Kafkas Univ Vet Fak Derg
19 (2): 271-276, 2013
DOI: 10.9775/kvfd.2012.7659

Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

RESEARCH ARTICLE

The Research of Clonal Relationship Among *Aeromonas* Strains Isolated from Human, Animal and Drinking Water by PFGE

Hanifi KÖRKOCA * Mustafa BERKTAŞ ** Rıza DURMAZ *** Nafia Canan GÜRSOY ****

* Department of Nursing, School of Health Sciences, Muş Alparslan University, TR-49100 Muş - TURKEY
** Department of Medical Microbiology, School of Medicine, Yüzüncü Yıl University, TR-65080 Van - TURKEY
*** Molecular Microbiology Research and Application Laboratory Microbiology Reference Laboratories Department Ministry of Health, Public Health Agency of Turkey, TR-06100 Ankara - TURKEY
**** Department of Medical Microbiology, School of Medicine, İnönü University, TR-44280 Malatya - TURKEY

Makale Kodu (Article Code): KVFD-2012-7659

Summary

Aeromonads infect human through potable water and causes various infections. Their existence in animal are being assessed as potential risk for human health. The aim of this study was to investigate clonal relationship among 52 *Aeromonas* strains isolated from human with diarrhea (14 strains), healthy food workers (2 strains), animals (24 strains) and drinking water (12 strains) by pulsed-field gel electrophoresis (PFGE). Clonal relation was determined between one diarrheic human isolate and one cattle isolate. No clonal relation was determined between drinking water and human isolates. Two fish isolates, *A. caviae* and *A. sobria*, were not distinguished PFGE patterns. Consequently no predominant clone was determined while clonal related strains were determined. Particularly, it is necessary to elicit the epidemiological importance of animals in respect of human *Aeromonas* infections and extensive studies are required for identification of environmental isolates.

Keywords: *Aeromonas spp., Human, Animal, Drinking Water, PFGE*

PFGE ile İnsan, Hayvan ve İçme Sularından İzole Edilen *Aeromonas* Suşları Arasında Klonal İlişkinin Araştırılması

Özet

Aeromonaslar insanlarda çeşitli enfeksiyonlara neden olmakta, içme suları ile insanlara bulaşabilmekte ve hayvanlardaki varlığı, insanlar için potansiyel risk olarak değerlendirilmektedir. Bu çalışmayla pulsed-field gel electrophoresis (PFGE) ile diyareli insanlardan (14 suş), sağlıklı gıda işçilerinden (2 suş), hayvanlardan (24 suş) ve içme sularından (12 suş) izole edilen 52 *Aeromonas* suşu arasındaki klonal ilişkinin araştırılması amaçlandı. Bir diyareli insan izolatı ve bir sığır izolatı arasında klonal ilişki tespit edildi. İnsan ve su izolatları arasında klonal ilişki tespit edilmedi. İki balık izolatı arasında (*A. caviae* ve *A. sobria*) ayırtedilemez PFGE paterni tespit edildi. Sonuç olarak, klonal ilişkili suşlar tespit edilmesine rağmen baskın bir klon tespit edilmedi. Özellikle hayvanların, insan *Aeromonas* infeksiyonları yönünden epidemiyolojik önemini aydınlatmaya, ayrıca çevresel izolatların identifikasyonuna yönelik kapsamlı çalışmalara ihtiyaç olduğu görüldü.

Anahtar sözcükler: *Aeromonas spp., İnsan, Hayvan, İçme Suyu, PFGE*

INTRODUCTION

Motile *Aeromonas* species may cause various infections in human mainly such as gastroenteritis, wound infection and systemic infection [1]. *Aeromonas* species exist in water, soil, food, human and animal feces [2]. It is possible that certain *Aeromonas* clones in the environment may predominate and cause human diseases more frequently than other clones [3].

Aeromonads are being transferred to food and other surfaces through the contaminated hands of food workers during manual processing and preparation [4]. Either the routes of contamination of food by *Aeromonas* or the role of contaminated food in the formation of *Aeromonas* infections has not been fully elicited. While it is being specified that

İletişim (Correspondence)
+90 436 2130013
hkorkoca@hotmail.com

food can be the source in respect of *Aeromonas* gastroenteritis by the determination of DNA hybridization group (HG) 4 and HG8 strains in food [5].

Aeromonads are a fairly heterogenous regarding to their biochemical, structural, and genetic features. The difficulties of correct identification of traditional *Aeromonas* biotypes are causing problem in epidemiological studies, in determination of disease spectrum and in determination of distribution of species in environment [6]. Especially, there are difficulties in phenotypic identification of environmental strains [7]. Pulsed-Field Gel electrophoresis (PFGE) is a very effective method to characterize clonal relationships among *Aeromonas* strains [6,8].

The aim of this study is to research of clonal relation in among *Aeromonas* strains isolated from human with diarrhea, healthy food workers, animals and potable water by PFGE.

MATERIAL and METHODS

Aeromonas Isolates

A total 52 epidemiologically unrelated *Aeromonas* strains that 14 isolates were from previous studies of Berktaş et al.[9] were investigated in this study. These 14 strains were isolated from stools of animals brought to Van municipality slaughterhouse and from stools of diarrheal patients samples collected from the laboratory Research Hospital of Yuzuncu Yil University. Other isolates, 24, were isolated from stools of diarrheic human of the same laboratory, gull strains from stools of gulls which inhabited in coast of Van Lake, fish strains from fish purchased from fisherman hunted in near the coast which inhabited of gulls and finally chicken strain from specimens collected from different hencoops in Van region. Also, drinking water and food worker isolates, 14, were isolated from specimens sent to Van Public Health Laboratory for analysis. Source, name and number of *Aeromonas* strains are given *Table 1*. Stock cultures were maintained frozen at -80°C in peptone water (1% peptone, 0.5% NaCl, [pH 7]) with 30% (vol/vol) glycerol.

Isolation and Identification of Motile Aeromonas spp. from Human and Animals

Fecal samples obtained from human (diarrheic and non-diarrheic), chickens and gulls via sterile swabs, and intestinal contents of fish (*Chalcalburnus tarichi* PALLAS 1811) were inoculated into alkaline peptone water (APW, pH 8.4). After incubation of samples overnight at 28°C within APW, 0.1 ml of inoculums was inoculated into 7% defibrine sheep blood agar (Blood Agar Base No.2, Oxoid CM0271) containing 10 µg/mL ampicillin. Oxidase test (Whatman filter papers no 1 soaked with N,N-Dimethyl-p-phenylen diammonium dichloride are used) was performed for suspicious colonies growing in medium. The passage of oxidase positive bacteria to Tryptone Soy Agar (TSA, Oxoid CM0131) was carried out and Gram staining of test microorganisms growing on TSA was performed. Catalase, motility, oxidation/fermentation test (O/F), growth in broth containing 6% NaCl, growth in saltless broth and acid from inositol tests were performed for Gram negative bacillus. When bacteria is motile, ferments glucose (O/F:+/+), no growth in broth including 6% NaCl, growing in saltless broth, not composing acid from inositol and resistant to vibriostatic agent O/129 (150 µg, Oxoid DD0015) were identified as *Aeromonas* spp. Test bacteria are identified by using BD phoenix™ (Becton, Dickinson and Company, Sparks, Maryland 21152 USA) panels at species level.

Isolation and Identification of Motile Aeromonas spp. from Drinking Water

Total 200 water samples (6 natural springs, 5 well waters, 189 public drinking water supply distribution systems) were collected in sterile colored bottles and then they were transferred to laboratory under cold chain and used to isolate *Aeromonas* bacteria. 100 ml from each water sample were filtered by vacuum through cellulose nitrate filters (Sartorius) with pore size 0.45 µm. Filters were placed on *Aeromonas* Medium Base (RYAN) (Oxoid) and incubated for

Table 1. *Resources of Aeromonas strains typed by PFGE in this study (*These strains were isolated at 2002 and other strains were isolated at 2005)*
Tablo1. *Bu çalışmada PFGE ile tiplendirilen Aeromonas suşlarının kaynakları (*Bu suşlar 2002 yılında, diğerleri 2005 yılında izole edildi)*

Resource	*A. hydrophila*	*A. caviae*	*A. sobria*	*A. veronii*	*A. media*	Total
Diarrhoeal patients	-	9*+5	-	-	-	14 (27%)
Nondiarrheic food workers	-	-	2	-	-	2 (3.8%)
Drinking waters	1	8	3	-	-	12 (23.1%)
Cattles	-	1*	1*	-	-	2 (3.8%)
Sheeps	-	2*	1*	-	-	3 (5.8%)
Fishes	-	1	2	3	1	7 (13.5%)
Chickens	-	-	1	-	-	1 (1.9%)
Gulls	-	11	-	-	-	11 (21.1%)
Total	1 (1.9%)	37 (71.2%)	10 (19.2%)	3 (5.8%)	1 (1.9%)	52 (100%)

24 h at 37°C. As it is mentioned above, dark green, opaque with darker centres suspicious colonies were identified at genus and species levels on RYAN (Oxoid) isolation plates.

Pulsed-Field Gel Electrophoresis

Stock cultures were inoculated on TSA plates and incubated for overnight at 37°C. A single colony of each isolate was inoculated in 2 ml tryptone soy broth and incubated at 37°C until blurring. Then, inoculated TSA plates via sterile swab were incubated for overnight at 37°C. Later, 1.5 mL inoculum inside 1.5 mL sterile cold PIV (10 mM Tris [pH 7.6]), 1 M NaCl) buffer were centrifuged in 1100 g for 15 min at 4°C. The pellet was diluted by PIV buffer and turbidity was set to 4 McFarland standard. 100 µL from the mixture, containing equal volume 3% agarose of low heat melting was distributed on plug molds placed previously on ice bars and kept for 30 min in order to provide solidification. Plugs were kept waiting inside 3 mL lysis buffer (6 mM Tris [pH 7.6], 1 M NaCl, 100 mM EDTA [pH 7.6], 0.5% Brij 58, 0.2 % sodium deoxycholate, 0.5% sodium lauryl sarcosine, 1mg/mL lysozyme) for 4 h at 37°C within shaking water bath. It was incubated for overnight at 50°C by placing proteinaze K solution (0.5 M EDTA [pH 8], 10% sodium lauryl sarcosine, 1 mg/mL proteinaze K) instead of lysis buffer. It was kept waiting for 5 min at room temperature, after placing 3 ml sterile distilled water instead of proteinase K. After aspiration of distilled water, plugs were washed four times with Tris-EDTA buffer (10 mM Tris [pH 7.6], 0.1 mM EDTA [pH 7.6]) and kept at 4°C. Genomic DNA was digested by adding 50 U *Xba*I as being incubated for overnight at 37°C. Electrophoresis was performed in 1%Saekem agarose (pulsed-field certified agarose, Bio-Rad Laboratories) by using the CHEF-DRII system (Bio-Rad Laboratories, Nazareth, Belgium) in 0.5x TBE buffer (45 mM Tris, 45 mM boric acid, 1 mM EDTA [pH 8.0]) at 14°C. The running parameters were 6 V/cm^2 for 8 h with 20-20 sec pulses and 12 h with 5 to15 sec pulse times. One *A. caviae* isolate (isolate GAC8) was run in multiple lanes of each gel owing to DNA global reference for standardizing run. DNA banding patterns were visualized with 0.5% ethidium bromide and digitally photographed. GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium) was used to compare genetic similarity among isolates and construct a similarity dendogram by using Dice coefficient and the UPGMA algorithm (unweighted pair-group method with aritmetic mean) with a position tolerance of 1%.

RESULTS

Total 38 *Aeromonas* strains were isolated and identified in this study. The percentage, count and source of isolates were as follows; 5% (5 *A. caviae*) from stool sample of 100 diarrheic patient, 2% from the non diarrheic samples of food workers (2 *A.sobria*), 6% from the drinking water samples (1. *A. hydrophila*, 8 *A. caviae*, 3 *A. sobria*), 7% from the intestinal content of fish (1 *A. caviae*, 2. *A. sobria*, 3 *A. veronii*, 1 *A. media*), 1% from the feces samples of chickens (1 *A. sobria*), 11% from the feces samples of gulls (11 *A. caviae*).

Digestion of genomic DNA's of 52 strains (38 new strains isolated in the current study, 14 strains isolated in previous study) with *Xba*I enzyme show that no PFGE pattern has been obtained for 7 strains (1 human *A.caviae*, 1 sheep *A. caviae*, 1 water *A. caviae*, 1 gull *A. caviae*, 2 water *A. sobria*, 1 chicken *A. sobria*) while PFGE profile was obtained for 45 strain out of 52 *(Fig.1)*. PFGE profiles show that 4-12 well-resolved genomic DNA bands were observed from test strains and PFGE profiles of 45 isolates were shown 44 distinct patterns. Particularly, two identical isolates (*A. caviae* [FAC] and *A. sobria* [FAS1]) were grouped showing one identical pattern.

The result of one-to-one comparison of DNA patterns of forty five isolates exhibited that one pair was indistinguishable (*A. caviae* and *A. sobria* fish isolate, band difference 0), two pairs were closely related (*A. caviae* human and cattle isolate, *A. caviae* well water and gull isolate; band differences respectively 3, 2) and one pair was possibly related (cattle *A. sobria* isolate and gull *A. caviae* isolate, band difference 4), and no clonal relation determined among 37 strains. Eight strains were determined to have clonal relation according to assessment criteria of Tenover et al.[10].

Two genetically indistinguishable fish isolates (*A. caviae* and *A. sobria*) were isolated at same time period from the same fish population. Drinking water isolate isolated at same period from a well close to the same location while two gull isolate, having close relation, were isolated from the gulls living in coast of Van Lake.

Genetically close related human-cattle strains and possible related gull-cattle strains were isolated at different isolation periods. However it determined that here is no clonal relation among strains isolated at same isolation period from gulls living in same population. Moreover, no clonal relation were determined among human strains (diarrheic human and healthy food worker isolates) isolated previously in our region and isolated in this study. Briefly, no predominant clone was determined in human and animal populations and in drinking water.

DISCUSSION

It is being thought that various enzymes of *Aeromonas* such as lipase, protease, β-lactamase, amilase, kitinase and nuclease significantly contribute to their distribution in nature and their adaptation to environmental changes [11]. Having ability to remain alive in chlorinated water [1] and determination of these bacteries in animals [12,13], reveals the difficulty of epidemiological characterization of *Aeromonas*.

Finding our study show that no clonal relation was determined between strains isolated from human and drinking water. Results of our study are in good agreement with Stephenson et al.[14] research that comparison of

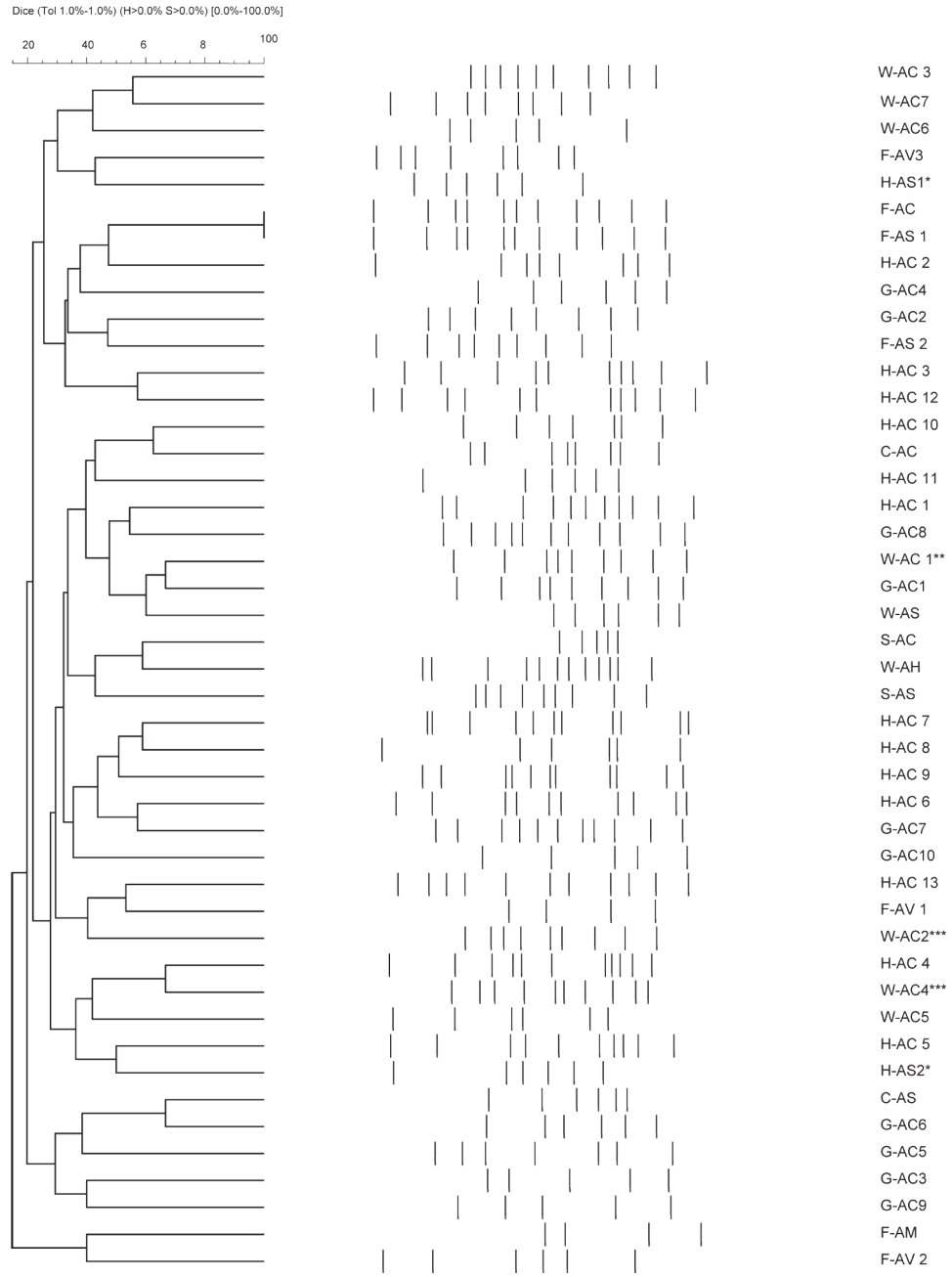

Fig 1. Pulsed-field gel electrophoresis patterns and similarity dendrogram of genomic DNA from *Aeromonas* spp. isolates from cattle (C), fish (F), gull (G), human (H), sheep (S) and water (W). AC: *A. caviae*; AV: *A. veronii*, AH: *A. hydrophila*, AS: *A. sobria*, AM: *A. media*. * nondiarrheic food worker isolates, ** well water isolate, *** natural spring isolates, other water isolates were isolated from public drinking water supply distribution system

Şekil 1. Sığırlardan (C), balıklardan (F), martılardan (G), insanlardan (H), koyunlardan (S) ve sulardan (W) izole edilen *Aeromonas* spp. izolatlarının genomik DNA'larının benzerlik dendogramı ve PFGE patternleri. AC: *A. caviae*; AV: *A. veronii*, AH: *A. hydrophila*, AS: *A. sobria*, AM: *A. media*. * Diyareli olmayan gıda çalışanı izolatları, ** Kuyu suyu izolatı, *** Doğal kaynak suyu izolatları, diğer su izolatları şebeke suyundan izole edilmiştir

protein profiles of 91 *Aeromonas* strains by the SDS-PAGE, were not similar human isolate stool and water isolates. Hanninen and Siitonen [15] reported that clinically significant *Aeromonas* hybridization groups are less in potable and soft water and there was a difference between *Aeromonas* hybridization groups isolated from potable water, chicken and cattle meat. Same investigators claim that contamination source of chicken and meat may not be water. However, Borchardt et al.[16] compared DNA patterns of 17 *Aeromonas* strains isolated from stools of diarrheic patients and 54 *Aeromonas* strains isolated from underground potable water by the PFGE. They stated that comparing stool and water of studied population were genetically irrelevant and gastrointestinal infections caused by *Aeromonas* were not due to exposure to underground water. Also, they clarified that it is necessary to determine at least a few stool and water isolates having closer relation than four band difference in order to claim that potable water is the source of *Aeromonas* infections in a population. Some researchers [17,18] suggested that the existence of epidemiological relation between *Aeromonas* infections and potable water are based on weak evidences. Burke et al.[17], found an epidemiological result owing to same biotypes *Aeromonas* bacteria isolated from children, causing infection, with well water. Millership and Want [18] compared protein profiles of 60 of *Aeromonas* strains isolated from human, food and water in London region, and data show that they might be similar strains in infected population, water and other environmental samples. These findings are supported by recent studies [3,19]. Bauab

et al.[19] ribotyped 88 *Aeromonas* strains isolated from human and environmental sources and extraintestinal infection isolates are within similar ribotypes by water isolates. Also they have informed that this finding verifies information regarding source of extraintestinal infections being water. However, Khajanchi et al.[3] searched 11 virulence gene and clonal relation of *Aeromonas* strains isolated from clinical samples and water of various regions of USA and world. In addition, they determined three pulsotypes, having same virulence genes and composed of epidemiologically relevant and irrelevant isolates. Humans are contaminated at least by *A. caviae-A. media* group living in water and due to exposing human to *Aeromonas* in water by virtue of these findings may cause gastroenteritis. Another research group reported that two *A.caviae* potable water isolate isolated in the same region at same period and one *A. caviae* isolate isolated from diarrheic human having indistinguishable PFGE patterns and identical virulence pattern indicated infection through water [20]. The same researchers reported that transmission through contaminated water of strains of the *A. caviae* group that can produce disease in humans at other their study [21].

There is no sufficient data based on strong evidences that the animals are reservoir for human infections as the existence of *Aeromonas* in animal being assessed as potential risk in respect of human infection. Maiti et al.[22] determined genetic variation between water, fish and clinical *Aeromonas* isolates by Random amplification of polymorphic DNA (RAPD) and enterobacterial repetitive intergenic consensus sequence (ERIC)-PCR. However Rahman et al.[23] asserted that specific fenotypic and genotypic stabile *Aeromonas* clones persisted for long time in treatment system and it may contaminate fishes produced for human consumption from hospitalized diarrheic children through sewage water treatment system. In our study, a clonal relation determined between strains isolated from two fish of same population (FAC and FAS1 isolate, indistinguishable, band difference 0), but no clonal relation found between human isolates and fish isolates. Moreover, no clonal relation determined between human isolates and gull isolates. Interestingly clonal relation determined between human and cattle isolates that are isolated at different period (HAC10 and CAC isolate, closely related, band difference 3).

Gulls are widespread coastal bird species and are being thought that they can contaminate coastal and lake water in world by their feces which is a major source of contamination [24]. In our study, a clonal relation determined between gull *A. caviae* strains living on the coast of Van Lake and well water *A.caviae* strain isolated from a close region (WAC1 and GAC1 isolate, close relation, band difference 2). Found from in a previous study in our region [25] that four *A. caviae* isolates (strains are not available now) isolated from gulls were determined to be identical by analyzing of result of typing with SDS-PAGE and concluded that these isolates might from same source and no genetic relation were determined between *A. caviae* isolates isolated from gulls living in same population. Moreover, clonal relation determined between gull isolate and cattle isolate isolated from same region at different time periods (CAS and GAC6 isolate, possible relation, band difference 4). Our findings are in parallel with Khajanchi et al.[3] study informing that isolates having weak epidemiological relation and isolated at different periods are same pulsotype and virulence group. They argued from this finding that very similar strains circulate in population and environment for a long period. On the contrary, Szcuka and Kaznowski [26] specified that they could not determine pathogenic clones among human living in different geographical regions despite clonal structure indication of *Aeromonas* species, and also they didn't determine reservoir of pathogenic strains in environment.

In our study, *A. caviae* ve *A. sobria* strains isolated from fish were grouped within same genotype. Our results are in good agreement with Bauab et al.[19] who specified that three different phenotypic types were within same ribotype. Øyvind et al.[7] compared the genetically and biochemical identification results of clinical and environmental *Aeromonas* isolates. Also, Øyvind et al.[7] claim that biochemical identification scheme being applied is developed based on data obtained from clinical isolates including only seven species, and thus that it is necessary to be careful while applying clinical data to environmental isolates and to use tests provided functional criteria for all *Aeromonas*. Moreover, same researchers reported that environmental isolates were more heterogenous than human stool isolates and it is accurate that biochemical profiles of environmental isolates is recognized less compared to clinical isolates, and consequently biochemical identification scheme being used in their study is not convenient for identifications of *Aeromonas* species, and that there is no sufficient current data to apply a full phenotypic identification for environmental strains.

Consequently, no predominant clone determined in this study. Findings of our study show that more extensive studies are required at three points. Firstly, more extensive studies, especially, on identification of environmental strains are required. Secondly, determination of clonal relation between a cattle isolate and a human isolate indicates that extensive studies are required regarding spread of these pathogens from animals to human. And third point is the determination of clonal relation between well water and a gull isolate being an indication of contamination of other water sources with swimming water by these birds.

REFERENCES

1. Brandi G, Sisti M, Giardini F, Schiavano GF, Albano A: Survival ability of cytotoxic strains of motile *Aeromonas* spp. in different types of water. *Lett Appl Microbiol,* 29, 211-215, 1999.

2. Abdullah AI, Hart CA, Winstanley C: Molecular characterization and distrubition of virulence-associated genes amongst *Aeromonas* isolates from Libya. *J Appl Microbiol*, 95, 1001-1007, 2003.

3. Khajanchi BK, Fadl AA, Borchardt MA, Berg RL, Horneman AJ, Stemper

ME, Joseph SW, Moyer NP, Sha J, Chopra AK: Distribution of virulence factors and molecular fingerprinting of *Aeromonas* species isolated from water and clinical samples: Suggestive evidence of water-to-human transmission. *Applied and Environmental Microbiology*, 76, 2313-2325, 2010.

4. Samakupa AP, Einarsson H, Eyþórsdóttir A: Hygiene indicators in a fish processing establishment- a case study in a white fish processing establishment, Fisheries Training Programme, Final Project 2003, The Unite Nations University. http://www.unuftp.is/static/fellows/document/alberthprf.pdf, *Accessed:* 01.08.2012.

5. Neyts K, Huys G, Uyttendaele M, Swings J, Debevere J: Incidence and idendification of mesophilic *Aeromonas* spp. from reatail foods. *Lett Appl Microbiol*, 31, 359-363, 2000.

6. Bonadonna L, Briancesco R, Ciccozzi M, Filetici E, Manuppella A, Pourshaban M, Semproni M, Shimada T: Biotyping, serotyping and genotyping of aeromonads from environmental and clinical samples. *World J Microbiol Biotechnol*, 17, 673-676, 2001.

7. Øyvind Ø, Einar GP, Jørgen L, Jose FM: Lack of agreement between biochemical and genetic identification of *Aeromonas* spp. *Apmis*, 113, 203-207, 2005.

8. Kariptaş E, Erdem B, Görgülü Ö: Protein profiles in different strains of *Aeromonas hydrophila* isolated from retail foods. *Kafkas Univ Vet Fak Derg,* 15 (6): 885-890, 2009.

9. Berktaş M, Körkoca H, Gülmez S: İnsan ve hayvanlardan izole edilen hareketli *Aeromonas* türlerinin SDS-PAGE yöntemi ile protein profillerinin araştırılması. Research Fund of Yüzüncü Yil University, Project No: 2002-TF.068 (unpublished study).

10. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B: Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol*, 33, 2233-2239, 1995.

11. Cahill MM: Virulence factors in motile *Aeromonas* species. *J Appl Bacteriol*, 69, 1-16, 1990.

12. Ceylan E, Ağaoğlu Z, Berktaş M: The occurrence and antibiotic resistance of motile *Aeromonas* in livestock. *Trop Anim Health Prod*, 41, 199-204, 2009.

13. Jindal.N, Garg SR, Kumar A: Comparison of *Aeromonas* spp.isolated from human, livestock and poultry faeces. *Isr J Vet Med*, 48, 80-83, 1993.

14. Stephenson JR, Millership SE, Tabaqchali S: Typing of *Aeromonas* species by polyacrylamide-gel electrophoresis of radiolabelled cell proteins. *J Med Microbiol*, 24, 113-118, 1987.

15. Hanninen ML, Siitonen A: Distrubition of *Aeromonas* phenospecies and genospecies among strains isolated from water, foods or from human clinical samples. *Epidemiol Infect*, 115, 39-50, 1995.

16. Borchardt MA, Stemper ME Standridge JH: *Aeromonas* isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis. *Emerg Infect Dis*, 9, 224-228, 2003.

17. Burke V, Robinson J, Cooper M, Beaman J, Partridge K, Peterson D, Gracey M: Biotyping and virulence factors in clinical and environmental isolates of *Aeromonas* species. *Appl Environ Microbiol*, 47, 1146-1149, 1984.

18. Millership SE, Want SV: Characterisation of strains of *Aeromonas* spp. by phenotype and whole-cell protein fingerprint. *J Med Microbiol*, 39, 107-113, 1993.

19. Bauab TM, Levy CE, Rodrigues J, Falcão DP: Niche-Specific association of *Aeromonas* ribotypes from human and environmental origin. *Microbiol Immunol*, 47, 7-16, 2003.

20. Pablos M, Remacha MA, Rodríguez-Calleja JM, Santos JA, Otero A, García-López ML: Identity, virulence genes, and clonal relatedness of *Aeromonas* isolates from patients with diarrhea and drinking water. *Eur J Clin Microbiol Infect Dis*, 29, 1163-1172, 2010.

21. Pablos M, Huys G, Cnockaert M, Rodríguez-Calleja JM, Otero A, Santos JA, García-López ML: Identification and epidemiological relationships of *Aeromonas* isolates from patients with diarrhea, drinking water and foods. *Int J Food Microbiol* 147, 203-210, 2011.

22. Maiti B, Raghunath P, Karunasagar I, Karunasagar I: Typing of clinical and environmental strains of *Aeromonas* spp. using two PCR based methods and whole cell protein analysis. *J Microbiol Meth*, 78, 312-318, 2009.

23. Rahman M, Huys G, Rahman M, Albert MJ, Kühn I, Möllby R: Persistence, transmission, and virulence characteristics of *Aeromonas* strains in a duckweed aquaculture-based hospital sewage water recycling plant in Bangladesh. *Appl Environ Microbiol*, 73, 1444-1451, 2007.

24. Lu J, Santo Domingo JW, Lamendella R, Edge T, Hill S: Phylogenetic diversity and molecular detection of bacteria in gull feces. *Appl Environ Microb*, 74, 3969-3976, 2008.

25. Körkoca H, Boynukara B: The characterization of protein profiles of the *Aeromonas hydrophila* and *A. caviae* strains isolated from gull and rainbow trout feces by SDS-PAGE. *Türk J Vet Anim Sci*, 27, 1173-1177, 2003.

26. Szczuka E, Kaznowski A: Typing of clinical and environmental *Aeromonas* sp. strains by random amplified palymorphic DNA PCR, 54 repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus sequence PCR. *J Clin Microbiol*, 42, 220-228, 2004.