Kafkas Univ Vet Fak Derg
21 (4): 585-592, 2015
DOI: 10.9775/kvfd.2015.12977

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

The Comparative Analysis of Phenotypic and Genotypic Properties of *Aeromonas sobria* Strains Isolated from Rainbow Trout (Oncorhynchus mykiss, Walbaum, 1972) [1]

Alper ÇİFTCİ [1] Ertan Emek ONUK [2] Gülay ÇİFTCİ [3]
Arzu FINDIK [1] Mehtap Ünlü SÖĞÜT [4] Timur GÜLHAN [1]

[1] *This work was supported by the Scientific Research Projects Commission of Ondokuz Mayıs University (Project No: PYO VET 1901.12.003)*

[1] Departments of Microbiology, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, TR-55210 Samsun - TURKEY
[2] Departments of Fisheries Diseases, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, TR-55210 Samsun - TURKEY
[3] Departments of Biochemistry, Faculty of Veterinary Medicine, University of Ondokuz Mayıs, TR-55210 Samsun - TURKEY
[4] High School of Health, University of Ondokuz Mayıs, TR-55210 Samsun - TURKEY

KVFD-2015-12977 Received: 09.01.2014 Accepted: 29.01.2015 Published Online: 17.02.2015

Abstract

Phenotypic and molecular characterization of *Aeromonas sobria (A. sobria)* isolates by antibiotyping, sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of whole cell proteins, random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) was aimed in this study. For this aim, thirty-six *A. sobria* isolates were analysed. Isolates were divided into 12 different antibiotypes and 4 proteotypes according to their antibiotic susceptibilities and SDS-PAGE patterns, respectively. Thirteen RAPD types were observed among all isolates. In conclusion, the use of double or triple combination of typing methods in this study was found to be more useful for discriminating the strains. The results obtained from this study may give information about phenotypic and genotypic variability of the *A. sobria* strains isolated from different regions of Turkey and can be helpful to control disease in fish through guiding the antibiotic therapy and giving information that will be useful to development vaccine.

Keywords: *Aeromonas sobria, Antibiotic resistance, Fish, RAPD, SDS-PAGE*

Gökkuşağı Alabalıklarından (Oncorhynchus mykiss, Walbaum, 1972) İzole Edilen *Aeromonas sobria* Suşlarının Fenotip ve Genotip Yönünden Karşılaştırmalı Analizi

Özet

Bu araştırmada *Aeromonas sobria (A. sobria)* izolatlarının antibiyotiplendirme, tüm hücre proteinlerinin sodyum dodesilsulfat-poliakrilamid jel electroforezi (SDS-PAGE) ile analizi, rastgele çoğaltılmış polimorfik DNA (RAPD) PCR ile genotiplendirme ile fenotipik ve genotipik karakterizasyonu amaçlanmıştır. Bu amaçla otuzaltı adet *A. sobria* izolatı incelenmiştir. İzolatlar antibiyotik duyarlılık sonuçlarına göre 12 farklı antibiyotip ve SDS-PAGE profiline göre 4 farklı proteotipe sahip oldukları belirlenmiştir. İzolatların 13 farklı genotipe sahip oldukları saptanmıştır. Sonuç olarak, Türkiye izolatı *A. sobria* suşlarının fenotipik ve genotipik olarak çeşitlilik gösterdiği ve çalışmada kullanılan tiplendirme metodlarının ikili veya üçlü kombinasyonlar halinde kullanılmasının suş ayrımında daha faydalı olduğu belirlenmiştir. Çalışmada elde edilen sonuçların balıklarda *A. sobria* infeksiyonlarının antibiyotik tedavi seçeneklerinin belirlenmesi konusunda faydalı olmakla birlikte, konu ile ilgili olarak yapılacak olan aşı ve teşhis kiti geliştirilmesi çalışmalarına öncülük edeceği düşünülmektedir.

Anahtar sözcükler: *Aeromonas sobria, Antibiyotik direnci, Balık, RAPD, SDS-PAGE*

INTRODUCTION

Motile Aeromonads which are *Aeromonas hydrophila, Aeromonas caviae* and *Aeromonas sobria*, all are small, Gram negative and rod-shaped bacteria are responsible for some troublesome diseases in pond fish [1]. These bacteria show wide-spread distribution in aquatic environment and several stress factors in aquatic culture systems predispose

İletişim (Correspondence)
+90 505 6150075
aciftci@omu.edu.tr

the fish to infections. The diseases caused by motile aeromonads are called as Motile Aeromonas Septicemia (MAS), Motile Aeromonad Infection (MAI), hemorrhagic septicemia, red pest or red sore. Whatever they are called, they are responsible for considerable economic losses [2]. Aeromonas septicemia is also fatal in reptiles, amphibians and humans. These organisms are considered to be a food and water borne pathogen causing an acute diarrheal disease human [3].

Motile Aeromonads that have multiple DNA groups (phenospecies) could not be differentiated from one another by biochemical tests readily and there are taxonomic complexities within the genus [4]. However, several reliable molecular *Aeromonas* identification methods have enabled new species such as *Aeromonas tecta* and *Aeromonas piscicola* to be discovered and also other known species associated to fish disease to be recognized such as *Aeromonas bestiarum, Aeromonas sobria, Aeromonas encheleia, Aeromonas veronii, Aeromonas eucrenophila* and *Aeromonas media* [5-8].

To characterize and type the bacterial isolates, conventional methods based on phenotypic characteristics such as biochemical properties and antimicrobial resistance, bacteriophage susceptibilities and rection to antisera together with molecular methods are offered as effective ways. Now, several genotypic methods such as polymerase chain reaction (PCR), pulsed-field gel electrophoresis (PFGE), DNA sequencing and restriction enzyme digestion with Southern blotting of chromosomal DNA and plasmids are used easily and rapidly. To determinate the genetic relatedness between bacterial strains by molecular methods are powerful and more commonly useful for bacterial typing [9]. Several PCR based protocols have been designed to identify and characterization of *Aeromonas* strains [10,11]. Moreover RAPD PCR with ERIC primer has been used for typing *Aeromonas* isolates [12,13]. Also some phenotypic and molecular methods have been used together to characterize *Aeromonas* strains and the results from these methods were evaluated comperatively [14,15].

In the current study, we aimed to characterize *A.sobria* isolates from rainbow trouts using both RAPD as a molecular method and phenotypic methods including SDS-PAGE analysis and antibiotyping.

MATERIAL and METHODS

Aeromonas sobria Strains

Total of 89 *Aeromonas* strains isolated between 2007-2011 from Rainbow trout *(Oncorhynchus mykiss)* were examined. To identify these isolates at the species level, strains were inoculated onto Tryptic Soy Agar (TSA) and incubated at 28°C. Macroscopically suspected colonies were selected. Strains were identified according to their Gram staining and biochemical characteristics based on Aerokey II group of tests for the identification of *Aeromonas* [16,17].

Identification of A. sobria Isolates by PCR

To confirm and genotype the *A. sobria* strains, DNA extractions of all isolates were performed using commercial DNA extraction kit (Invitrogen, Canada). The concentrations of extracted DNAs were measured and equalized to 10 ng/ml. To identify and confirm the strains as *A. sobria*, PCR was carried out according to Das et al.[10] with *asa*1 gene-specific primers F (5'-TAA AGG GAA ATA ATG ACG GCG-3') and R (5'-GGC TGT AGG TAT CGG TTT TCG-3'). The expected size of the PCR product was 249 bp. *A. sobria* ATCC 43979 was used as standard control strains in this study.

Genotyping of A. sobria Isolates by RAPD-PCR

ERIC-2 (5'-AAG TAA GTG ACT GGG GTG AGC G-3') primer was used to determine RAPD patterns of *A. sobria* strains. Amplification was performed according to the method reported by Onuk et al.[18] and amplification products were analysed by 1.5% agarose gel electrophoresis. DNA was visualized by staining with ethidium bromide. Dendogram of these patterns was obtained using UPGMA (Unweighted Pair Group Method with Arithmetic Averages). The genetic relatedness between the strains was determined applying the 70% similarity index.

Antibiotyping

To determine the resistance of the isolates against several antibiotics using in fish farming, Kirby-Bauer Disc Diffusion Method was used [19]. The antimicrobial agents were tested including nalidixic acid (30 µg), gentamycin (10 µg), sulfamethoxazole + trimethoprim (1.25 µg/23.75 µg), ampicillin (10 µg), oxolinic acid (2 µg), flumequine (30 µg), erythromycin (15 µg), oxytetracycline (30 µg), neomycin (10 µg), kanamycin (30 µg), florfenicol (30 µg), amoxicillin (25 µg), enrofloxacin (5 µg) (Bioanalyse, Turkey). Results were evaluated as sensitive, intermediate and resistant according to NCCLS [19]. A dendogram was created using UPGMA. The relatedness between the strains was determined applying the 70% similarity index.

Determination of SDS-PAGE Profiles

Whole cell protein profiles of *A. sobria* strains were analysed by SDS-PAGE [20,21]. Strains were propoagated onto Brain Heart Infusion Agar at 37°C for 24 h and after incubation period colones were harvested from agar plate by washing physiological saline and cells were inactivated by incubating at 60°C for 30 min. Inactivated bacterial suspension was centrifugated at 10.000 rpm for 15 min and supernatant was decanted. After pellet was washed three times it was resuspended in 0,01M Tris-HCl (pH 7.4) and optical density (OD) was adjusted to 5.0 at 470 nm spectrophotometrically. Before loading samples into gel for SDS-PAGE, samples were mixed with the sample buffer

in 2:1 (v/v) and sonicated for 5 min. Then mixture was incubated at 100°C in a water bath for providing protein denaturation. Twenty microliter samples were loaded into each well of 10% polyacrilamide gel. After electrophoresis at 200 V for 20 min, Blue Silver staining method was used for visualizing the protein bands. In electrophoresis, a molecular weight standard (205-6,6 kDa, Sigma S8445) was used for determining and calculating the molecular weights of protein bands.

Determination of Typeability, Reproducibility, Discrimination Power and Confidence Intervals of Typing Methods

To determine the typeability, a formula, T = Nt/N where Nt is the number of isolates assigned a type and N is the number of isolates tested, was used. The reproducibility was determined by using the formula: R = Nr/N, where Nr is the number of isolates assigned the same type on repeat testing and N is the number of isolates tested [22]. Discriminatory indices and confidence intervals of the typing methods were determined according to the formulas described previously [23,24].

RESULTS

Identification of A. sobria

Fourty five of eighty nine *Aeromonas* isolates from rainbow trout were identified as *A. sobria* by conventional cultural tests. Thirty six of 45 isolates were confirmed by PCR as *A. sobria*. *A. sobria* isolates including a reference *A. sobria* strain gave a specific 249 bp band. Further analyzes were carried out with using the 36 isolates which were commonly identified as *A.sobria* by conventional tests and PCR.

RAPD-PCR Profiles of A. sobria Strains

All *A. sobria* strains (n=36) were able to typed by this method. The strains showed genetic variability and given the 70% similarity indices, they were grouped into 13 RAPD types. One of them, R4 was a cluster including two subtypes (R4a and R4b) and the others were unique types *(Fig. 1)*. Most of the isolates (33.33%) were assigned to R13 type and the second predominant RAPD type, R1 included 8 (22.22%) isolates.

Antibiotyping

According to the antibiogram results, *A. sobria* strains (n=36) were grouped into 12 antibiotypes depending upon their susceptibilities to 13 different antibiotics. Dendogram of antibiotyping of these isolates is shown in *Fig. 2*. These antibiotypes (A1-A12) included four clusters (A3, A4, A10 and A12) and eight unique types. Cluster A3 and A12 were differentiated into 8 and 9 subtypes, respectively. All isolates except for one (97.2%) were resistant against oxolinic acid and most of the isolates (91.7%) were susceptible to florfenicole. More than 60% of isolates were resistant against oxytetracycline, amoxycillin and ampicilline. The predominant types, A3 and A12 included 11 and 12 isolates, respectively. While in A3 cluster, dominant pattern was susceptible to the most of antibiotics (at least seven), in A12 cluster most of the isolates were resistant against at least 5 antibiotics. The percentages of isolates that were susceptible, intermediate or resistant to 13 antibiotics were given in the *Table 1*.

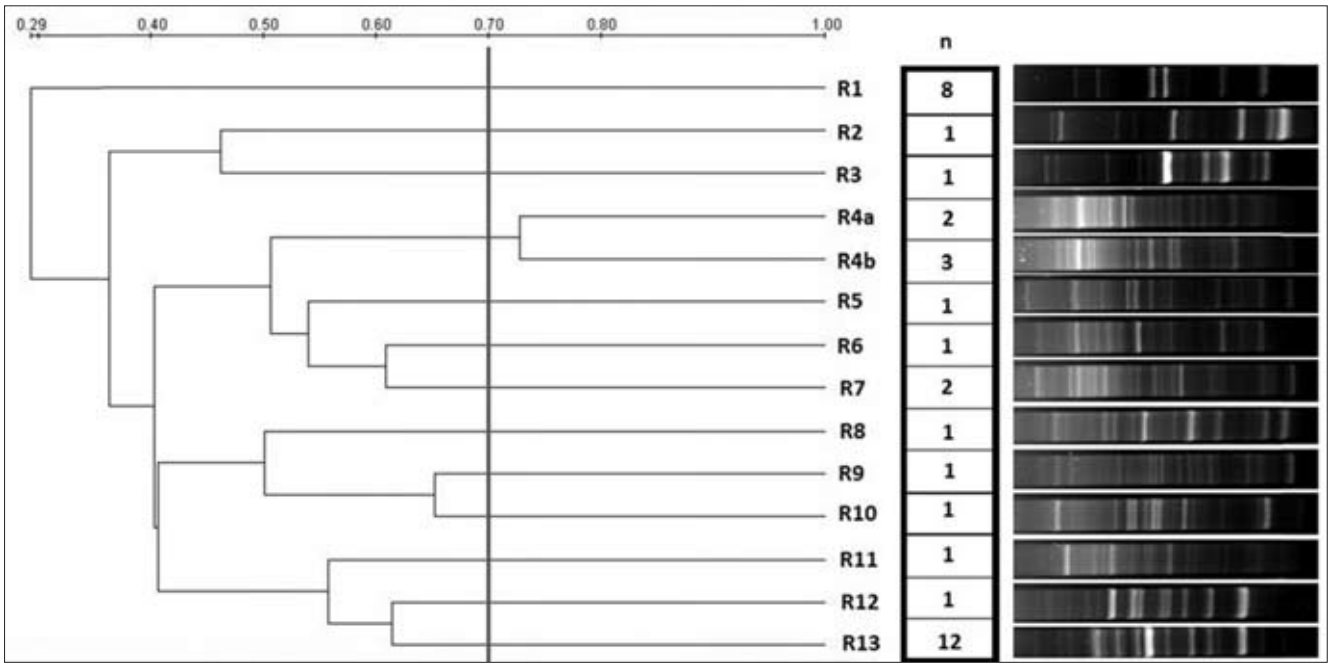

Fig 1. The phylogenetic tree of *A. sobria* strains with RAPD-PCR patterns
Şekil 1. RAPD-PCR profiline göre *A. sobria* suşlarının filogenetik ağacı

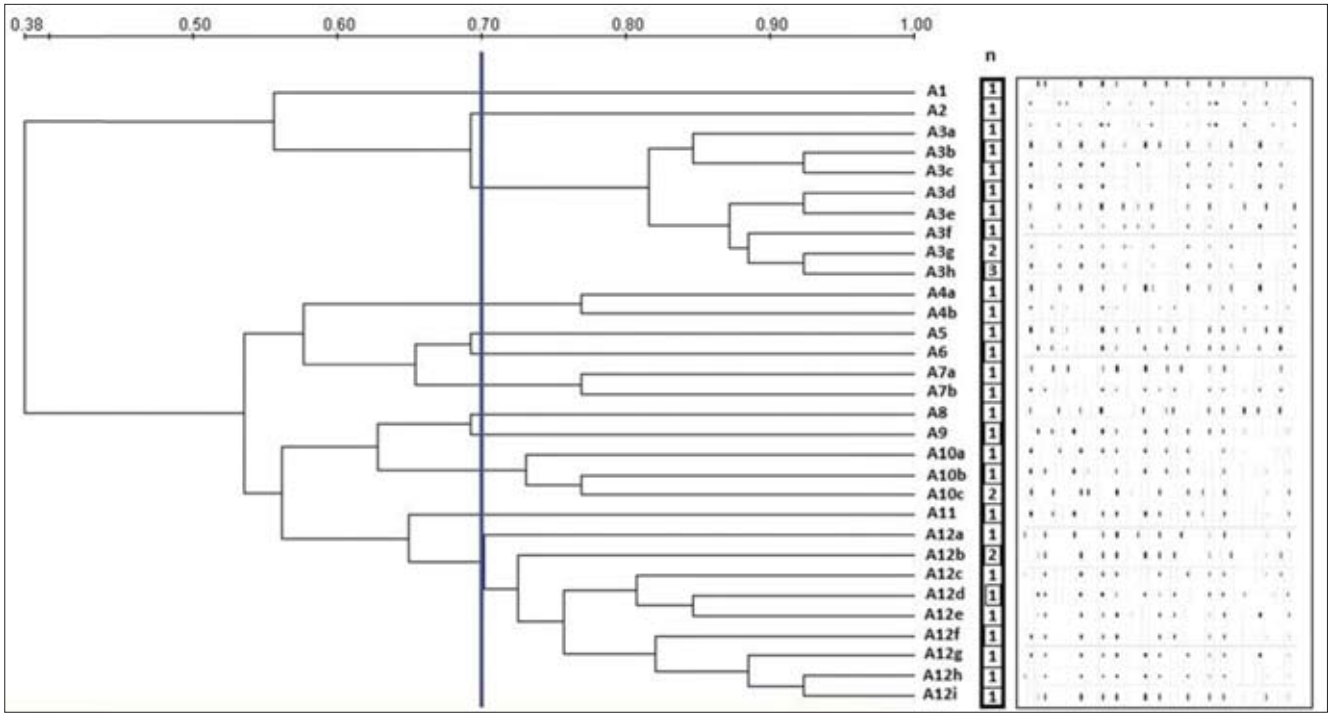

Fig 2. Antibiotyping dendogram of *A. sobria* isolates depending upon susceptibilities to 13 antibiotics
Şekil 2. *A. sobria* suşlarının 13 antibiyotiğe karşı duyarlılıklarına gore antibiyotip dendogramı

Table 1. *Antibiotic susceptibilities of A. sobria isolates*
Tablo 1. *A. sobria izolatlarının antibiyotik duyarlılıkları*

Antibiotic Discs	Resistance Profiles					
	R		I		S	
	n	%	n	%	n	%
Oxolinic acid (2 µg)	35	97.2	1	2.8	0	0
Oxytetracycline (30 µg)	25	69.5	4	11.1	7	19.4
Amoxycillin (25 µg)	23	63.8	2	5.6	11	30.6
Ampicilline (10 µg)	22	61.1	2	5.6	12	33.3
Nalidixic acid (30 µg)	17	47,2	12	33.3	7	19.5
Flumequine (30 µg)	16	44.4	14	38.9	6	16.7
Erythromycin (15 µg)	15	41.7	11	30.6	10	27.7
Neomycin (10 µg)	8	22.2	13	36.1	15	41.7
Sulphamethoxazole-trimetoprim (25 µg)	6	16.7	0	0	30	83.3
Enrofloxacin (5 µg)	6	16.7	11	30.6	19	52.7
Kanamycin (30 µg)	5	13.9	6	16.7	25	69.4
Gentamycin (10 µg)	2	5.6	8	22.2	26	72.2
Florfenicol (30 µg)	0	0	3	8.3	33	91.7

R: *Resistance* ***I:*** *Intermedier resistance.* ***S:*** *Susceptibility*

Protein Profile Analysis by SDS-PAGE

All thirty six *A. sobria* isolates were able to typed according to their whole cell protein profiles by SDS-PAGE. Strains were clustered at 70% similarity level using UPGMA method. Strains were grouped into two cluster (S2 and S4) and 2 unique types. Most of the isolates (66.66%) were assigned to S2 cluster and the similarity level between two subtypes (S2a and S2b) within this cluster was 75%. Besides the second predominant cluster including seven (19.44%) isolates included two subtypes, S4a and S4b and 87% of similarity was shown between these subtypes. A dendogram illustrating SDS-PAGE protein patterns of *A. sobria* isolates is shown in *Fig. 3*.

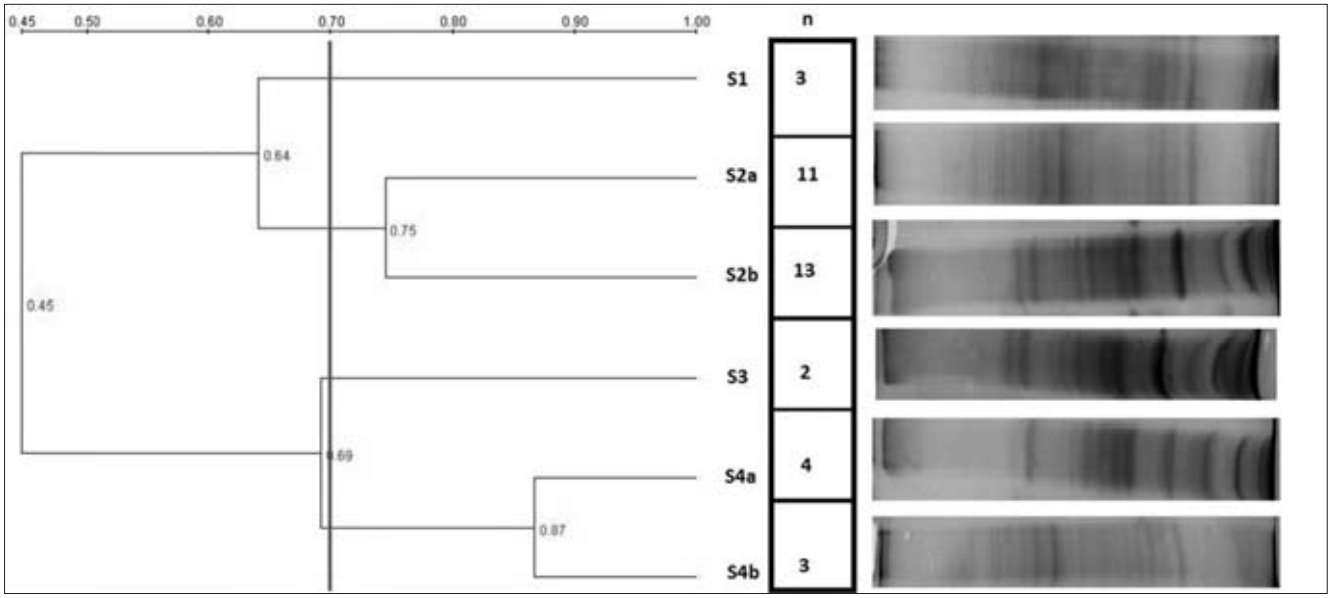

Fig 3. Dendogram based on whole cell protein patterns of *A. sobria* strains
Şekil 3. *A. sobria* suşlarının tüm hücre protein profillerine göre dendogramı

Table 2. *Discriminatory indices (with Confidence intervals) of the typing methods used in this study (70% of cut-off value)*
Tablo 2. *Çalışmada kullanılan tiplendirme metodlarının (%70 eşik değeri) ayrım güçleri (güven aralıkları ile)*

Method	No. of Types	Discrimination Power	Confidence Intervals (%)
Antibiotyping	12	0.83	78-88
SDS-PAGE	4	0.52	38-66
RAPD-PCR	13	0.83	80-87
Antibiotyping+ SDS-PAGE+ RAPD-PCR	22	0.95	93-97

The Typeability, Reproducibility, Discriminatory Power and Confidence Intervals of Typing Methods

All strains (n=36) were typeable and methods used to type *A. sobria* strains phenotypically and genotypically had good reproducibility. Discriminatory indices (D) and confidence interval (CI) of the methods and their combinations were given in *Table 2*.

DISCUSSION

Motile mesophilic *Aeromonas* species including *A. sobria* are Gram negative, ubiquitious bacteria which are frequently isolated from various foods such as fish, shellfish, raw meat and raw milk, vegetables. However, seafood was the most favorable environment for proliferation of these bacteria. FDA considers that motile *Aeromonads* are re-emerging enteric pathogens [25]. These bacteria are also producing warmwater fish diseases [26]. Some factors such as poor water quality, overcrowding and rough handling causing stress are the most common cause the susceptibility of fish to these organisms [27].

Over time, new species has been added to *Aeromonas* genus and however it has been proved that aeromonads were the member of *Aeromonadaceae* family by means of genetic studies. Futhermore, multiple hybridization groups (HGs) were revealed within each of mesophilic *Aeromonas* including *A. sobria* by *DNA* hibridization studies [28]. According to the last edition of Bergey's Manual of Systematic Bacteriology [17], 17 HGs or "genomospecies" and 14 "phenospecies" which refers to a single heterogeneous species (such as *A. sobria*) containing multiple HGs within it are described. Although taxonomic confusions has been tried to solve, it is difficult to see the harmony between phenotypic and genotypic characteristics of *Aeromonas* species. Much of phenotypical methods especially based on biochemical characteristics of *Aeromonas* species are not good enough to characterize and differentiate the species due to their complex taxa. Therefore multiple molecular techniques are required for accurate characterization [3,18]. Some phenotyping methods other than the methods based on biochemical characteristics such as antibiotyping and SDS-PAGE protein analysing have been used to type and/or characterize *Aeromonas* spp [4,8,10]. Das et al.[25] have reported that all *A. sobria* isolates from fresh water fish, frozen fish and fish pickle were 100% resistance to ampicillin. The percentages of resistance against nalidixic acide and neomycin were 12% and 80%, respectively. Awan et al.[29] and Guz and Kozinska [30] have also found that all *A. sobria* strains isolated from food and environmental samples and diseased fish, respectively were resistant against ampicillin. Motyl et al.[31] have also found that all *A. sobria* isolates of human origin were resistant to ampicillin. Guz and Kozinska [30] have also reported that these strains (100%) were sensitive to trimethoprim-sulphamides, oxolinic acid and flumequine. However in this study, 61.1% of *A. sobria* strains were resistant to ampicillin and

the susceptibilies to nalidixic acid and neomycin were also different from their reports. The percentages of resistance against nalixic acid and neomycin were found as 47.2% and 22.2%, respectively. Resistance of our *A. sobria* strains against nalidixic acid were relatively higher in those in other studies [25,32]. Other notable differences in the results between Guz and Kozinka's study [30] and this study were the susceptibilities to flumequine and oxolinic acid. Namely, our *A. sobria* strains were found to be resistant against flumequine (44.4%) and oxolinic acid (97.2%). However while the less percentage (16.7%) of resistance against trimetoprim-sulphamethaxasole was found, resistance against oxytetracycline (69.5%) was higher in this study. Other researchers [29,31] have reported the higher percentages of the susceptibility to tetracycline compared to our results. As for the resistance of the strains against erythromycine, our strains were less resistant (41.7%) than those isolated in other studies [29,30]. The most strains (94.40%) in this study were susceptible to gentamicin as such in other studies [29,31]. Nalidixic acid, flumequine and oxolinic acid which are the first generation quinolons and also oxytetracycline are widely used in farm fish. Resistance against these quinolones especially against oxolinic acid and against oxytetracycline seems to be significant in terms of the difficulties in fish therapy. Similarly resistance against ampicillin (although found to be less than in some other studies) was also noticible. Although antimicrobial therapy is an effective way to control fish diseases, widespread and improper antibiotic use and also other factors such as genetic mutations have all resulted in antibiotic resistance [32-34]. Therefore more effective antimicrobial agents and also vaccines such an alternative way to control disease should be developed.

Whole-cell protein fingerprinting is one of the typing methods used for both taxonomy and differentiation of strains within a species [35]. Several studies have performed to analyse the protein profiles of *Aeromonas* spp and widely varying patterns have been obtained [14,36]. Körkoca and Boynukara [37] have firstly analysed SDS-PAGE protein profiles of *A. hydrophila* and *A. caviae* strains in Turkey. However, studies are seen to be limited and especially no study is available on characterization of protein profiles of *A. sobria* isolates from diseased fish. We analysed the whole cell protein profiles of 36 *A. sobria* strains in order to type and characterize them. Although no very high diversity was found among strains, all of them were typeable by SDS-PAGE analyse and clustered by UPGAMA method. We obtained two clusters (S2 and S4) and 2 unique types and most of the isolates were assigned to S2 cluster. Discriminatory power of SDS-PAGE method determining whole cell profiles was found low (0.52) however, when it was used in combination with another phenotypic method (antibiotyping) and RAPD typing discriminatory power become high (0.95). So using whole cell profiling in combination with other typing techniques may be useful to type and characterize the strains. Maiti et al.[14] have also found that discriminatory power of whole cell profiling was low. Furthermore some researchers [38,39] have reported that although protein fingerprinting has the potential to differentiate *Aeromonas* species, whole cell protein profiles were unsuitable for the characterization of strains within a species because of the low qualitative variation.

Although several phenotypic methods that also mentioned above are effectively used to type different bacterial strains signly or together with another phenotypic and/or genotypic methods, several genotypic techniques such as AP-PCR (arbitrarily primed PCR) or RAPD-PCR, PFGE (Pulsed Field Gel Electrophoresis), DNA sequencing and restriction enzyme digestion with Southern blotting of chromosomal DNA and plasmids are considered to be more powerful and more commonly used to characterize the strains and determine the genetic relatedness between them. However, some criteria such as reproducibility, typeability, discriminatory power, speed, simplicity, ease of interpretation and cost should be considered to optimize these molecular techniques [40-42]. This method has considered to be cheap, simple, speed and to need less equipment. We attempted to determine the genetic relatedness among *A. sobria strains* isolated from diseased fishes by a RAPD-PCR method using ERIC primer. All the strains were able to type and made a cluster analysis. Only one cluster including two subtypes and 12 unique types were found based on 70% similarity indices. Although most of the strains, 33.33% and 22.22% of the strains were assigned to cluster R4 and R1, respectively, the strains showed wide variation among themselves. This may due to their different geophragical origins. Yousr et al.[13] have used RAPD-PCR and ERIC-PCR for molecular typing of *Aeromonas* species and they have found these strains also including *A. sobria* were very diverse. They have grouped *A. sobria* strains into one significant cluster grouped the three strains from the same source and six single isolates at the similarity of 40%. In several studies [12,38], numerous *Aeromonas* isolates from different geographical, environmental and clinical origins have been tried to distinguish and characterize using RAPD-PCR. Although RAPD-PCR has been proved to be useful tool for epidemiological investigation and population genetic analysis of *Aeromonas* spp.[38], RAPD fingerprinting has been reported to allow the identification of strains; but, because of the high variability its potential as an aiding method for species identification was limited [12]. On the other hand being able to detect diversities by this method may allow advantage for vaccine development trials. There is no available study on the phenotypic and genetic diversity of *A.sobria* isolates from diseased fish in Turkey.

In conclusion, we were able to type *A. sobria* strains isolated from diseased fish in different regions of Turkey through both phenotypically based on their antibiotic susceptibilities and whole cell protein profiles and geno-

typically using RAPD-PCR. While high diversitiy among the strains were found by antibiotyping and RAPD-PCR fingerprinting, in SDS-PAGE whole cell protein analysis, less variation were observed in their protein profiles. Compared the discriminatory powers of all three typing methods, RAPD-PCR and antibiotyping was superior to SDS-PAGE. However when used in combination with other techniques SDS-PAGE analysis may contributes the typing goal. Likewise the discriminatory power of triple combination of typing methods we used was higher than when used individulally. The results obtained from anti-biotyping should be guided the antimicrobial therapy efforts in terms of any emergence of antibiotic resistance. The results obtained from this study may give information about variability of the *A. sobria* strains isolated from different regions of Turkey and can be helpful to control disease in fish through guiding the antibiotic therapy and giving information that will be useful to development vaccine.

REFERENCES

1. Roberts R: Motile aeromonad septicemia. **In,** Inglis V, Roberts RJ, Bromage NR (Eds): Bacterial Diseases of Fish. 143-155. Blackwell Scientific Publications, Oxford, 1993.

2. Camus AC, Durborow RM, Hemstreet WG, Thune RL, Hawke JP: *Aeromonas* bacterial infections-motile aeromonad septicemia. SRAC Publication No. 478, 1998.

3. Ghenghesh KS, Ahmed SF, El-Khalek RA, Al-Gendy A, Klena J: Aeromonas-associated infections in developing countries. *J Infect Dev Count*, 2, 81-98, 2008. DOI: 10.3855/jidc.277

4. Abulhamd A: Genetic diversity and antimicrobial susceptibility of motile aquatic aeromonads. *Int J Chem Eng Appl*, 1 (1): 90-95, 2010.

5. Beaz-Hidalgo R, Alperi A, Buján N, Romalde JL, Figueras MJ: Comparison of phenotypical and genetic identification of *Aeromonas* strains isolated from diseased fish. *Syst Appl Microbiol*, 33, 149-153, 2010. DOI: 10.1016/j.syapm.2010.02.002

6. Beaz-Hidalgo R, Figueras MJ: Molecular detection and characterization of furunculosis and other aeromonas fish infections. **In,** Carvalho E (Ed): Health and Environment in Aquaculture. *InTech,* 2012. DOI: 10.5772/29901

7. Kozińska A: Dominant pathogenic species of mesophilic aeromonads isolated from diseased and healthy fish cultured in Poland. *J Fish Dis*, 30, 293-301, 2007. DOI: 10.1111/j.1365-2761.2007.00813.x

8. Li Y, Cai SH: Identification and pathogenicity of *Aeromonas sobria* on tail-rot disease in juvenile tilapia *Oreochromis niloticus. Current Microbiol*, 62, 623-627, 2011. DOI: 10.1007/s00284-010-9753-8

9. Ghori SA, Grubb WB: Applications of molecular methods for typing bacteria. **In,** Ahmed N, Qureshi FM, Khan OY (Eds): Industrial and Environmental Biotechnology. 157-174. Horizon Scientific Press, UK, 2001.

10. Das A, Rathore A, Janani C, Hemanth C, Balakrishnan RA: Diagnosis of motile *Aeromonas sobria* from cat fishes with septicemia by PCR. *IOSR J Agricult Vet Sci*, 2 (6): 87-91, 2013.

11. Lee C, Cho JC, Lee SH, Lee DG, Kim SJ: Distribution of *Aeromonas spp.* as identified by 16S rDNA restriction fragment length polymorphism analysis in a trout farm. *J Appl Microbiol*, 93, 976-985, 2002. DOI: 10.1046/j.1365-2672.2002.01775.x

12. Szczuka E, Kaznowski A: Typing of clinical and environmental *Aeromonas* sp. strains by random amplified polymorphic DNA PCR, repetitive extragenic palindromic PCR, and enterobacterial repetitive intergenic consensus sequence PCR. *J Clin Microbiol*, 42, 220-228, 2004. DOI: 10.1128/JCM.42.1.220-228.2004

13. Yousr AH, Napis S, Rusul GRA, Alitheen NB, Son R: Molecular typing of *Aeromonas* species using RAPD and ERIC-PCR fingerprinting. *Int Food Res J*, 17, 691-698, 2010.

14. 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 Method*, 78, 312-318, 2009. DOI: 10.1016/j.mimet.2009.07.009

15. Sinha S, Shimada T, Ramamurthy TSK, Bhattacharya T, Yamasaki S, Takeda Y, Balakrish NG: Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. *J Med Microbiol*, 53, 527-534, 2004.

16. Carnahan AM, Behram S, Joseph SW: Aerokey II: A flexible key for identifying clinical *Aeromonas* species. *J Clin Microbiol*, 29, 2843-2849, 1991.

17. Martin-Carnahan E, Joseph WS: *Aeromonas*. **In,** Brenner DJ, Krieg NR, Staley JT, Garrity GM (Eds): Bergey´s Manual of Systematic Bacteriology. 2nd ed., Vol. 2, 556-578, Springer-Verlag, New York, 2005.

18. Onuk EE, Çiftci A, Fındık A, Çiftci G, Altun S, Balta F, Özer S, Çoban AY: Phenotypic and molecular characterization of *Yersinia ruckeri* isolates from rainbow trout *(Oncorhynchus mykiss*, Walbaum, 1792) in Turkey. *Berlin Munch Tierarzt Wochen*, 124 (7-8): 320-328, 2011.

19. CLSI: M02-A11 and M100-S23 Package-Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard Eleventh Edition & Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Third Informational Supplement, 2013.

20. Laemmli K: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685, 1970. DOI: 10.1038/227680a0

21. Uysal H, Ciftci G, Ciftci A: Determination of antigenic glycoproteins of *Enterococcus faecalis* strains distinguished with aggregation substance, gelatinase and cytolysine. *Rev Méd Vét*, 164, 374-381, 2013.

22. Strueleus MJ: Consensus guidelines for appropriate use and evaluation of microbiologic typing system. *Clin Microbiologic Infect*, 2, 2-11, 1996. DOI: 10.1111/j.1469-0691.1996.tb00193.x

23. Grundmann H, Hori S, Tanner G: Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. *J Clin Microbiol*, 39, 4190-4192, 2001. DOI: 10.1128/JCM.39.11.4190-4192.2001

24. Hunter PR, Gaston MA: Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. *J Clin Microbiol*, 26 (11): 2465-2466, 1988.

25. Das A, Viyanesree V, Santhosh CR, Sree HS: Surveillance of *Aeromonas sobria* and *Aeromonas hydrophila* from commercial food stuffs and environmental sources. *J Exper Sci*, 3 (9): 36-42, 2012.

26. Rahimi E, Raissy M, Razzaghimanesh M, Dastgerdi AA, Shahraki MM: Occurrence of *Aeromonas hydrophila* in fish, shrimp, lobster and crab in Iran. *Kafkas Univ Vet Fak Derg*, 20, 691-696, 2014. DOI: 10.9775/kvfd.2014.10892

27. Cipriano RC: *Aeromonas hydrophila* and motile aeromonad septicemias of fish. 25, Fish Disease Leaflet 68. U.S. Dept. of the Interior, Fish and Wildlife Service, Division of Fishery Research, Washington, DC, 2001.

28. Janda M, Abbott SL: The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *J Clin Microbiol*, 23, 35-73, 2010. DOI: 10.1128/CMR.00039-09

29. Awan MB, Maqbool A, Bari A, Krovacek K: Antibiotic susceptibility profile of *Aeromonas* spp. isolates from food in Abu Dhabi, United Arab Emirates. *New Microbiol*, 32, 17-23, 2009.

30. Guz L, Kozińska A: Antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp *(Cyprinus carpio). Bull Vet Inst Pulawy*, 48, 391-395, 2004.

31. Motyl MR, McKinley G, Janda JM: *In vitro* susceptibilities of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* to 22 antimicrobial agents. *Antimicrob Agents Chemother*, 28, 151-153, 1985. DOI: 10.1128/AAC.28.1.151

32. Durmaz Y, Türk N: Alabalık işletmelerinden motil aeromonasların izolasyonu ve antibiyotiklere duyarlılıklarının saptanması. *Kafkas Univ Vet Fak Derg*, 15, 357-361, 2009. DOI: 10.9775/kvfd.2008.121-A

33. Korun J, Toprak HB: Kültürü yapılan gökkuşağı alabalıkları (Oncorhynchus mykiss)'nın barsağından izole edilen hareketli aeromonas suşlarının antibiyotik hassasiyetleri üzerine NaCl'ün etkisi. *Kafkas Univ Vet Fak Derg*, 16, 193-198, 2010. DOI: 10.9775/kvfd.2009.453

34. Aravena-Román M, Inglis TJJ, Henderson B, Riley TV, Changa BJ: Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents. *Antimicrob Agents Chemother*, 56, 1110-1112, 2012. DOI: 10.1128/AAC.05387-11

35. Millership SE, Want SW: Characterisation of strains of *Aeromonas* spp. by phenotype and whole-cell protein fingerprint. *J Med Microbiol*, 39, 107-113, 1993. DOI: 10.1099/00222615-39-2-107

36. Szczuka E, Kaznowski A: Characterization of *Aeromonas caviae* and *Aeromonas veronii* by standardized cellular protein electrophoretic patterns. *Folia Micro*biol, 52, 65-69, 2007. DOI: 10.1007/BF02932140

37. 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. *Turk J Vet Anim Sci*, 27, 1173-1177, 2003.

38. Delamare APL, Artico LD, Grazziotin FG, Echeverrigaray S, Pinto da Costa SO: Total protein electrophoresis and RAPD fingerprinting analysis for the identification of Aeromonas at the species level. *Braz J Microbiol*, 33, 358-362, 2002. DOI: 10.1590/S1517-83822002000400016

39. Wilcox MH, Cook AM, Thickett KJ, Eley A, Spencer RC: Phenotypic methods for speciating clinical *Aeromonas* isolates. *J Clin Pathol*, 45, 1079-1083, 1992.

40. Altun S, Onuk EE, Çiftci A, Büyükekiz AG, Duman M: Phenotypic, genotypic characterisation and antimicrobial susceptibility determination of *Lactoccous garvieae* strains. *Kafkas Univ Vet Fak Derg*, 19, 375-381, 2013. DOI: 10.9775/kvfd.2012.7754

41. Wei LS, Musa N, Weel W, Seng CT, Shazili NAM, Shaharom F: Review on RAPD-PCR as a Discriminative Tool for Fish Bacteria. www.paper.edu.cn/en.../200806-333, 2008. *Accessed:* 01.09.2014.

42. Körkoca H, Berktaş M, Durmaz R, Gürsoy NC: The research of clonal relationship among aeromonas strains isolated from human, animal and drinking water by PFGE. *Kafkas Univ Vet Fak Derg*, 19, 271-276, 2013. DOI: 10.9775/kvfd.2012.7659