

Pathogenic bacteria for human and fish isolated from fish farm in Kastamonu, Turkey

Abstract

The danger of zoonotic diseases is increasing day by day for human. A lot of bacteria lives high concentrate in the water environment. Also the bacterial flora of the aquatic organisms is determined by the environment in which it lives. According to, this the objective of the study was to evaluate, examine, research and monitoring the condition of the pathogenic bacteria for human and fish in the fish farms of Kastamonu, Turkey. *Aeromonashydrophila*, *Lactococcusgarviae*, *Vibrio anguillarum* and were isolated from fish samples, also *Burkholderiacepacia*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were isolated from water samples. *Aeromonashydrophila*, *Burkholderiacepacia*, *Pseudomonas aeruginosa* and *Vibrio fluvialis* are zoonotic bacteria, and *Lactococcusgarviae* is a circuitously dangerous for human health. According to this study results, fish producers must pay attention to hygiene rules and monitoring the aquaculture environment.

Keywords: Zoonotic bacteria, Fish pathogens, Water microbiology

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Introduction

Aquaculture production has showed a rapid growth in the last years. To provide increasing amounts of aquaculture for the world's growing population, many major fish farms are already at maximum capacity; fish are often cultured in limited spaces such as ponds and net cages under high densities, under parameters that depend on the species stocked and prevailing environmental conditions. However, these overcrowding trends adversely affect the health of cultured fish by making them susceptible to infectious diseases.¹

As in every living organism, human beings are producing a number of waste products while continuing their life, and diseases such as fish and aquatic products are emerging due to the pollution caused by these products, and some of these diseases may show zoonotic characteristics.² Zoonotic diseases can be transmitted to humans through drinking water and, consumption of raw and undercooked fish or similar aquatic products. For these reasons zoonotic organisms are an important for human health.

Enterobacteriaceae spp. can be transmitted to human by consumption of the aquatic organisms and contaminated water. Also *Bacillaceae* spp. are widely found in fish.^{3,4} *Vibrionaceae* spp. lives extensively in almost every water source, especially in salty and brackish water. Besides these bacteria are frequently isolated from fish, other aquatic products, and shellfish.^{2,5} Many bacteria of *Vibrionaceae*, especially non-epidemic isolates of *Vibrio vulnificus*, *Vibrio parahaemolyticus* and *Vibrio cholera* cause the infections for human due to consumption of raw and/or undercooked aquatic products. In contrast, the epidemic *Vibrio cholerae* isolates are transmitted from environments to water and human.⁶ The most common species of the *Vibrionaceae* is the *Vibrio parahaemolyticus* strains.⁷ *Aeromonadaceae* spp. are more often contaminated to human by water and food.^{8,9} *Aeromonadaceae* spp. were isolated in 11.1% of the samples taken from diarrhea patients in Japan between 1985-1987. It has been found that younger human who eat fish products infected with *Aeromonadaceae* spp. may develop diarrhea and, chronic enterocolitis performs in elderly people, as well as inflammation and vomiting in people contaminating with these

bacteria.^{10,11} *Salmonella* spp. are isolated from fish and other aquatic organisms. Infections of *Salmonella* spp. are mostly transmitted to human by contaminated water and food. It has been reported that 7% of *Salmonella* spp. infections in the United States are related to seafood.^{12,13}

Importance of zoonotic diseases are day by day increasing owing to its risks and effects. For this reason, careful evaluation, examination and monitoring of foodstuffs in terms of human health is important. The microbial flora of processed and fresh aquatic organisms should be carefully examined in the evaluating stage. Also the bacterial flora of the aquatic organisms is determined by the environment in which it lives. According to, this the objective of the study was to evaluate, examine, research and monitoring the condition of the pathogenic bacteria for human and fish in the fish farms of Kastamonu, Turkey.

Materials and methods

There are 11 fish farm in Kastamonu, which are total production capacities 419, 50 t per year, but 5 of them made fish production at all seasons. For this, sample collected from 5 fish farms for the bacteriological analyses at 2015 and 2016. Farm I, II and II based in different streams, Farm IV and V established in a dam lake. Dissolved oxygen, pH, salinity, temperature, conductivity and resistivity are measured in these farm with Hach Lange HQ 40 D multimeter and were given Table 1. Ten fish and water samples were taken in duplicate from each fish farm for the bacteriological investigations. Bacteria were isolated from samples with dilution-plate methods.¹⁴ All of the bacteriological media used in this research was from Merck (Merck, Germany). Individual colonies were then identified.^{15,16} Gram staining, catalase, oxidase and motility were using for presumptive identification of the bacterial strains. Biochemical characteristics of totally 21 bacteria were determined with API 20 E, API 20 STREP and API 50 CH tests (BioMerieux S.S., France) at 25±0.5 °C for 48 h.^{17,18}

Results and discussion

There are no find any parasitic infestation in fish farms. *Aeromonashydrophila*, *Lactococcusgarviae*, *Vibrio*

anguillarum and *Vibrio fluvialis* were isolated from fish samples, also *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were isolated from water samples. Distribution of the bacteria were given Table 2 in the fish farms. API 20 E, API 20 STREP and API 50 CH tests results were given Table 3-5 respectively.

Aeromonas hydrophila can be find in fresh water, aquatic plants, fish and fish eggs, which is a pathogenic bacterium for both human and fish. It causes high mortality and heavy economically losses in fish farms and also gastroenteritis, vomiting, fever, epigastric pain, septicemia, arthritis, meningitis and peritonitis in humans.^{16,19}

Table 1 Water parameters in the fish farms

Parameters	Farm I	Farm II	Farm III	Farm IV	Farm V
Dissolved O ₂ (mg L ⁻¹)	8.60	8.56	8.62	9.58	9.50
pH	8.96	8.78	8.82	8.68	8.65
Temperature (°C)	10.70	10.40	10.50	17.50	17.30
Salinity (mg L ⁻¹)	0.22	0.22	0.22	0.21	0.21
Conductivity (ms cm ⁻¹)	462	465	453	434	430
Resistivity (Ω)	2165	2154	2161	2054	2050

Table 2 Isolated bacteria species in the fish farms

Parameters	Farm I	Farm II	Farm III	Farm IV	Farm V
<i>Aeromonas hydrophila</i>	+		+		
<i>Burkholderia cepacia</i>					+
<i>Lactococcus garvieae</i>				+	+
<i>Pseudomonas aeruginosa</i>	+	+		+	
<i>Pseudomonas fluorescens</i>				+	+
<i>Vibrio anguillarum</i>					
<i>Vibrio fluvialis</i>			+		

+: positive (isolated)

Table 3 Isolated bacteria from fish farms according to API 20 E tests results

Properties	Farm I <i>A.hydrophila</i>	<i>P.aeruginosa</i>	Farm II <i>P.aeruginosa</i>	Farm III <i>A.hydrophila</i>	<i>V.fluvialis</i>	Farm IV <i>P.fluorescens</i>	<i>P.aeruginosa</i>	Farm V <i>V.anguillarum</i>	<i>B.cepacia</i>
Gram stain	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+
Oxidase	+	+	+	+	-	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+
ONPG	+	-	-	+	+	-	-	+	-
ADH	+	+	+	+	+	-	+	+	+
LDC	+	-	-	+	+	-	-	-	+
ODC	-	-	-	-	-	-	-	-	+
CIT	+	+	+	+	+	+	+	+	+
H ₂ S	-	-	-	-	+	-	-	-	-
URE	-	+	+	-	-	-	+	-	-
TDA	-	-	-	-	-	-	-	-	-
IND	+	-	-	+	-	-	-	-	-
VP	+	-	-	+	+	-	-	+	-
GEL	+	+	+	+	+	-	+	+	-
GLU	+	-	-	+	+	-	-	+	-
MAN	+	-	-	+	+	-	-	+	-
INO	-	-	-	-	-	-	-	+	-
SOR	+	-	-	+	+	-	-	+	-
RHA	-	-	-	-	-	-	-	-	-
SAC	+	-	-	+	+	-	-	+	-
MEL	-	-	-	-	-	-	-	-	-
AMY	+	-	-	+	+	-	-	-	-
ARA	+	-	-	+	+	-	-	+	-
OX	+	+	+	+	-	+	+	+	+

ONPG, β-galactosidase; ADH, Arginine DiHydrolase; LDC, Lysine DeCarboxylase; ODC, Ornithine DeCarboxylase; CIT, CITrate utilization; H₂S, H₂S production; URE, UREase; TDA, Tryptophane De Aminase; IND, INDole Production; VP, VogesProskauer; GEL, GELatinase; GLU, GLUcose Fermentation / oxidation; MAN, MANnitro Fermentation / Oxidation; INO, INOsitro Fermentation / Oxidation; SOR, SORbitro Fermentation / Oxidation; RHA, RHAmnose Fermentation / Oxidation; SAC, SACcharose Fermentation / Oxidation; MEL, MELibiose Fermentation / Oxidation, AMY, AMYgdalin Fermentation / Oxidation; ARA, ARABinose Fermentation / Oxidation; OX, cytochrome-Oxidase

Table 4 Isolated bacteria from two farms according to API 20 STREP tests results

Properties	Farm IV <i>Lactococcus garvieae</i>	Farm V <i>Lactococcus garvieae</i>
Gram stain	+	+
Motility	-	-
Oxidase	-	-
Catalase	-	-
VP	+	+
HIP	-	-
ESC	+	+
PYRA	+	+
α -GAL	-	-
β -GUR	-	-
β -GAL	-	-
PAL	-	-
LAP	+	+
ADH	+	+
RIB	+	+
ARA	+	+
MAN	+	+
SOR	-	-
LAC	-	-
TRE	+	+
INU	-	-
RAF	-	-
AMD	-	-
GLYG	-	-
β -Hemolysis	-	-

Abbreviations: VP, Voges Proskauer; HIP, acid HIPpurique; ESC, ESCuline; PYRA, PYRrolidonyl Arylamidase; α -GAL, α -GALactosidase; β -GUR, β -GIUcRonidase; PAL, Phosphatase ALcaline; LAP, Leucine AminoPeptidase; ADH, Arginine DiHydrolase; RIB, RIBose Acidification; ARA, ARAbinose Acidification; MAN, MANnitrol Acidification; SOR, SORbitol Acidification; LAC, LACtose Acidification; TRE, TREhalose Acidification; INU, INUline Acidification; RAF, RAFFinose Acidification; AMD, AMiDon Acidification; GLYG, GLYcoGene Acidification

Table 5 Isolated bacteria from two farms according to API 50 CH tests results

Properties	Farm IV <i>L. garvieae</i>	Farm V <i>L. garvieae</i>	Properties	Farm IV <i>L. garvieae</i>	Farm V <i>L. garvieae</i>
GLY	-	-	SAL	+	+
ERY	-	-	CEL	+	+
DARA	-	-	MAL	+	+
LARA	-	-	LAC	+	+
RIB	+	+	MEL	-	-
DXYL	-	-	SAC	-	-
LXYL	-	-	TRE	+	+
ADO	-	-	INU	-	-
MDX	-	-	MLZ	-	-
GAL	+	+	RAF	-	-
GLU	+	+	AMD	-	-
FRU	+	+	GLYG	-	-
MNE	-	-	XLT	+	+
SBE	-	-	GEN	-	-
RHA	-	-	TUR	-	-
DUL	-	-	LYX	+	+
INO	+	+	TAG	-	-
MAN	-	-	DFUC	-	-
SOR	-	-	LFUC	-	-
MDM	-	-	DARL	-	-
MDG	+	+	LARL	-	-
NAG	+	+	GNT	-	-
AMY	+	+	2KG	-	-
ARB	+	+	4KG	-	-
ESC	-	-			

Abbreviations: GLY, GLYcerol, ERY, ERYthritol, DARA, D-ARAbinose, LARA, L-ARAbinose, RIB, D-RIBose, DXYL, D-XYLose, LXYL, L-XYLose, ADO, D-ADOnitol, MDX, Methyl- β D-Xylopyranoside, GAL, D-GALactose, GLU, D-GLUcose, FRU, D-FRUctose, MNE, D-MaNNosE, SBE, L-SorBosE, RHA, L-RHAMnose, DUL, DULcitol, INO, INOsitol, MAN, D-Mannitol, SOR, D-SORbitol, MDM, Methyl- α D-Mannopyranoside, MDG, Methyl- α D-Glucopyranoside, NAG, N-AcetylGlucosamine, AMY, AMYgdaline, ARB, ARButine, ESC, ESCuline, SAL, SALicine, CEL, D-CELLobiose, MAL, D-MALTose, MEL, D-MELibiose, SAC, D-SACcharose, TRE, D-TREhalose, INU, INUline, MLZ, D-MeLeZitose, RAF, D-RAFFinose, AMD, AMiDon, GLYG, GLYcoGene, XLT, XyLiToL, GEN, GENtiobiose, TUR, D-TURanose, LYX, D-LYXose, TAG, D-TAGatose, DFUC, D-FUCose, LFUC, L-FUCose, DARL, D-ARabitoL, LARL, L-ARabitoL, GNT, potassium GlucoNaTe, 2KG, potassium 2-CetoGluconate, 5KG, potassium 5-CetoGluconate.

Pseudomonas aeruginosa were isolated from Farm I, II and IV. This pathogen causes various lesions and fever in the body for human and fish.^{16,20} *Vibrio fluvialis*, a zoonotic pathogen that can cause zoonotic infections, is a type of bacterium that is found intensely in a normal environment. It may be dangerous for human health with produces toxins. It is known that this bacterium, which causes gastroenteritis, diarrhea, lesions and bacteremia in humans, causes diseases in shellfish and their eaters. There are contamination and disease risks with consumption of raw and/or undercooked aquatic products.^{6,21-23} *V. fluvialis* was isolated from Farm III.

Pseudomonas fluorescens bacteria isolated from the production water in Farm IV is among the bacterium commonly found in the waters. It is known to be pathogenic in many species of fish, especially in carp.^{16,24}

Lactococcus garvieae isolated from Farm IV and V is known to be a species of pathogenic bacterium that causes heavy economically losses in various fish species all over the world.¹⁶ It is also an important species in terms of threatening human health. Bacterium was first time isolated from a bovine mastitis case in England.^{25,26} *Vibrio anguillarum* isolated from fish in Farm V is a pathogenic bacterium causing vibrios infection in many fish species and high rate of losses in aquaculture. This bacterium, which can be found especially in salty and brackish waters, is causing an outbreak infection in fish farms.^{16,18,27}

Burkholderia cepacia was isolated from water in the Farm V. This bacteria, which is pathogenic for human, can be found in the water and sediment, also cause cystic fibrosis in human.²⁸ Diler²⁹ reported that Gram positive coccus, *Coryneform* and Gram negative bacteria were isolated from rainbow trout microflora.

Similarly, *Achromobacter* spp., *Acinetobacter* spp., *Aeromonas* spp., *Bacillus* spp., *Brevundimonas* spp., *Comamonas* spp., *Enterobacter* spp., *Flavobacterium* spp., *Hafnia* spp., *Klebsiella* spp., *Kluyvera* spp., *Micrococcus* spp., *Moraxella* spp., *Oligella* spp., *Pantoea* spp., *Pseudomonas* spp., *Proteus* spp., *Rahnella* spp., *Salmonella* spp., *Shigella* spp., *Sphingomonas* spp., *Staphylococcus* spp., *Stenotrophomonas* spp., *Streptococcus* spp., *Vibrio* spp. and *Yersinia* spp. were isolated from fish farm in Turkey.^{17,27,30-33} The results of these study have a similarity with this research finding.

Conclusion

Unsurprisingly, *Aeromonas hydrophila*, *Lactococcus garvieae*, *Vibrio anguillarum*, *Vibrio fluvialis*, *Burkholderia cepacia*, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were isolated from these farms on account of its natural living environments. It is thought that, this condition is caused by the microbial flora of these water and also this results supported that the production water of the fish is directly related to the bacterial flora of fish. Some of them these bacteria can be cause zoonotic infection for human. For this reason, fish producers must pay attention to hygiene rules and monitoring the aquaculture environment.

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Conflicts of Interest

The authors declare no conflict of interest.

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