reported high levels of resistance to tetracycline (23.3%), penicillin (16.5%), and cephalothin (16.5%) in *Listeria* spp. isolates from 862 raw fish and environmental samples of fish markets and open-air fish outlets in Iran.

Conclusion

The presence of antibiotic-resistant microorganisms in foods and their transfer to humans and the environment is one of the most important concerns worldwide. To conclude, finfish and shrimp tested in this study contained pathogenic *Vibrio* and *Aeromonas* species that are resistant to many antibiotics may pose risk for public health and they could serve as a vehicle for the transfer of these microorganisms to consumers. Hence, continuous monitoring should be implemented and required control programs for these bacteria in seafood to ensure hygienic fish handling and marketing facilities.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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An ethical statement was received from the author that the data, information and documents presented in this article were obtained within the framework of academic and ethical rules and that all information, documents, evaluations and results were presented in accordance with scientific ethics rules.

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