ANALYSIS OF TETRACYCLINE RESISTANCE CHARACTERISTICS OF *Edwardsiella piscicida* ISOLATED FROM CULTURED OLIVE FLOUNDER *Paralichthys olivaceus* AND STARRY FLOUNDER *Platichthys stellatus* IN JEJU ISLAND

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ABSTRACT

In Jeju Island, the number of olive flounder (*Paralichthys olivaceus*) farms declined from 284 in 2015 to 224 in 2024, representing a 22% decrease. In contrast, starry flounder (*Platichthys stellatus*) and turbot (*Scophthalmus maximus*) aquaculture farms showed steady growth, increasing from 15 in 2015 to 60 in 2024, a 300% increase. This study aimed to analyze and compare the tetracycline resistance characteristics of *Edwardsiella piscicida* isolated from olive flounder and starry flounder cultured in Jeju Island.

A total of 51 *E. piscicida* isolates from olive flounder and 10 *E. piscicida* isolates from starry flounder were collected from 2023 to 2025. Colonies were further cultured in tryptic soy broth (TSB; Difco, USA) with 2% NaCl for 18–24 h. For isolates were plated on salmonella shigella (SS; Difco, USA) and incubated at 28°C for 24 h to observe colony morphology. Isolates were stored at −80°C in TSB supplemented with 20% glycerol (Sigma, USA) until further use. Genomic DNA was extracted using the Higene™ Genomic DNA prep kit (BIOFACT, Korea) and stored at −20°C. Species identification was confirmed by polymerase chain reaction (PCR) targeting the *E. piscicida* specific gene (Costa et al., 2022). Antimicrobial susceptibility was assessed using tetracycline class antibiotics, including tetracycline (Tc), oxytetracycline (OT), and doxycycline (DXT), by the disk diffusion test and broth microdilution methods. Tetracycline resistance genes were detected by PCR (Jun, 2003; 2010).

All isolates successfully amplified the expected 423 bp fragment of the *E. piscicida* specific gene, confirming species identity. Analysis of tetracycline resistance genes in olive flounder revealed 1 *tet*(A) gene and 36 *tet*(D) genes, while in starry flounder 3 *tet*(D) genes were identified.

Introduction

Olive flounder (*Paralichthys olivaceus*) is one of the most important aquaculture species in Korea, accounting for approximately 50% of total aquaculture production (45,801 tons out of 90,545 tons in 2022). More than half of this production (51.38%) is concentrated on Jeju Island, highlighting the region as a major center of flatfish aquaculture (KOSIS, 2025). In recent years, the aquaculture production of starry flounder (*Platichthys stellatus*) has also increased due to accumulated disease-related losses and production instability in olive flounder farming.

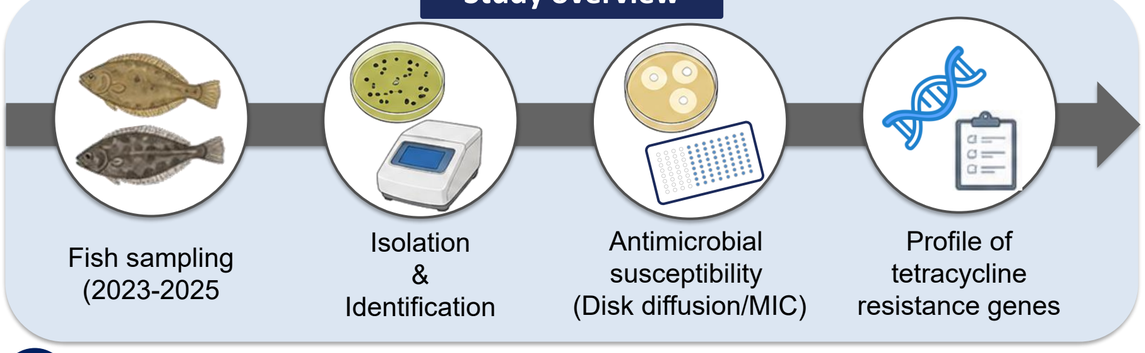
Both olive flounder and starry flounder are highly susceptible to *Edwardsiella piscicida*, a major bacterial pathogen responsible for edwardsiellosis in marine aquaculture. Experimental infection studies have reported cumulative mortality rates of up to 87% within 16 days in olive flounder infected with *E. piscicida* (Hu et al., 2019). The disease causes severe economic losses in aquaculture through mass mortality and reduced productivity.

Disease management in aquaculture relies heavily on antimicrobial agents, particularly tetracycline-class antibiotics such as oxytetracycline. However, the long-term and intensive use of these antibiotics has resulted in the widespread emergence of antimicrobial-resistant bacteria. In particular, the dissemination of tetracycline resistance genes has become a global concern due to their potential transfer among bacterial populations.

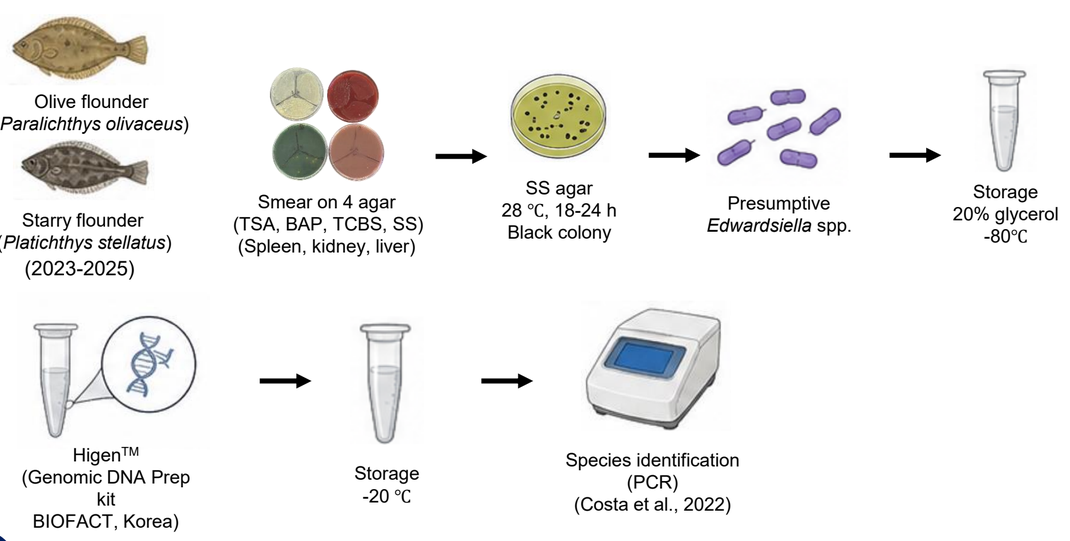
Despite Jeju Island being a major aquaculture region in Korea, information regarding tetracycline resistance patterns in *E. piscicida* remains limited. Therefore, this study aimed to investigate the prevalence, level, and patterns of tetracycline resistance in *E. piscicida* isolates obtained from cultured olive flounder and starry flounder in Jeju Island, and to provide fundamental data for improving antimicrobial management and treatment strategies in aquaculture.

Materials & Methods

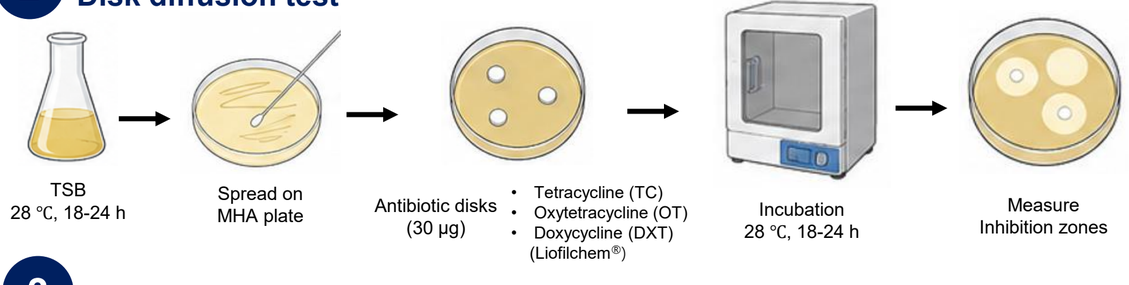
Study overview



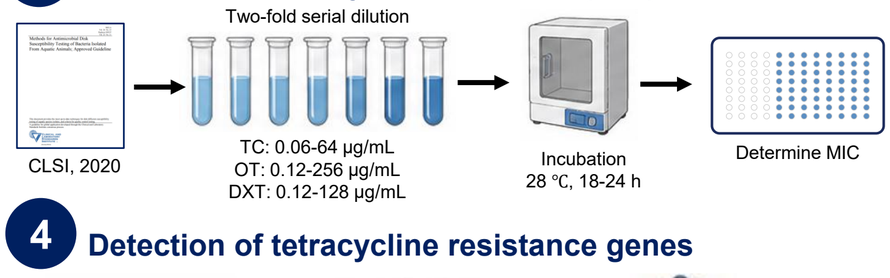
1 Isolation and identification of bacteria



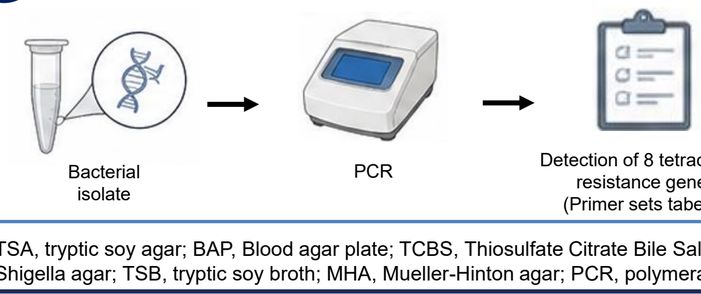
2 Disk diffusion test



3 Minimum inhibitory concentration (MIC)



4 Detection of tetracycline resistance genes



TSA, tryptic soy agar; BAP, Blood agar plate; TCBS, Thiosulfate Citrate Bile Salt Sucrose Agar; SS, Salmonella-Shigella agar; TSB, tryptic soy broth; MHA, Mueller-Hinton agar; PCR, polymerase chain reaction

Results

Table 1. Primers used for PCR

Purpose	Target	Primer	Oligonucleotide sequences (5' to 3')	Size (bp)	Reference
Detection	*E. tarda*	FTP7	GGCGATTACCAGCTCTTTATC	282	Coast et al., 2022
		RTP7	GCGAGAGGGAAGCGTATTC		
	E. piscicida	FSP4	ACTTTATCTGCGATCCCACG	423	
		RSP4	CGGTGATATAGCCCTGAGAG		
	E. anguillarum	FAP2	TCCCAGTATTGCCGAAGTTG	621	
		RAP2	GCTGTAGAGAGTAAAGCCGC		
tet gene	*tet*(A) to *tet*(G)	TETF	GCGCTNTATGCGTTGATGCA		
	tet(A)	TAR2	ACAGCCCGTCAGGAAATT	387	Jun, 2003
	tet(B)	TBR	TGAAAGCAAACGGCCTAA	171	
	tet(C)	TCR3	CGTGCAAGATTCCGAATA	631	
	tet(D)	TDR3	CCAGAGGTTTAAGCAGTGT	489	
	tet(E)	TER	ATGTGTCCTGGATTCCT	246	
	tet(G)	TGR3	ATGCCAACACCCCCGGCG	803	
	tet(M)	TMF	GAATCTGAACAATGGGAT	1,099	Jun, 2010
		TMR	CTAACAATTCTGTTCCAGC		
	tet(S)	TSF	CATAGACAAGCCGTTGACC	667	
		TSR	ATGTTTTTGGAACGCCAGAG		

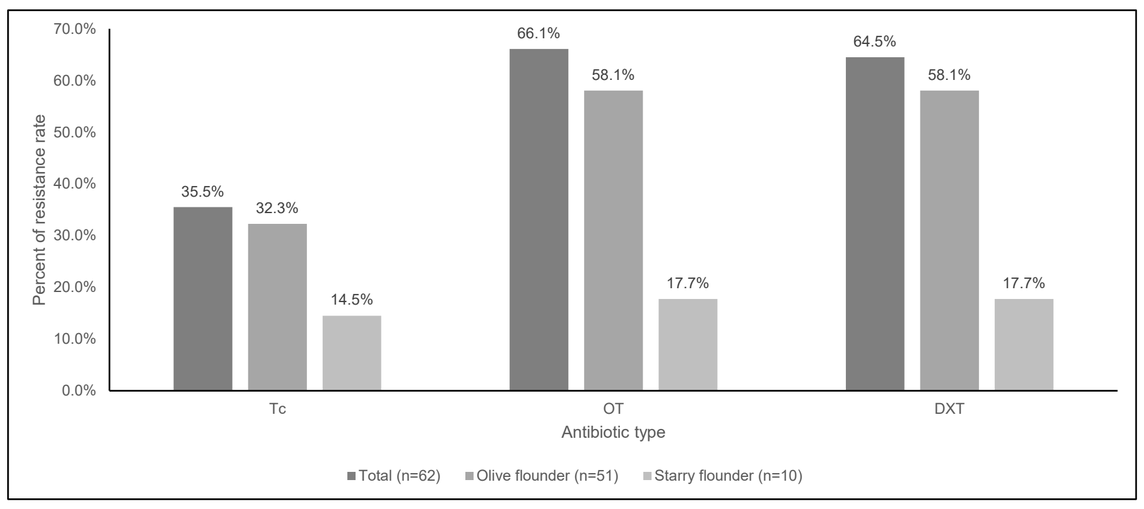


Fig. 1. Resistance rates of *E. piscicida* isolates from olive flounder and starry flounder based on disk diffusion test.

Resistance confirmed with agar diffusion test (diameter ≤ 6 mm)
Tc, tetracycline; OT, oxytetracycline; DXT, doxycycline

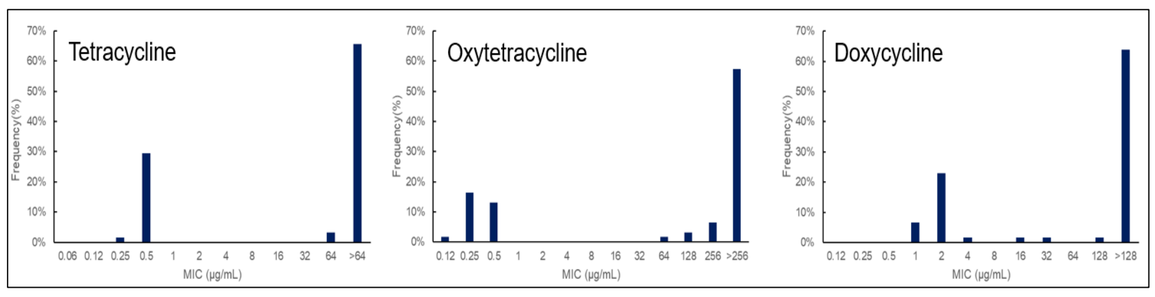


Fig. 2. Distribution of MIC values of *E. piscicida* (n=61) determined by the broth microdilution method.

Table 2. *tet* gene analysis results

	tet gene							
	tet(A)	*tet*(B)	*tet*(C)	*tet*(D)	*tet*(E)	*tet*(G)	*tet*(M)	*tet*(S)
E. piscicida (n=61)	1	-	-	39	-	-	-	-

Table 3. Tetracycline MIC distribution and antibiotic resistance genes in *E. piscicida* isolates

		MIC (µg/mL)											
Strain	*tet* gene	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64
E. piscicida (n=61)	*tet*(A) (n=1)											1	
	tet(D) (n=39)				1							1	37
	ND (n=21)			1	17								3

Discussion & Conclusion

This study investigated the antimicrobial resistance characteristics of *E. piscicida* isolated from cultured olive flounder and starry flounder in Jeju Island between 2023 and 2025. All isolates presumptively identified as *Edwardsiella* spp. were confirmed as *E. piscicida* by PCR analysis.

Disk diffusion assays showed that most isolates remained susceptible to Tc, whereas approximately 60% of the isolates exhibited reduced susceptibility or resistance to OT and DXT. MIC analysis further demonstrated elevated resistance levels to tetracycline-class antibiotics in several isolates, and tetracycline resistance genes were predominantly detected in isolates showing high MIC values (Fig. 1, 2).

Previous studies by Jun et al. (2004) reported similar detection frequencies of *tet*(A) and *tet*(D), including isolates harboring both resistance genes simultaneously. In contrast, the present study demonstrated a predominance of *tet*(D), while *tet*(A) was detected in only a single isolate. Furthermore, no isolates carrying combined *tet*(A) and *tet*(D) genes were identified. These findings suggest that the tetracycline resistance patterns of *E. piscicida* in Jeju aquaculture environments may have changed over time (Table 2, 3).

Although antimicrobial resistance in *Streptococcus parauberis* has been extensively investigated in Korean aquaculture, studies focusing on *E. piscicida* remain limited. Therefore, the present study provides fundamental information regarding tetracycline resistance profiles and resistance gene distribution in *E. piscicida*, which may contribute to improved antimicrobial management and treatment strategies in aquaculture.

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