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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Current Status of Antimicrobial Resistance in the Major Bacterial Pathogens of Rainbow Trout

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Abstract: In this study, the antimicrobial resistance of three important bacterial fish pathogens (*Lactococcus garvieae*, *Vibrio anguillarum,* and *Yersinia ruckeri*) was determined to antibiotics used mainly in rainbow trout farms.The Kirby-Bauer disc diffusion technique was used for determination of antibiotic susceptibility of each pathogen. The minimum inhibitory concentration (MIC) values were determined with E-test strips which provide rapid and quantitative results for selected chemotherapeutics. *L.garvieae* strains showed resistance to most of the selected antimicrobial agents, although *V.anguillarum* and *Y.ruckeri* strains were determined resistant to clindamycin, ampicillin, and penicillin G. The treatable and untreatable results for each antibiotics were determined and the results are pioneer for detecting effective antibiotic doses by using E-test strips for fish pathogens. The results of this study will be representative of the effective treatment and preventive misuse of antibiotics in the aquaculture industry.

Keywords: Antibiogram, E-test, MIC, *Lactococcus garvieae*, *Vibrio anguillarum*, *Yersinia ruckeri*.

Gökkuşağı Alabalığında Görülen Başlıca Bakteriyel Patojenlerin Mevcut Antimikrobiyal Durumu

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Öz: Bu çalışmada, önemli üç bakteriyel balık patojeninin (*Lactococcus garvieae, Vibrio anguillarum, Yersinia ruckeri*) gökkuşağı alabalığı çiftliklerinde yaygın olarak kullanılan antibiyotiklere karşı oluşturdukları antimikrobiyal direnç belirlenmiştir. Her bir patojenin antibiyotik duyarlılığının belirlenmesi için Kirby-Bauer disk difüzyon tekniği kullanıldı. Seçilen kemoterapötikler için hızlı ve kantitatif sonuçlar sağlayan E-test şeritleri ile minimum inhibitör konsantrasyon (MIC) değerleri belirlendi. *L. garvieae* suşlarının birçok antimikrobiyel ajana karşı direnç oluşturduğu, *V. anguillarum* ve *Y. ruckeri* suşlarının ise klindamisin, ampisilin ve penisilin G'ye karşı dirençli olduğu tespit edilmiştir. Her antibiyotik için tedavi edilebilir ve tedavi edilemez sonuçlar belirlendi ve sonuçlar, balık patojenleri için E-test şeritleri kullanılarak etkili antibiyotik dozlarının saptanmasında öncü oldu. Bu çalışmadan elde edilen bulgular su ürünleri yetiştiriciliğinde etkili tedavi ve yanlış antibiyotik kullanımının önlenmesi için örnek oluşturacaktır.

Anahtar kelimeler: Antibiyogram, E-testi, MİK, *Lactococcus garvieae*, *Vibrio anguillarum*, *Yersinia ruckeri*.

INTRODUCTION

Antibiotic use has been the major treatment of bacterial infectious diseases in the twentieth century, and antimicrobial resistance has been increasing due to the excessive use of chemotherapeutics (Igbinosa, 2014; Sharma et al., 2017). Antibiotic resistance is developed by encoding the resistance genes which imitate the inhibitory effect of the antibiotics (Blair et al., 2015; Khan et al., 2019). It is important to determine whether an antibiotic is susceptible or not to prevent multidrug resistance, which could present the causative agent with harsher symptoms than its predecessors and prevent its rapid diagnosis (Khan et al., 2019). Accurate and rapid antimicrobial tests have been more important in practicing appropriate prophylaxis and antimicrobial chemotherapy (Hughes et al., 1993). Furthermore, the misuse of antibiotics causes the development of the resistance mechanism and limits the effects of antibiotics. Recent studies have shown that more than 70% of infected bacteria have developed resistance to at least one of the generally used antibiotics (Mathew et al., 2007; Allerberger & Mittermayer, 2008; Jilani et al., 2008; Shahriar et al., 2019).

Aquaculture production in the EU has been predicted to be around 1.3 million tonnes in 2015 (EUROSTAT, 2019) and Turkey is one of the notable producers of farmed fish among European countries. Rainbow trout (*Oncorhynchus mykiss*) is the second most produced species in the country (TUIK, 2020), in addition, Aegean region, especially the Muğla province, has the highest production amount for farmed fish (rainbow trout, sea bass, and sea bream) considering other regions (Balcı-Akova, 2015). Recent developments in the amount of production have led to challenges with different fish diseases, especially those caused by bacterial pathogens. The main disease-causing pathogens of rainbow trout were established as *Vibrio anguillarum*, *Yersinia ruckeri*, *Lactococcus garvieae*, *Flavobacterium columnaris*, *Aeromonas hydrophila*, *Pseudomonas fluorescens*, and *Flavobacterium psychrophilum* (Toranzo, 2004).

Along with the development of the aquaculture industry and the prevalence of different bacterial diseases, the continuous and occasional use of antibiotics has been recognized as a serious concern for leading mutagenic and multidrug resistant pathogens that can cause infectious diseases (Vignesh et al., 2011). Antibiotics used in both fish and human infections reduce the efficiency of antibiotics over time and resistance limits disease treatment (Kathleen et al., 2016). Furthermore, antimicrobial resistance in aquaculture leads to global spread through international trade and maritime transport (Lulijwa et al., 2020).

In this study, the main bacterial fish pathogens that were isolated from different rainbow trout farms located in the Southern Aegean Region of Turkey were tested with different antimicrobial agents to determine antibiotic resistance and appropriate antimicrobial chemotherapy. However, E-test results in the review in detail the available information on the MIC values of selected antibiotics for each bacterial pathogen. It is important to determine the relevant antibiotic treatment and the effective dose to reduce antibiotic resistance and provide safe production for the environment and human consumption.

MATERIAL AND METHOD

The antibiotic susceptibility of bacterial fish pathogens was utilized by using the Kirby-Bauer disc diffusion technique under aseptic conditions (Figure 1) (Stokes et al., 1993). The total 12 identified bacterial strains (*Lactococcus garvieae*, *Vibrio anguillarum*, *Yersinia ruckeri*) were obtained from Izmir Katip Celebi University Faculty of Fisheries Fish Disease and Biotechnology Laboratory culture collection. ALG4 (MT875309), GLG1 (MT876413), ILG1 (MT875319) and A3LG22 (OP420800) represent *L. garvieae*, AVA1 (MT875321), BVA1 (MT876113), EVA1 (MT876427) and C5VA22 (OP422224) represent *V. anguillarum*, AYR1 (MT876430), BYR1 (MT876477), FYR1 (MT876522) and B3YR22 (OP420869) represent *Y.ruckeri* strains in this study. Fresh bacteria suspensions were prepared in tubes containing Tyriptic Soy Broth (TSB, Merck, Germany) then incubated until the bacteria suspension was equivalent to the McFarland 0.5 standard (approximately 1.5x 10^{8} CFU/mL) and then spread with a sterile glass baguette on Mueller Hinton Agar (MHA, Merck, Germany). Twenty different antimicrobial susceptibility discs (Oxoid, UK) were placed on the agar with sterile forceps and incubated at 21 ºC for 48 hours (Table 1). The diameters of the inhibition zone were measured in millimeters and compared with the interpretive criteria of the Clinical and Laboratory Standards Institue. To obtain statistically reliable data and minimize the margin of error, each antibiogram test was redoubled three times to take the arithmetic mean of the values.

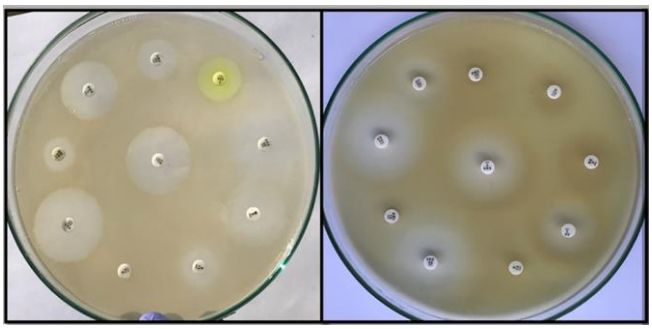

Figure 1. Determination of the susceptibility of antibiotics to bacterial fish pathogens by the Kirby-Bauer disc diffusion technique.

Table 1. The selected antimicrobial susceptibility test discs, their codes, and concentrations.

Antimicrobial material	Code	Concentration
Trimethoprim/Sulfamethoxazole 1:19	SXT	25 µg
Oxytetracycline	OT	30 µg
Fosfomycin	FOS	50 µg
Streptomycin	S	10 µg
Enrofloxacin	ENR	5 µg
Amoxicillin/Clavulanic Acid	AMC	30 µg
Florfenicol	FFC	30 µg
Amoxicillin	AML	10 µg
Clindamycin	DA	2 µg
Flumequine	UB	30 µg
Doxycycline	DO	30 µg
Ampicillin	AMP	10 µg
Erythromycin	E	15 µg
Trimethoprim	W	5 µg
Oxolinic Acid	OA	2 µg
Polymyxin B	PB	300U
Chloramphenicol	C	30 µg
Penicillin G	P	10U
Kanamycin	K	50 µg
Nitrofurantoin	F	300 µg

The minimum inhibitory concentration (MIC) values of the identified bacteria were determined by performing E-test (BioMerieux, France) (Figure 2). The E-test strips were chosen according to the susceptibility of the bacteria stated in EUCAST and Austin and Austin, (2007) (Table 2). Before performing the tests, E-test strips were taken from -20 ºC and kept at room temperature for 30 minutes. The surface with the MIC gaps was placed on top and the antibiotic-impregnated back of the strip was placed crosswise with sterile forceps to contact the MHA. The E-test strips were evaluated by measuring ellipsoid inhibition zones after incubation at 21ºC for 48 hours (Table 2). The minimum inhibitory concentration values determined where antibiotic agents no longer inhibited the growth of bacteria on the epsilometer strip.

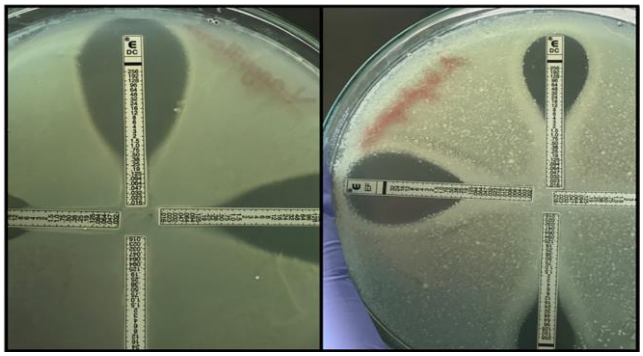

Figure 2. Determination of the minimum inhibitory concentration (MIC) of bacterial fish pathogens with E-test strips.

Table 2. The selected E-test strips, their codes, and MIC values.

Antimicrobial material	Code	MIC values (µg/ml)
Amoxicillin	AC	0.016-256
Ampicillin*Sulb 2/1	AB	0.016-256
Benzylpenicillin	PG	0.016-256
Clindamycin	CM	0.016-256
Doxycycline	DC	0.016-256
Enrofloxacin	EF	0.002-32
Moxifloxacin	MX	0.002-32
Nitrofurantoin	NI	0.032-512
Oxacillin	OX	0.016-256
Tetracycline	TC	0.016-256
Trimethoprim/sulfamethoxazole	TS	0.002-32

RESULTS

The microbial sensitivity was determined by the Kirby-Bauer disc diffusion technique with trimethoprim/sulfamethoxazole, oxytetracycline, fosfomycin, streptomycin, enrofloxacin, amoxicillin/clavulanic acid, florfenicol, amoxicillin, clindamycin, flumequine, doxycycline, ampicillin, erythromycin, trimethoprim, oxolinic acid, polymyxin B, chloramphenicol, penicillin G, kanamycin and nitrofurantoin against strains of *L. garvieae*, *V. anguillarum* and *Y. ruckeri* isolated from rainbow trout farms. The antimicrobial sensitivity and resistance percents of important bacterial fish pathogens (*L. garvieae*, *V. anguillarum*, and *Y. ruckeri*) were presented to clarify the antibiotic use (Figure 3; Table 3). *L. garvieae* strains showed resistance to most of the antibiotics used, however *V. anguillarum* and *Y. ruckeri* strains were determined 100% resistant to clindamycin and penicillin G (Figure 3). All *V. anguillarum* and *Y. ruckeri* strains were sensitive to trimethoprim/sulfamethoxazole, florfenicol, trimethoprim and chloramphenicol (Table 3).

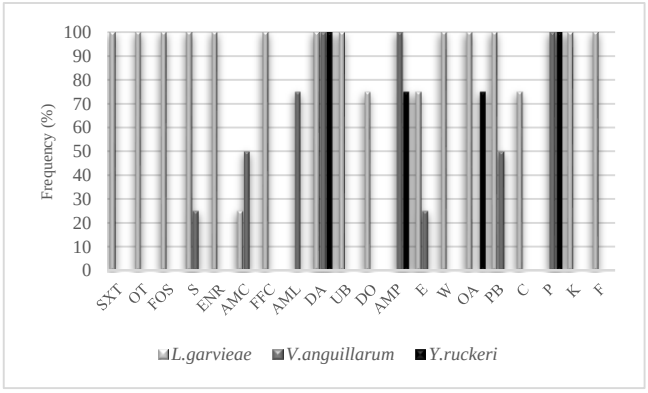

Figure 3. The resistance percents of bacterial fish pathogens towards different antibiotics.

All the analysed *L. garvieae* strains determined resistance to various antibiotics such as trimethoprim/sulfamethoxazole, oxytetracycline, fosfomycin, streptomycin, florfenicol, clindamycin, flumequine, erythromycin, trimethoprim, oxolinic acid, polymyxin B, kanamycin, and nitrofurantoin. The MIC values of tetracycline, amoxicillin, ampicillin, benzylpenicillin, moxifloxacin, and doxycycline against strains of *L. garvieae* are presented in Table 4. The minimum inhibitory concentration of amoxicillin, ampicillin, benzylpenicillin, doxycycline, moxifloxacin and nitrofurantoin were detected 0.25, 0.25, 0.75, 0.25, 0.125, 12, and 0.75 µg/mL, respectively.

The antimicrobial susceptibility of *V. anguillarum* strains showed 100% resistance to clindamycin, ampicillin, and penicillin G. Furthermore, the 100% sensitivity of isolates to trimethoprim/sulfamethoxazole, fosfomycin, florfenicol, flumequine, trimethoprim, and chloramphenicol was determined. The results of the E-test for amoxicillin, ampicillin, benzylpenicillin, clindamycin, doxycycline and tetracycline showed different MIC values between 0.25 and 32 µg/ml (Table 5).

Table 3. Antimicrobial susceptibility of bacterial fish pathogens**.**

	ALG4	GLG1	ILG1	A3LG22	AVA1	BVA1	EVA1	C5VA22	AYR1	BYR1	FYR1	B3YR22	
SXT	R	R	R	R	S	S	S	S	S	S	S	S	
OT	R	R	R	R	I	S	I	I	I	I	I	S	
FOS	R	R	R	R	S	S	S	S	S	S	S	I	
S	R	R	R	R	R	I	I	I	I	I	I	I	
ENR	R	R	R	R	S	I	S	S	S	S	S	S	
AMC	R	I	I	I	R	I	I	R	I	I	S	S	
FFC	R	R	R	R	S	S	S	S	S	S	S	S	
AML	I	I	I	I	R	R	I	R	I	I	I	S	
DA	R	R	R	R	R	R	R	R	R	R	R	R	
UB	R	R	R	R	S	S	S	S	I	I	S	S	
DO	R	I	R	R	S	I	I	I	I	I	I	I	
AMP	I	I	I	I	R	R	R	R	R	R	I		R
E	R	R	R	I	I	R	I	I	I	I	I	I	
W	R	R	R	R	S	S	S	S	S	S	S	S	
OA	R	R	R	R	S	I	S	S	R	R	R	I	
PB	R	R	R	R	R	R	I	I	I	I	I	I	
C	R	R	R	I	S	S	S	S	S	S	S	S	
P	I	I	I	I	R	R	R	R	R	R	R	R	
K	R	R	R	R	I	I	I	I	I	S	I	S	
F	R	R	R	R	S	S	I	S	S	S	S	S	

Resistant **Intermediate** **Sensitive**

*ALG4, GLG1, ILG1, A3LG22: *L. garvieae,* AVA1, BVA1, EVA1, C5VA22: *V. anguillarum,* AYR1, BYR1, FYR1, B3YR22: *Y. ruckeri*

The antibacterial resistance of *Y. ruckeri* was less determined than other bacterial pathogens studied in this current study. *Y. ruckeri* strains were found resistant to clindamycin, penicillin G, ampicillin, oxolinic acid, and sensitive to trimethoprim/sulfamethoxazole, enrofloxacin, florfenicol, chloramphenicol. The results of the E-test revealed the effective doses of the MIC values for amoxicillin, ampicillin, doxycycline, moxifloxacin and trimethoprim/sulfamethoxazole as 1.5, 0.5, 0.25, 0.094 and 0.012 µg/mL, respectively. (Table 6).

Table 4. Minimum inhibitory concentration (MIC) of different antibiotics against *L. garvieae* by E-test.

Code	MIC range (µg/mL)	ALG4 (µg/mL)	GLG1 (µg/mL)	ILG1 (µg/mL)	A3LG22 (µg/mL)
AC	**0.016-256**	0.25	0.50	1.0	0.75
AB	**0.016-256**	0.25	0.75	0.75	0.75
PG	**0.016-256**	0.75	0.75	2.0	0.75
DC	**0.016-256**	0.25	0.50	0.38	0.50
MX	**0.002-32**	0.50	0.125	0.25	0.38
NI	**0.032-512**	16	12	16	12
TC	**0.016-256**	0.75	0.75	1.5	1.0
TS	**0.002-32**	>32	>32	>32	>32

* ALG4, GLG1, ILG1, A3LG22: *L. garvieae*

Table. 5 Minimum inhibitory concentration (MIC) of different antibiotics against *V. anguillarum* by E-test.

Code	MIC range (µg/mL)	AVA1 (µg/mL)	BVA1 (µg/mL)	EVA1 (µg/mL)	B3YR22 (µg/mL)
AC	**0.016-256**	6	24	16	16
AB	**0.016-256**	8	32	12	24
PG	**0.016-256**	24	12	32	16
CM	**0.016-256**	1.0	4	2	8
DC	**0.016-256**	0.75	1.5	1.0	1.0
TC	**0.016-256**	0.50	0.75	0.38	0.75

*AVA1, BVA1, EVA1, C5VA22: *V. anguillarum*

Table 6. Minimum inhibitory concentration (MIC) of different antibiotics against *Y. ruckeri* by E-test.

Code	MIC range (µg/mL)	AYR1 (µg/mL)	BYR1 (µg/mL)	FYR1 (µg/mL)	C5VA22 (µg/mL)
AC	**0.016-256**	8	2	1.5	1.5
AB	**0.016-256**	4	0.50	3	2
DC	**0.016-256**	0.25	0.75	0.50	1.5
MX	**0.002-32**	0.25	0.19	0.094	0.50
OX	**0.016-256**	>256	128	64	192
TS	**0.002-32**	0.012	0.064	0.064	0.094

*AYR1, BYR1, FYR1, B3YR22: *Y. ruckeri*

The results of the E-test give the proper MIC values for the bacterial fish pathogens, and the frequencies are presented in Figure 4.

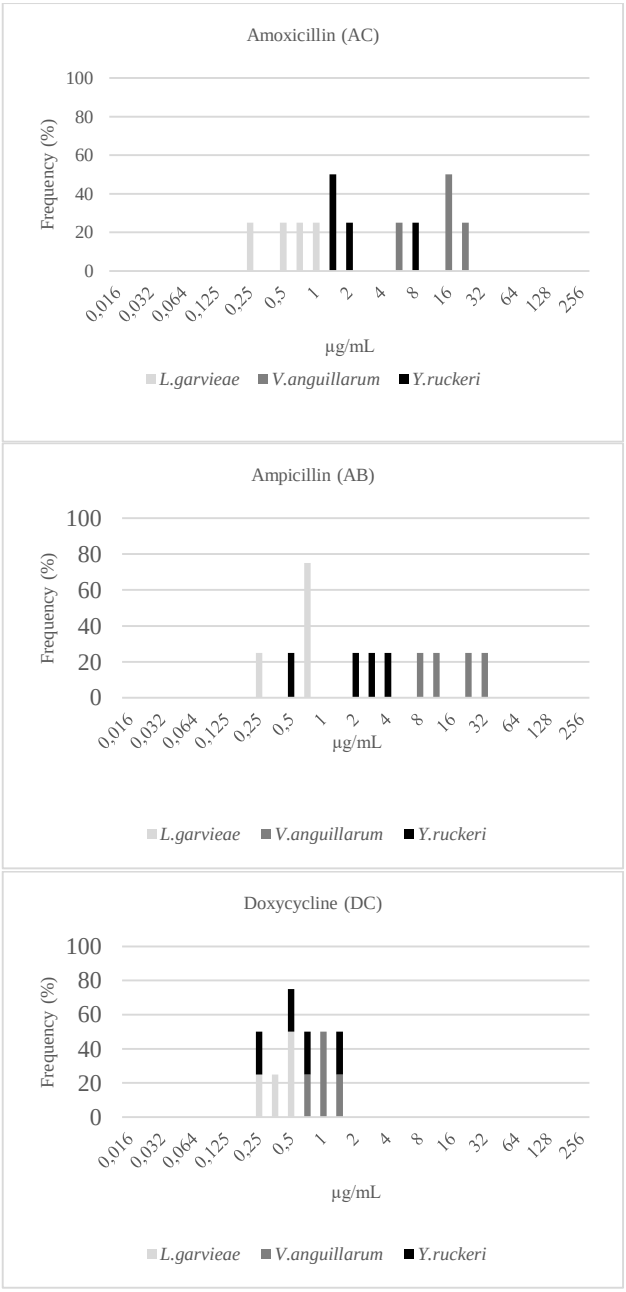

Figure 4. Frequency of *L. garvieae*, *V. anguillarum*, and *Y. ruckeri* MIC values.

DISCUSSION AND CONCLUSION

Nowadays, antibiotics are commonly used for the treatment of fish diseases in aquaculture by oral or bath treatment. However, high concentrations of antibiotic use can cause various physiological disorders such as kidney and liver damage, digestive system problems, and also environmental hazards (Terech-Majewska, 2016). Additionally, chemotherapeutics have a negative effect on immune system cells and the cellular and humoral defense mechanisms of fish (Lunden et al., 2002; Sieroslawska et al., 2004; Terech-Majewska & Siwicki, 2006; Kum & Sekkin, 2011; Terech-Majewska, 2016). It is of great importance to determine the antimicrobial resistance and the proper MIC values for effective treatment and environmentally friendly production.

In this study, the antimicrobial susceptibility of *L.garvieae* strains isolated from rainbow trout in the Southern Aegean region of Turkey showed resistance to most of the antibiotics used. Similarly, Raissy and Ansari, (2011) reported the susceptibility of 52 different strains of *L. garvieae* isolated from diseased rainbow trout, and determined that resistance to several antibiotics ranged between 25-100%. Kubilay et al., (2005) stated that nine different isolates of *L. garvieae* were found resistant to penicillin, enrofloxacin, kanamycin, clindamycin, flumequine, oxolinic acid, trimethoprim / sulfamethoxazole and streptomycin, as well as Diler et al. (2002) reported resistance of *L. garvieae* strains against clindamycin, penicillin, and ceftriaxone. Raissy and Moumeni, (2016) determined the microbial resistance of *L.garvieae* strains isolated from rainbow trout farms in western Iran to ampicillin (87.5%), erythromycin (37.5%) and tetracycline (79.1%). These results are attributed to the extensive use of antibiotics for Lactococcosis in rainbow trout farms, which results in the development of resistance against a wide range of antibiotics.

V. anguillarum strains were neither fully resistant nor sensitive to all antibiotics used. Furthermore, the MIC values of the results of the E-test ranged from 0.25 to 32 µg/ml. Pedersen et al., (1995) compared 520 *V. anguillarum* strains that were isolated from diseased fish and environmental sources and noted that all strains were sensitive to spectinomycin, neomycin, flumequine, and oxolinic acid. Balta and Dengiz-Balta, (2017) have demonstrated the antimicrobial sensitivity of strains of *V. anguillarum* that were isolated from the Eastern Blacksea region of Turkey and reported resistance to 100% sulfamethoxazole, 90.6% ampicillin, 71.9% erythromycin, 62.5% oxytetracycline and 46.9% streptomycin. In addition, enrofloxacin, florfenicol and oxolinic acid were determined sensitive and could be practicable for the treatment of the disease. Akaylı et al., (2013) noted that *V. anguillarum* strains were resistant to chloramphenicol, kanamycin, erythromycin sulfamethoxazole, and sensitive to flumequine, oxytetracycline florfenicol, ciprofloxacin, and furazolidone.

In this study, the antimicrobial susceptibility of *Y. ruckeri* strains was found to be more sensitive than other bacterial fish pathogens. Balta et al., (2016) compared 75 different strains of *Y. ruckeri* isolated from rainbow trout farms in the Eastern Black Sea Region and antimicrobial test results revealed that the strains were resistant to ampicillin (97.5%), oxytetracycline (62.0%) and streptomycin (22.3%). The antimicrobial resistance of the strains was associated with the lack of quarantine management and the fish transfer process of the farms. Akaylı et al., (2013) stated that *Y. ruckeri* strains isolated from rainbow trout were resistant to erythromycin and furazolidone. Despite this, the isolates were sensitive to flumequine, chloramphenicol, kanamycin, sulfamethoxazole, oxytetracycline, florfenicol, and ciprofloxacin. Orozova et al., (2014) point out that all *Y. ruckeri* strains isolated from rainbow trout farms in Bulgaria were determined to be sensitive to nalidixic acid, sulfamethoxazole / trimethoprim, and oxolinic acid. Furthermore, strains were found resistant to ampicillin and oxacillin. The authors noted that it is necessary to highlight the control of antibiotic use in the treatment of Yersiniosis.

The most commonly used antibiotic groups in aquaculture are aminoglycosides, quinolones, sulfonamides, and tetracycline, which are wide-spectrum antibiotics, and oxytetracycline (OTC) is one of the most important antibiotics that are effectively used against many microorganisms such as *Flexibacter* sp., *Yersinia* sp., *Aeromonas* sp., *Vibrio* sp. and *Edwardsiella* sp. (Ingram, 1980; Lunden & Bylund, 2000; Terech-Majewska & Siwicki, 2006; Wojtacka, 2007; Terech-Majewska, 2016). Lulijwa et al., (2020) reviewed current antibiotic use in aquaculture across 15 major producers and concluded that 67 antibiotic compounds were used in 11 of them, mostly oxytetracycline, florfenicol and sulfadiazine. According to recent reports, antibiotics used in aquaculture do not completely decompose and are excreted in urine or feces (Muziasari et al., 2014; Manage, 2018). The inappropriate use of antibiotics causes the development of resistance in pathogenic microorganisms, thus making difficulties for the treatment and the control of diseases (Kırhan et al., 2006; Austin & Austin, 2012; Roberts, 2012; Akaylı et al., 2013). The treatment of diseases caused by bacterial fish pathogens should be carried out by determining the antimicrobial susceptibility of the bacteria.

The E-test is an alternative, relatively simple method for the determination of antibiotic susceptibility using a single strip with different concentrations of antibiotics (Miftahussurur et al., 2020). This technique

provides to retain some principles of the agar dilution method for the detection of quantitative MIC values (Sader & Pignatari, 1994). Furthermore, the E-test is stated to be an easy to use, rapid, and routinely used method that allows us to work with fastidious organisms for several antimicrobial drugs at the same time under the same conditions (Benkova et al., 2020). The MIC values of this study represent the treatable and untreatable forms of bacterial pathogens with susceptible and resistant results. The E-test is not routinely used in the aquaculture industry, and these results will be a pioneer for fish disease specialists in detecting effective antibiotic doses using E-test strips. It is recommended to use this antimicrobial gradient method for the detection of MIC values of bacterial fish pathogens. Furthermore, the findings of this study could be evidence of the excessive use of antibiotics in fish farms. Reduce antibiotic use and the need for surveys in aquaculture facilities are required to fill the gap in antibiotic consumption (Lulijwa et al., 2020).

In conclusion, this research could be applicable to antibiotic use management strategies to avoid escalation of antibiotic resistant bacteria. Today, in many aquaculture facilities, treatment against bacterial diseases is carried out with broad-spectrum antibiotics without detecting the causative pathogen and determining the appropriate antibiotic for the pathogen. Additional research should be carried out in different regions of Turkey to generate an antibiotic resistance map of fish farms.

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CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this document.

AUTHOR CONTRIBUTIONS

All authors contributed equally to the article as conceptualization, methodology, research, writing, and editing. All authors approved the final draft.

ETHICS APPROVAL

No specific ethical approval was necessary for this study.

DATA AVAILABILITY STATEMENT

Data supporting the findings of the present study are available from the corresponding author on reasonable request.

REFERENCES

Akaylı, T., Ürkü, Ç. & Çanak, Ö. (2013). Antimicrobial Susceptibilities of Gram-negative Bacteria Isolated from Cultured Rainbow Trout (*Oncorhynchus mykiss*, Walbaum 1792) (in Turkish with English abstract). *Biyoloji Bilimleri Araştırma Dergisi, **6***(2), 17-22.

Allerberger, F. & Mittermayer, H. (2008). Antimicrobial stewardship. *Clinical Microbiology & Infection, **14***(3), 197-199, 2008. DOI: 10.1111/j.1469-0691.2007.01929.x

Austin, B. & Austin, D.A. (2007). *Bacterial fish pathogens: Disease of farmed and wild fish, 4th ed.*, Chichester, UK: Springer Dordrecht Publishers. DOI: 10.1007/978-1-4020-6069-4

Austin, B. & Austin, D.A. (2012). *Bacterial fish pathogens: Disease of farmed and wild fish 5th ed.*, New York, USA: Springer Dordrecht Publishers. DOI: 10.1007/978-94-007-4884-2

Balcı-Akova, S. (2015). Aquaculture and its distribution in Turkey. *Journal of Aquaculture Engineering & Fisheries Research*, ***1***(4), 160-190. DOI: 10.3153/jaefr15018

Balta, F., Dengiz-Balta, Z., Özgümüş, O.B. & Çağırgan, H. (2016). The Antimicrobial resistance and investigation of *Yersinia ruckeri* from rainbow trout (*Oncorhynchus mykiss*) farms in the Eastern Black Sea Region (in Turkish with English abstract). *Journal of Anatolian Environmental and Animal Sciences*, ***1***(3), 72-76. DOI: 10.35229/jaes.280741

Balta, F. & Dengiz-Balta, Z. (2017). Serotyping, genetic characterization and antimicrobial susceptibility determination of *Vibrio anguillarum* strains isolated from farmed rainbow trout (*Oncorhynchus mykiss*) in the eastern Black Sea (in Turkish with English abstract). *Ankara Universitesi Veteriner Fakültesi Dergisi*, ***64***, 321-328. DOI: 10.1501/Vetfak_0000002816

Benkova, M., Soukup, O. & Marek, J. (2020). Antimicrobial susceptibility testing: currently used methods and devices and the near future in clinical practice. *Journal of Applied Microbiology*, ***129***(4), 806-822. DOI: 10.1111/jam.14704

Blair, J.M., Webber, M.A., Baylay, A.J., Ogbolu, D.O. & Piddock, L.J. (2015). Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, ***13***(1), 42-51. DOI: 10.1038/nrmicro3380

Diler, Ö., Altun, S., Adiloglu, A.K., Kubilay, A. & Isıklı, B. (2002). First occurrence of Streptococcosis affecting farmed rainbow trout (*Oncorhynchus*

mykiss) in Turkey. *Bulletin-European Association of Fish Pathologist*, ***22***(1), 21-26.

EUROSTAT. (2019). Aquaculture statics. https://ec.europa.eu/eurostat/statisticsexplained

Hughes, J.H., Biedenbach, D.J., Erwin, M.E. & Jones, R.N. (1993). E test as susceptibility test and epidemiologic tool for evaluation of *Neisseria meningitidis* isolates. *Journal of Clinical Microbiology*, ***31***(12), 3255-3259. DOI: 10.1128/jcm.31.12.3255-3259.1993

Igbinosa, I.H. (2014). Antibiogram profiling and pathogenic status of *Aeromonas* species recovered from Chicken. *Saudi Journal of Biological Sciences*, ***21***(5), 481-485. DOI: 10.1016/j.sjbs.2014.06.003

Ingram, G.A. (1980). Substances involved in the natural resistance of fish to infection–a review. *Journal of Fish Biology*, ***16***(1), 23-60. DOI: 10.1111/j.1095-8649.1980.tb03685.x

Jilani, M.S.A., Murshed, M., Sultana, L. & Hasan, Z. (2008). Common clinically important aerobic bacteria and their antibiotic resistance pattern of Dhaka city and its vicinity. *Bangladesh Medical Collage Journal*, ***14***, 66-71.

Kathleen, M.M., Samuel, L., Felecia, C., Reagan, E.L., Kasing, A., Lesley, M. & Toh, S.C. (2016). Antibiotic resistance of diverse bacteria from aquaculture in Borneo. *International Journal of Microbiology*, 2164761. DOI: 10.1155/2016/2164761

Khan, Z.A., Siddiqui, M.F. & Park, S. (2019). Current and emerging methods of antibiotic susceptibility testing. *Diagnostics*, ***9***(2), 49. DOI: 10.3390/diagnostics9020049

Kırhan, Ş., Göksoy, E.Ö., Kaya, O. & Tekbıyık, S. (2006). In-vitro antimicrobial susceptibility of pathogenic bacteria in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Turkish Journal of Veterinary & Animal Sciences*, ***30***(3), 337-341.

Kubilay, A., Altun, S., Uluköy, G. & Diler, Ö. (2005). The Determination of Antimicrobial Susceptibilities of *Lactococcus garvieae* Strains (in Turkish with English abstract). *Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi*, ***1***(1), 39-48.

Kum, C. & Sekkin, S. (2011). The immune system drugs in fish: immune function, immunoassay, drugs. In: Aral, F. & Doğu, Z. (Eds.), *Recent advances in fish farms* 169-210p, IntechOpen., Croatia. DOI: 10.5772/26869

Lulijwa, R., Rupia, E.J. & Alfaro, A.C. (2020). Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. *Reviews in Aquaculture*, ***12***(2), 640-663. DOI: 10.1111/raq.12344

Lunden, T. & Bylund, G. (2000). The influence of in vitro and in vivo exposure to antibiotics on mitogen-induced proliferation of lymphoid cells in rainbow trout (*Oncorhynchus mykiss*). *Fish Shellfish Immunology*, ***10***(5), 395-404. DOI: 10.1006/fsim.1999.0247

Lunden, T., Lilius, E.M. & Bylund, G. (2002). Respiratory burst activity of rainbow trout (*Oncorhynchus mykiss*) phagocytes is modulated by antimicrobial drugs. *Aquaculture*, ***207***(3-4), 203-212. DOI: 10.1016/s0044-8486(01)00760-8

Manage, P.M. (2018): Heavy use of antibiotics in aquaculture; emerging human and animal health problems–a review. *Sri Lanka Journal of Aquatic Sciences*, ***23***(1), 13-27. DOI: 10.4038/sljas.v23i1.7543

Mathew, A.G., Cissell, R. & Liamthong, S. (2007). Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathogens & Disease*, ***4***(2), 115-133. DOI: 10.1089/fpd.2006.0066

Miftahussurur, M., Fauzia, K.A., Nusi, I.A., Steiawan, P.B., Syam, A.F., Waskito, L.A., Doohan, D., Ratnasari, N., Khomsan, A., Adnyana, I.K. & Yamaoka, Y. (2020). E-test versus agar dilution for antibiotic susceptibility testing of *Helicobacter pylori*: a comparison study. *BMC Research Notes*, ***13***(1), 1-6. DOI: 10.1186/s13104-019-4877-9

Muziasari, W.I., Managaki, S., Parnanen, K., Karkman, A., Lyra, C., Tamminen, M., Suzuji, S. & Virta, M. (2014). Sulphonamide and trimethoprim resistance genes persist in sediments at Baltic Sea aquaculture farms but are not detected in the surrounding environment. *PLoS One*, ***9***, e92702. DOI: 10.1371/journal.pone.0092702

Orozova, P., Chikova, V. & Sirakov, I. (2014). Diagnostics and antibiotic resistance of *Yersinia ruckeri* strains isolated from trout fish farms in Bulgaria. *International Journal of Development Research*, ***4***(12), 2727-2733.

Pedersen, K., Tiainen, T. & Larsen, J. L. (1995). Antibiotic resistance of *Vibrio anguillarum*, in relation to serovar and plasmid contents. *Acta Veterinaria Scandinavica*, ***36***(1), 55-64. DOI: 10.1186/bf03547702

Raissy, M. & Ansari, M. (2011). Antibiotic susceptibility of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) in Iran fish farms. *African Journal of Biotechnology*, ***10***(8), 1473-1476.

Raissy, M. & Moumeni, M. (2016). Detection of antibiotic resistance genes in some *Lactococcus garvieae* strains isolated from infected rainbow trout. *Iranian Journal of Fisheries Sciences*, ***15***(1), 221-229.

Roberts, R.J. (2012). *Fish pathology 4th ed.*, Wiley-Blackwell Publishers, London, UK. DOI: 10.1111/jfd.12118

Sader, H.S. & Pignatari, A.C.C. (1994). E test: a novel technique for antimicrobial susceptibility testing. *Sao Paulo Medical Journal*, ***112***(4), 635-638. DOI: 10.1590/s1516-31801994000400003

Shahriar, A., Akter, T., Kobra, A.T., Emran, T.B., Mallick, J. & Dutta, M. (2019). Isolation of Pathogenic and Non-pathogenic Microbial Stains from Different Types of Sea Fish Samples and their Quality Assessment with Antibiogram Properties. *Journal of advences in Microbiology*, ***19***(1), 1-10, 2019. DOI: 10.9734/jamb/2019/v19i130185

Sharma, I., Kumar, A. & Pramanik, A.K. (2017). Antibiotic sensitivity test of Aeromonads isolated from foods of animal origin including fish. *Assam University Journal of Science and Technology*, ***5***(1), 43-47.

Sieroslawska, A., Terech-Majewska, E. & Siwicki, A.K. (2004). Impact of chemotherapeutics on the fish immune system. In: Siwicki, A. K., Antychowicz, J. & Szweda, W. (Eds.), *Current Challenges in Fish Disease Prevention and Treatment*, 227-235p, Wyd, IRS, Olsztyn.

Stokes, E.J., Ridgway, G.L. & Wren, M.W.D. (1993). Laboratory control of antimicrobial chemotherapy. *Clinical Microbiology*, ***7***, 237-51.

Terech-Majewska, E. & Siwicki, A.K. (2006). Influence of oxytetracycline on the metabolic and phagocyte activity of macrophages and the proliferative response of lymphocytes in Carp and European Catfish. *Medycyna Weterynaryjna*, ***62***(12), 1431-1434.

Terech-Majewska, E. (2016). Improving disease prevention and treatment in controlled fish culture. *Fisheries and Aquatic Life*, ***24*** (3), 115-165. DOI: 10.1515/aopf-2016-0013

Toranzo, A.E. (2004). Report about fish bacterial diseases: Mediterranean Aquaculture Laboratories, In: Alvarez-Pellitero, P., Barja ,J. L., Basurco, B., Berthe, F. & Toranzo, A. E. (Eds.), *Mediterranean aquaculture diagnostic laboratories* 49-89p, Santiago de Compostela, Spain.

TUIK. (2020). https://data.tuik.gov.tr/Bulten/Index?p=Fishery-Products-2020-37252

Vignesh, R., Karthikeyan, B.S., Periyasamy, N. & Devanathan, K. (2011). Antibiotics in aquaculture: an overview. *South Asian Journal of Experimental Biology*, ***1***(3), 114-120. DOI: 10.38150/sajeb.1(3).p114-120

Wojtacka, J. (2007). The impact of oxytetracycline and lysozyme dimer (KLP-602) on immune processes and the pathomorphology of selected internal organs of Siberian sturgeon (*Acipenser baeri*, Brandt 1869) (in Polish). *Doctoral dissertation*, UWM Olsztyn, Poland.