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RESEARCH ARTICLE

Determination of Phenotypic, Serotypic and Genetic Diversity and Antibiotyping of *Yersinia ruckeri* Isolated from Rainbow Trout [1]

Soner ALTUN * Ertan Emek ONUK ** Alper ÇİFTCİ ***
Muhammed DUMAN * Ayşe Gül BÜYÜKEKİZ *

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* Uludağ Üniversitesi, Veteriner Fakültesi, Su Ürünleri Hastalıkları Anabilim Dalı, TR-16059 Bursa - TÜRKİYE
** Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Su Ürünleri Hastalıkları Anabilim Dalı, TR-55139 Samsun - TÜRKİYE
*** Ondokuz Mayıs Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı, TR-55139 Samsun - TÜRKİYE

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Summary

In this study, 15 *Yersinia ruckeri* isolates that had been isolated from rainbow trout farms and 2 reference strains (serotype 1 and serotype 2) were examined in terms of phenotypic, serotypic and genotypic characteristics. Conventional microbiological and API 20 E tests were used to determine phenotypic characteristics of *Y. ruckeri* isolates and it was determined that the *Y. ruckeri* showed significantly homogenous profile by microagglutination test performed by using serotype 1 and serotype 2 immunsera, and 11 of 15 isolates were serotyped as serogroup 1 while the rest 4 were serotyped as serogroup 2. In Random Amplified Polymorphic DNA (RAPD) analysis, *Y. ruckeri* isolates were genotyped within 2 separate clusters according to 70% similarity coefficient index and it was detected that first cluster includes 2 genotypes (YR1 and YR2) and second cluster includes 3 genotypes (YR3, YR4 and YR5). Furthermore, antibiotic resistance profiles of *Y. ruckeri* strains were determined and it was found that they were resistance to florfenicol, erythromycin, oxytetracycline and trimethoprim-sulphamethoxazole which have been licensed for fish health in Turkey. It is considered that findings obtained will form a basis to develop diagnosis kit and/or vaccination for *Y. ruckeri*.

Keywords: *Oncorhynchus mykiss, Yersinia ruckeri, API 20 E, Microagglutination, RAPD-PCR*

Gökkuşağı Alabalıklarından İzole Edilen *Yersinia ruckeri* Suşlarının Fenotipik, Serotipik ve Genetik Farklılıklarının Belirlenmesi ve Antibioyotiplendirilmesi

Özet

Bu çalışmada gökkuşağı alabalığı işletmelerinden izole edilmiş olan 15 adet *Yersinia ruckeri* izolatının 2 adet referans suşla (serotip 1 ve serotip 2) karşılaştırmalı olarak fenotipik, serotipik ve genotipik özellikleri bakımından incelenmesi amaçlanmıştır. *Y. ruckeri* izolatlarının fenotipik özelliklerinin belirlenmesinde klasik mikrobiyolojik ve API 20 E testleri kullanılmış ve bu testlerde bakterinin oldukça homojen bir yapı gösterdiği belirlenmiştir. Serotip 1 ve serotip 2 immunserumlar kullanılarak yapılan mikroaglütinasyon testinde ülkemizden izole edilmiş olan 15 suştan 11'nin serotip 1, 4'ünün ise serotip 2 özellikte olduğu belirlenmiştir. Rastgele Çoğaltılmış Polimorfik DNA (RAPD) analizinde *Y. ruckeri* izolatları %70 benzerlik katsayısına göre 2 ayrı küme içerisinde gruplanmış, kümelerden birincisinin 2 (YR1 ve YR2), ikincisinin ise 3 genotip (YR3, YR4 ve YR5) içerdiği saptanmıştır. Ayrıca bu çalışmada *Y. ruckeri* izolatlarının antibiyotik duyarlılıkları belirlemiş ve bu izolatların ülkemizde balıklarda ruhsatlı olan florfenikol, eritromisin, oksitetrasiklin ve trimetoprim-sulfamethoxazole karşı direnç geliştirmiş oldukları saptanmıştır. Çalışma sonucunda elde edilen bulguların *Y. ruckeri* için teşhis kiti ve/veya aşı geliştirme çalışmalarına temel teşkil edeceği düşünülmektedir.

Anahtar sözcükler: *Oncorhynchus mykiss, Yersinia ruckeri, API 20 E, Mikroaglütinasyon, RAPD PCR*

İletişim (Correspondence)
+90 224 2941264
saltun@uludag.edu.tr

INTRODUCTION

Yersinia ruckeri is the ethiological agent of the diseases known as yersiniosis or "enteric red mouth" which causes significant economical losses in fish production [1]. Although the disease is detected on salmonid fish in particular, it also effects other fish species [2]. The disease is now widely distributed and it has been reported in North America, Australia, South Africa and European countries [1]. In Turkey, *Y. ruckeri* was first isolated from a rainbow trout farming facility in İzmir in 1990 by Cagirgan and Yurekliturk [3].

Although there are serological differences between *Y. ruckeri* strains, it is mentioned that they have very similar serotypes with regards to biochemical characteristics [4-6]. However, it is reported that differences may be detected on methyl red (MR), voges proskauer (VP), lysine decarboxylase, arginine dihydrolase and lactose fermentation tests results in some strains [7]. It is specified that the ability to fermentate sorbitol can vary among *Y. ruckeri* strains and serotype 2 strains can be differed from other strains by the ability to fermentate sorbitol [1,2].

Conventional tests, API 20 E test kit and selective medium are used for identification and to determine the phenotypic characteristics of *Y. ruckeri* strains [5,6,8]. Nowadays, rapid and easy diagnosis of *Y. ruckeri* can be performed by Polymerase Chain Reaction (PCR) and this method is used as alternative to traditional identification methods [9,10]. The RAPD-PCR method which is a DNA based technique are used frequently to determine differences among the strains due to high discriminatory power, reproducibility, and low cost relatively [11-13].

In this study, it were determined the phenotypic and serologic properties of *Y. ruckeri* isolates isolated from rainbow trout in different geographical regions of Turkey and genetic diversity among the isolates by RAPD-PCR. Efficiency of the technique was also evaluated for epidemiological studies.

MATERIAL and METHODS

Bacterial Strains and Phenotypic Characterizations

Fifteen *Y. ruckeri* isolates obtained from different locations in Turkey and 2 reference strains belonging to serotype 1 (Genk 3281) and serotype 2 (Denmark 1850621-1116B) were used in this study. Origins and sources of the strains used in the study were presented in *Table 1*.

Isolates were identified as *Y. ruckeri* by gram staining, oxidation-fermentation (O/F), Simmons citrate, gelatin hydrolysis, cytochrome oxidase, catalase, growth in 1%, 3% and 7% NaCl, motility at 22°C and 37°C, indole, MR, VP, acid production from sorbitol, maltose, glucose [5,8]. Additionally, tween 80 hydrolysis ability was determined by examining the colony morphology on Shotts-Waltman Agar (SWA) [14].

Table 1. *Yersinia ruckeri strains in the study*
Tablo 1. *Çalışmada kullanılan Y. ruckeri suşları*

Strain No	Origin	Source	Isolation Year
1	Isparta	Rainbow Trout	2009
2	Isparta	Rainbow Trout	2009
3	Denizli	Rainbow Trout	2009
4	Afyon	Rainbow Trout	2009
5	Sakarya	Rainbow Trout	2010
6	Bursa	Rainbow Trout	2010
7	Bilecik	Rainbow Trout	2010
8	Kütahya	Rainbow Trout	2010
9	Denmark 1850621-1116B	Rainbow Trout	-
10	Muğla	Rainbow Trout	2010
11	Muğla	Rainbow Trout	2010
12	Bursa	Rainbow Trout	2010
13	Kütahya	Rainbow Trout	2010
14	Muğla	Rainbow Trout	2010
15	Kütahya	Rainbow Trout	2010
16	Bilecik	Rainbow Trout	2010
17	Genk 3281 (Belgium)	Rainbow Trout	-

Isolates were inoculated on strips according to API 20 E (Biomerieux) rapid diagnostic kit directory and incubated at 26°C for 24 h and identification was performed on API WEB.

PCR

Y. ruckeri isolates identified conventionally were confirmed by PCR. For this purpose, DNA was extracted with a commercial kit (Omega Bio-tek, Inc.) according to the manufacturer's instructions. DNA concentrations were measured on 260 nm and 280 nm and equalized to 50 ng/µl.

PCR was performed by using ruck1 (5'-CAGCGGAAAGTA GCTTG-3') and ruck2 (5'-TGTTCAGTGCTATTAACACTTAA-3') primers according to the method described by Lejune and Rurangirwa [10]. After amplification, 409 bp PCR products were identified as *Y. ruckeri*.

Genotyping

RAPD method was used for genotyping of the isolates. For this purpose, ERIC-2 primer (5'-AAGTAAGTGACTGGGGT GAGCG-3') was used. Amplification step was performed by modifying the method reported by Versalovic et al.[15]. At this stage, 25 µl RAPD master mixture including DEPC-treated water, 1XPCR Buffer, 2.5 mM $MgCl_2$, 200 µM each dNTP, 2.5U Taq DNA polymerase, 25 pmol primer and 5 µl DNA was prepared. After initial denaturation of DNA at 95°C for 1 min, 25 amplificates cycles compose denaturation at 94°C for 1 min, annealing at 40°C for 1 min and from extension at 72°C for 1 min were followed. Amplification products were visualised by electrophoresis in 1.5% agarose gel including ethidium bromyde (2 mg/ml).

The phylogenetic relatedness of isolates was evaluated with dendogram of RAPD patterns. The dendogram was drawn by UPGMA (Unweighted Pair Group Method with Arithmetic Averages) with CHEF-DR® III, Quantity One® software (Bio-Rad Laboratories, Hercules, CA). To determine reproducibility of RAPD analysis, 5 strains from each bacteria species were selected randomly and RAPD analysis were repeated for 3 times subsequently.

Serological Characterization

- *Preparation of Immunsera:* In this study, 3 month-old, 8 healty New Zelland rabbits (2 positive and 2 negative controls for each serotype) taken from Uludag University Experimental Animals Breeding and Research Center were used. *Y. ruckeri* reference strains Genk 3281 (*Y. ruckeri* serotype 1) and Denmark 1850621-1116B (*Y. ruckeri* serotype 2) were inoculated into Triptic soy broth, incubated at 22°C for 48 h, centrifuged and inactivated by addition of formol (final concentration 0.3%). The suspension was kept for 2 h at room temperature and subsequently over-night at 4°C. Thereafter, the bacteria were washed 3 times in phosphate buffer saline (PBS) and the bacterial concentration was calibrated to McFarland no: 7 [16]. Prepared inoculums were inoculated subcutaneously to each test groups animals on 1st, 5th, 9th and 13th day in the quantity of 0.2, 0.4, 0.8 and 1.0 ml respectively. For negative control group, the same amount of steril PBS was inoculated on the same intervals. On the 15th day of the last inoculation, blood samples (20 ml) were collected from ear veins of rabbits and the sera preparated and aliquoted before freezing at -20°C. Microplate agglutination test was applied to detect antibodies [17,18].

- *Preparation of Antigen:* To prepare the antigen; before the isolates were incubated at 22°C for 48 h and collected in Phosphate Buffer Solution (PBS) then autoclaved at 100°C for 30 min to obtain the heat-stable O antigen. After, bacterial cultures were centrifuged at 5.000 rpm for 15 min. The sediment was removed and diluted with PBS to have suspension (antigen) at Optic Density (OD) of 0.65 at 525 nm wavelength on the spectrophotometer. This antigen was stored at -20°C until used for slide and microplate agglutination tests as somatic O antigen [19,20].

- *Microplate Agglutination Test: Y. ruckeri* isolates were serotyped by microplate agglutination test. For this purpose, an antiserum diluted with PBS on 2:1 proportion (50 ml PBS:25 ml antiserum) was put to wells of microplate. Then, antigen solution was added into these wells (25 µl) and mixed well. They were left at room temperature for 2 h and then at 4°C for overnight [21-23]. Positive and negative sera were put into the last and penultimate wells of each microplate for test control. Test results and agglutination titers were assessed according to $\log_2$ base [21,23,24].

Antimicrobial Susceptibility Test

All the isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute (CLSI). The following antibiotic discs were used: neomycin (10 µg, Oxoid), gentamicin (120 µg Oxoid), oxytetracycline (30 µg Oxoid), florfenicol (30 µg Oxoid), erythromycin (15 µg, Oxoid), sulfamethoxazole+trimethoprim (25 µg, Oxoid), doxycycline (30 µg, Oxoid), lincomycin (2 µg, Oxoid) and amoxicillin (25 µg, Oxoid). At 24th and 48th h of the incubation, incubation zone diameters were measured and evaluated [25].

RESULTS

Phenotypic Characteristics of Y. ruckeri Strains

Conventional microbiological tests and API 20 E test kits were used to determine phenotypic characteristics of *Y. ruckeri* isolates and results were presented in *Table 2* and *3*. It was observed that *Y. ruckeri* strains has created green colonies on SWA and a zone with an ice glass appearance due to precipitation of calcium salts and hydrolysis of tween 80 around these colonies.

By API 20 E and conventional tests, phenotypic characteristics of *Y. ruckeri* isolates were found significantly similar. It was determined that 5, 6, 8, 9 and 10 numbered isolates were positive for sorbitol test (both on API 20 E and conventional tests).

PCR was used for molecular confirmation of *Y. ruckeri* isolates. All of isolates and reference strains gave the final PCR product of 409 bp *(Fig. 1)*.

Genotyping

By RAPD analysis, *Y. ruckeri* isolates gave 5 different patterns. Isolates were grouped within 2 separate clusters according to 70% similarity index and it was detected that first cluster includes 2 genotypes (YR1 and YR2) and second cluster includes 3 genotypes (YR3, YR4 and YR5). Genotype YR2 was predominant and included 5 isolates (29.4%); and each of the genotypes YR3, YR4 and YR5 composed of 3 isolates. Furthermore, it was detected that isolates within genotypes YR4 and YR5 were closely related species.

Serological Characterization

It was detected that isolates 5, 6, 8, 9 and 10 numbered isolates have the characteristics of serotype 2 by microplate agglutination tests and other isolates have the characteristics of serotype 1. It was observed that antigenic structures of 15 isolates and two reference strains were not shown cross reaction against to serotype 1 and serotype 2 immune serums. Furthermore, 1:256 dilution of antigens prepared from isolates either serotype 1 or serotype 2 reacted with specific immunsera.

Antimicrobial Susceptibility Profiles

Seventeen *Y. ruckeri* isolate were examined for antibiotic sensitivity against to 9 different antibiotics and results were presented in *Table 4*. It was determined that all *Y. ruckeri*

Table 2. *Phenotypic characteristic of Y. ruckeri isolates with conventional microbiological tests*
Tablo 2. *Y. ruckeri izolatlarının konvansiyonel mikrobiyolojik testler kullanılarak belirlenen fenotipik özellikleri*

Phenotypic Characters	Isolates No																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Gram Staining	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Simons Citrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
O/F	F*	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F	F
Growth in 3% NaCl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in 7% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 30°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility at 22°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Motility at 37°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SWA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid from																	
Mannitol	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

** **F:** fermentative*

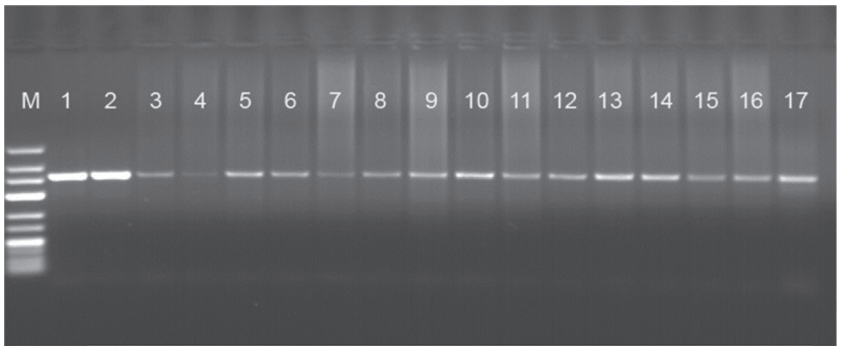

Fig 1. *Y. ruckeri* specific PCR, 409 bp. **M;** Molecular weight standard (25-700 bp), 9 (Denmark 1850621-1116B) and 17 (Genk 3281 [Belgium]) reference strains, others are sample strains

Şekil 1. *Y. ruckeri* spesifik PCR, 409 bp. M; Moleküler ağırlık standardı (25-700 bp), 9 (Denmark 1850621-1116B) ve 17 (Genk 3281 [Belgium]) referans suşlar, diğerleri saha suşları

isolates were resistant to neomycin, lincomycin, amoxicillin (except isolate 11) and erythromycin, 5 (33.33%) isolates to gentamicin and doxycyclin, 2 (13.33%) isolates to oxytetracycline, 3 (20%) isolates to florfenicol, and 1 (6.66%) isolate to sulphamethoxazole-trimetoprim.

DISCUSSION

In this study, it has shown that *Y. ruckeri* have serologic differences among isolates and such these strains are found very similar in terms of phenotypes [2,5,8,26].

Many typing strategies have been performed until today based on the phenotypic characteristics of *Y. ruckeri* strains. *Y. ruckeri* strains have been divided into two biotypes according to sorbitol fermentation ability [1], and five biotypes according to heat-stable O antigen [27].

In this study, no difference was detected between the strains in terms of gram staining, O/F, cytochrome oxidase, catalase, H_2S production, VP, indol production, glucose, inositol,

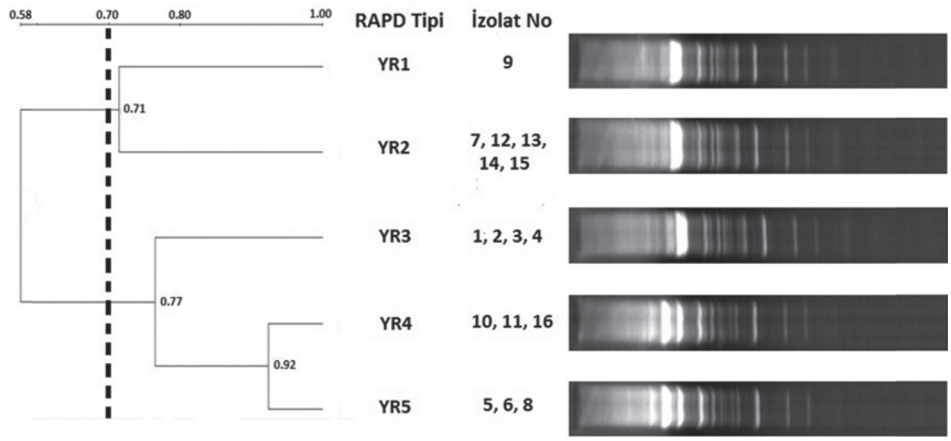

Fig 2. The RAPD band profile of *Y. ruckeri* isolates and phylogenetic tree of genotypes

Şekil 2. *Y. ruckeri* isolatlarının RAPD bant profilleri ve genotiplere ait filogenetik ağaç

Table 3. *Phenotypic properties of Y. ruckeri isolates with API 20E tests*
Tablo 3. *Y. ruckeri izolatlarının API 20E kullanılarak belirlenen fenotipik özellikleri*

Phenotypic Characters	Isolates No																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
ONGP	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
LDC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ODC	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Urease production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TDA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatin Hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Glucose*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inositol*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sorbitol*	-	-	-	-	+	+	-	+	+	+	-	-	-	-	-	-	-
Rhamnose*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melibiose*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amygdalin*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sitokrom Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

ONPG: *b - Galaktosidaz,* ***ADH:*** *Arginine dihydrolase,* ***LDC:*** *Lysine decarboxylase,* ***ODC:*** *Ornithine decarboxylase,* ***TDA:*** *Tryptophan deaminase,* ***VP:*** *Voges proskauer reaction, * acid from*

maltose, mannitol, citrate utilization, gelatin hydrolysis, growth in 1%, 3% and 7% of NaCl, motility at 22°C and 37°C and pigment production. Although there were differences among the isolates on sorbitol fermentation test, a homogenous structure was seen between all studied *Y. ruckeri* isolates. These results are found similar *Y. ruckeri* isolates isolated from Turkey [6,28,29].

Some *Y. ruckeri* strains could not hydrolyse tween 80 [4,26,28]. Davies and Frerichs [26] announced that nonmotile *Y. ruckeri* strains cannot hydrolyse tween 80 and Bush [4] reported that there were no relation between motility and tween 80 hydrolyse. Çağırgan and Tanrıkul [28] provided evidence that all *Y. ruckeri* strains that he studies with were motile, however some of these strains could not hydrolyse tween 80. In our study, all 15 *Y. ruckeri* strains were motile and able to hydrolyse tween 80.

API 20 E test kits are one of the most common methods used to determine phenotypic characteristics of bacterial

Table 4. *Antibiotic susceptibility profiles of Y. ruckeri isolates*
Tablo 4. *Y. ruckeri izolatlarının antibiyotik duyarlılık profilleri*

Antibiotic Disc	Isolates No																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Gentamicin	I	R	I	I	R	R	R	R	I	I	I	I	I	I	I	I	I
Neomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Lincomycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Oxytetracycline	I	R	I	I	R	I	I	I	I	I	I	I	I	I	I	I	I
Amoxicillin	R	R	R	R	R	R	R	R	R	R	I	R	R	R	R	R	R
Florfenicol	R	I	R	I	R	I	I	I	I	I	I	I	S	I	I	I	I
Erythromycin	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Doxycycline	R	R	R	I	I	I	I	I	R	I	I	I	R	I	I	I	I
Sulfamethoxazole-Trimethoprim	S	I	S	I	I	R	S	I	I	I	I	S	I	S	S	I	S

S: *Sensitive,* ***I:*** *Intermediate,* ***R:*** *Resistance*

agents. The researchers reported that *Y. ruckeri* strains might be shown variety in terms of arginin dihydrolase production, gelatin hydrolysis, VP and sorbitol fermentation due to incubation periods (24, 48, 72 h) 20°C, 22°C and 25°C by API 20 E test kits [20,26,29,30]. Also, many researchers mentioned that there was a risk to confuse with *Hafnia alvei* for diagnosis with API 20 E [5,8,20,26,29-31]. While no difference was observed among *Y. ruckeri* strains for arginin dihydrolase test, mannitol fermentation, citrate utilization, gelatin hydrolysis and VP as a result of incubation at 26°C for 24 h in our study, the differences were determined in sorbitol tests. Differences in sorbitol fermentation were detected in only serotype 2 isolates. Candan and Yazıcı [29] have reported isolates negative gelatin hydrolysis (2 isolates), positive VP (9 isolates), negative citrate utilization (2 isolates) after an incubation at 22°C for 48 h in their research. They have studied in 15 *Y. ruckeri* strains to determine the most suitable temperature and time for diagnosis of *Y. ruckeri* by using API 20 E test. Observation of differences in gelatin hydrolysis tests differently from conventional microbiological tests in our research results related with API 20 E test complies with the results of other researchers who had studied on this subject [29,31-33].

Although it has been reported that *Y. ruckeri* might be confused with *H. alvei* in identifications by using API 20 E test, it was observed that while *Y. ruckeri* was positive β-galactosidase, gelatin hydrolysis and negative rhamnose, arabinose at 37°C, *H. alvei* was negative β-galactosidase, gelatin hydrolysis and positive rhamnose, arabinose results at 37°C [5,20,34]. Furthermore, *Y. ruckeri* may be differed from other bacteria easily by help of specific colony morphologies on SWA [6].

Serotypic scheme of *Y. ruckeri* has continuously modified by introduction of new antisera [35,36] or creation of new antigenic schemes [7,20,27,37] since first occurrence of yersiniosis. All these studies revealed that *Y. ruckeri* presented a complex structure for serology and antigenic characteristics. When serotyping of 15 *Y. ruckeri* strains in our country was performed by using microplate agglutination technique, it was detected that isolates 5, 6, 8, 10 and reference strain 9 presented serotype 2 and other ten strains presented serotype 1 characteristics. During agglutination test, "O" antigen, serotype 1 and serotype 2 hyperimmune (rabbit) sera were used. It was reported in studies performed with European isolates that *Y. ruckeri* strains have presented serotype O1 characteristics in common [6,20,26,36].

Diagnosis of *Y. ruckeri* has been performed successfully since 1992 by PCR [38]. Besides, PCR based genotyping methods such as PCR-RFLP ribotyping, REP-PCR, ERIC-PCR, AP-PCR and DNA amplification fingerprint has played important role on bacterial typing scheme for the last twenty years [39]. However, these techniques have been used very rarely for fish pathogens in compared with human and animal pathogens [40]. Plasmid profile analysis, ribotyping [41,42], PFGE [40], PCR based fingerprinting [40,43,44] have been used successfully as alternative to phenotypic techniques of *Y. ruckeri* strains. RAPD typing is one of the techniques where single oligonucleotides selected optionally [45]. ERIC2 and M13 primers are used featly as universal primers for typing various bacteria [46]. RAPD-PCR is advantageous since molecular preliminary information of the species is not necessary and clonal relatedness may be determined rapidly and cheaply. In this study, ERIC2 primer was used in RAPD-PCR analysis of *Y. ruckeri* isolates [15,40,47-49]. In this study, *Y. ruckeri* isolates were classified within 5 groups according to 70% similarity index by RAPD typing with using ERIC 2 primer. It was determined that *Y. ruckeri* strains grouped within YR1 and YR 2 have presented a 71% similarity among themselves and strains grouped within YR 3, YR 4 and YR 5 have presented 77% similarity among themselves. Onuk et al.[12] have grouped *Y. ruckeri* strains within 6 groups by RAPD method and reported that strains grouped as RE1 and RE2 have presented 83% genetic similarity and RAPD types that were specified within the same cluster except RE1 and RE2 have presented a genetic similarity between 27% and 53%. Same researchers have reported that strains which cause

epidemics within Central Anatolia and Black Sea regions were dominant types and isolates within RA, RB, RC and RC groups isolated from Mediterranean and Aegean regions were significantly different from isolates within Black Sea and Central Anatolia regions. In our research, in line with Onuk et al.[12], it was observed that *Y. ruckeri* isolates typed with RAPD were distributed among regions. This situation shows that there might be fish transfer among fish farms.

Y. ruckeri strains were classified by performing an antibiotyping according to their resistance to antibiotic [12,50]. Determination of antibiotic resistance profiles of bacteria can provide important information to take specific control preventions. De Grandis and Stevenson [50] have determined antibiotic susceptibilities of 50 *Y. ruckeri* strains and reported that all these strains have presented uniform susceptibility profile against many antibiotics. Onuk et al.[12] reported that 97 *Y. ruckeri* strains had presented similar antibiotic susceptibility profile against oxytetracyclin and trimethoprim while weak resistance to erythromycin and De Grandis and Stevenson [50] have reported that *Y. ruckeri* had presented an acquired resistance against oxytetracycline and sulphanomide although it is resistant to many antibiotics. Kırkan et al.[51] reported that 17 *Y. ruckeri* isolates were resistant to tetracycline with 30% and 40% by agar dilution and E- test, respectively. Furthermore, Kırkan et al.[52] found that all 8 *Y. ruckeri* strains were resistant to erythromycin and ampicillin. While in the present study all 17 *Y. ruckeri* isolates were resistant to neomycin, lincomycin and erythromycin, 16 strains were resistant to amoxicillin, 5 strains were resistant to gentamicin, 3 strains were resistant to florfenicol and doxycyclin, 2 strains were resistant to oxytetracycline and 1 strain was resistant to trimetoprim-sulphamethoxazole. Florfenicol, erythromycin, oxytetracycline and sulphamethoxazole-trimetoprim are antibiotics which have been licensed for bacterial fish diseases and used most frequently in Turkey. According to this study, it may be concluded that a resistance against oxytetracycline, florfenicole and sulphamethoxazole-trimetoprim has developed or is being developed (because of susceptibility of strains intermediately according to antibiogram results) among *Y. ruckeri* isolates.

Consequently, it was found that phenotypic characteristics of *Y. ruckeri* strains were very similar both of conventional microbiological tests and API 20 E rapid diagnosis kit and SWA agar has provided successful results for identification of *Y. ruckeri* strains and control of purity of the colonies, in this study. It was determined that 4 isolates have serotype 2 characteristics similarly with reference 9 (serotype 2) and other strains have presented a serotype 1 characteristics as a result of serotyping of *Y. ruckeri* strains with microplate agglutination test by using reference serotype 1 and serotype 2 strains. It was detected that *Y. ruckeri* has developed resistance to oxytetracycline, florfenicol and sulphamethoxazole-trimethoprim which have been used to treat bacterial diseases in Turkey (licensed for fish). RAPD analysis was discriminative for determination of genetic diversity among *Y. ruckeri* isolates and has provided rapid and safe results. This result showed that RAPD analysis is a useful epidemiological method for determination of genetic differences on fish pathogens. We consider that results obtained in this study can be useful for preparation of effective vaccination formulations against yersiniosis and for further studies that will be performed related with this subject.

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