Vietnam Journal of Agricultural Sciences

p-ISSN 2588-1299
e-ISSN 3030-4520

VJAS 2025; 8(4): 2703-2714
https://doi.org/10.31817/vjas.2025.8.4.03

Antimicrobial Resistance and Minimum Inhibitory Concentration Profiles of *Flavobacterium oreochromis* Isolated from Tilapia Cultured in Northern Vietnam

Doan Thi Nhinh[1], Tran Thi Trinh[1], Dang Thi Hoa[1], Nguyen Van Tuyen[1], Nguyen Thi Huong Giang[2] & Truong Dinh Hoai[1]*

[1]Faculty of Fisheries, Vietnam National University of Agriculture, Hanoi 12400, Vietnam
[2]Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Hanoi 12400, Vietnam

Abstract

Flavobacterium oreochromis is a major bacterial pathogen that causes columnaris disease in tilapia (*Oreochromis* spp.) and other freshwater fish species. This study assessed the antibiotic resistance profiles and minimum inhibitory concentrations (MICs) of *F. oreochromis* isolates obtained from infected tilapia cultured in Northern Vietnam. A total of 51 isolates retrieved from diseased fish collected in Hai Duong (n = 23), Bac Ninh (n = 16), and Hoa Binh (12 isolates) were used in the study. The bacteria were cultured on TYES agar, identified through morphological and biochemical analyses, and confirmed by PCR. Antimicrobial susceptibility testing employed the disk diffusion method against 15 antibiotics, while the MICs of six frequently used antibiotics were determined via the broth microdilution method. The antimicrobial susceptibility test revealed that the isolates exhibited high resistance to oxacillin (100%) and neomycin (76.5%), and moderate resistance to sulfamethoxazole-trimethoprim (37.3%), doxycycline (35.3%), and oxytetracycline (41.2%). Resistance values to the remaining antibiotics were below 12%. Notably, all the isolates were multidrug-resistant (MDR), exhibiting resistance to 2-10 antibiotics, and 14 distinct resistance phenotypes were identified. The MIC values ranged from 0.063-1 µg mL^{-1} for amoxicillin, 0.125-8 µg mL^{-1} for erythromycin, and 0.25-8 µg mL^{-1} for florfenicol. For oxytetracycline, doxycycline, and sulfamethoxazole-trimethoprim, the MICs ranged from 0.016-1 µg mL^{-1}, 0.125-2 µg mL^{-1}, and 0.5-16 µg mL^{-1}, respectively. These findings highlight a decline in the efficacy of commonly used antibiotics against *F. oreochromis*, underscoring the necessity for routine antimicrobial susceptibility testing to promote rational antibiotic use in aquaculture.

Keywords

Flavobacterium oreochromis, tilapia, antimicrobial resistance, MIC

Received: July 15, 2025
Accepted: October 22, 2025

Correspondence to
Truong Dinh Hoai
tdhoai@vnua.edu.vn

ORCID
Truong Dinh Hoai
https://orcid.org/0000-0002-2271-849X

Introduction

Tilapia (*Oreochromis* spp.) is one of the most widely farmed fish species globally (Prabu *et al*., 2019) and plays a particularly important role in the aquaculture sector of Vietnam (MARD, 2019). Due to its rapid growth rate, strong adaptability to adverse farming conditions, and low production costs (Wang & Lu, 2016), tilapia has become a dominant cultured species in various provinces of Vietnam, particularly under intensive and semi-intensive farming systems. However, the expansion of farming areas and increased stocking densities have led to more frequent and severe disease outbreaks, particularly bacterial infections. These diseases represent major challenges in tilapia farming, resulting in significant production losses and increased costs for disease prevention and treatment (Haenen *et al*., 2023). Among these, infections caused by *Flavobacterium columnare*, the pathogen responsible for columnaris disease, have emerged as one of the most serious threats. *F. columnare* affects a wide range of cultured fish, including both catfish and non-catfish species (Soto *et al*., 2008; Barony *et al*., 2015; Dong *et al*., 2016). Recently, LaFrentz *et al*. (2022) demonstrated that *F. columnare* is comprised of four distinct species, namely *F. columnare*, *F. covae*, *F. davisii*, and *F. oreochromis*, based on polyphasic and phylogenomic analyses. Of these, *F. oreochromis* is the species responsible for disease outbreaks in tilapia. This disease typically affects small fish reared under high-density conditions and poor water quality (Figueiredo *et al*., 2005; Declercq *et al*., 2013b). Infections of *F. columnare* in tilapia have been reported in several provinces of Vietnam, causing mortality rates ranging from 5% to 35% in affected culture systems. The disease can occur year-round but is most prevalent and causes the greatest losses during the winter–spring season (Nhinh *et al*., 2023).

To control bacterial infections, antibiotics are commonly used in aquaculture, including in tilapia farming (Vignesh *et al*., 2011; Chen *et al*., 2020). However, improper or overuse of antibiotics has contributed to the rise of antibiotic-resistant bacteria, reduced treatment efficacy, and has increased the risk of antibiotic residues in aquatic products (Romero *et al*., 2012; Chen *et al*., 2020). Serious antibiotic resistance has been reported in several bacterial pathogens affecting tilapia, such as *Aeromonas hydrophila*, *Edwardsiella ictaluri*, and *Streptococcus agalactiae* (Nhinh *et al*., 2021; Truong Thi My Hanh *et al*., 2024). However, data on the antibiotic resistance of *F. oreochromis* in tilapia remain limited, especially in Vietnam. In addition, determining the minimum inhibitory concentration (MIC) of antibiotics can provide essential quantitative information for evaluating the efficacy of antimicrobial agents and guiding rational dosage selection.

This study aimed to investigate the antibiotic resistance profiles of *F. oreochromis* isolates obtained from diseased tilapia cultured in northern Vietnam and to determine the MIC distribution of antibiotics commonly used in Vietnamese aquaculture. The findings will inform effective treatment strategies and guide appropriate dosage decisions in managing columnaris disease in farmed tilapia.

Materials and Methods

Materials

The study used 51 *F. oreochromis* isolates, which were obtained from diseased tilapia (*Oreochromis* spp.). Sixteen antibiotics belong to 11 antibiotic classes/subclasses were selected for antimicrobial susceptibility testing. Bacterial culture media, reagents, and other laboratory consumables used for bacterial isolation, identification, and the antibiotic resistance assays were prepared and applied according to standardized laboratory protocols.

Recovering bacteria from frozen glycerol stock and identification

A total of 51 bacterial isolates retrieved from infected tilapia (*Oreochromis* spp.) exhibiting

clinical signs of columnaris disease, including skin lesions, fin erosion, and gill necrosis, were used in this study. Samples were collected between 2023 and 2024 from tilapia farms in three northern provinces of Vietnam: Hai Duong (23 isolates), Bac Ninh (16 isolates), and Hoa Binh (12 isolates) (**Table 1**). The samples of tilapia exhibiting signs of *F. oreochromis* infection ranged in body weight from 35 to 302.6g, but the disease was most prevalent in fish weighing less than 100g. Outbreaks occurred at water temperatures ranging from 21 to 29°C in various culture systems, including earthen ponds, concrete ponds, and floating cages in rivers and reservoirs. Stocking densities varied widely, from 20-50 fish m^{-3} in floating cages and from 3-10 fish m^{-2} in earthen and concrete ponds.

All isolates were preserved in glycerol at -- 80 °C at the Aquatic Pathology Laboratory, Faculty of Fisheries (VNUA). The bacteria were recovered on TYES agar (Merck, Darmstadt, Germany) at 28°C, following the guidelines of Cain & LaFrentz (2007), and initially identified based on morphological and biochemical characteristics, followed by PCR confirmation.

The morphological and biochemical characteristics of *F. oreochromis* were assessed following the protocols described by Bernardet & Grimont (1989). Colony morphology was examined on agar plates, while cell shape was determined via Gram staining. Bacterial motility was observed using wet mounts under a light microscope. Congo red absorption was tested using Congo red reagent (Merck, Darmstadt, Germany), and the presence of flexirubin-type pigments was evaluated using 20% KOH (Merck, Darmstadt, Germany), according to the procedures described by Bernardet & Bowman (2006).

Genomic DNA was extracted from all isolates using the InstaGen DNA extraction kit (Bio-Rad, USA), following the manufacturer's instructions. Extracted DNA was stored at -80 °C for molecular identification.

PCR identification of *F. oreochromis* was conducted using species-specific primers targeting the ISR gene region, with the forward primer F: TGCGGCTGGATCACCTCCTTTCTAGAGA CA and the reverse primer R: TAATYRCTAAAGATGTTCTTTCTACTTGT TTG, as described by Welker *et al*. (2005), producing an amplicon of approximately 470bp. PCR reactions were carried out in a total volume of 20µL, consisting of 10µL GoTaq Green Master Mix (Promega), 1.5µL of each primer (10µM), 3µL of DNA template, and 4µL of nuclease-free water. Amplification was performed using a thermal cycler (Eppendorf, Massachusetts, USA) under the cycling conditions outlined by Welker *et al*. (2005). *F. columnare* ATCC 23463 served as the positive control, and nuclease-free water was used as the negative control. PCR products were analyzed by electrophoresis on a 1.3% agarose gel stained with Redsafe (Intron, Korea) in 1× TBE buffer. The bands were visualized using an Analytik Jena imaging system (Upland, USA).

Determination of antimicrobial resistance by the disk diffusion method

The antibiotic resistance and susceptibility profiles of the *F. oreochromis* isolates were determined using the antibiotic disc diffusion method, following the guidelines provided in CLSI (2020) and CLSI (2015). Bacterial suspensions were adjusted to a turbidity equivalent to the 0.5 McFarland standard and inoculated onto diluted Mueller-Hinton agar (DMHA, 4 g L^{-1}) plates using sterile cotton swabs. After 36 hours of incubation, the diameters of the inhibition zones were measured.

A total of 15 antibiotics representing 11 classes/subclasses were tested, namely: two penicillins: oxacillin (Ox, 1µg) and amoxicillin (Ax, 10µg); one β-lactam/β-lactamase inhibitor combination: amoxicillin-clavulanic acid (Ac, 20/10µg); three cephalosporins: cefotaxime (Ct, 30µg), cefuroxime (Cu, 30µg), and ceftriaxone (Cx, 30µg); one macrolide: erythromycin (Er, 15µg); one quinolone: nalidixic acid (Na, 30µg); one sulfonamide: sulfamethoxazole/trimethoprim (ST, 23.75/1.25µg); one aminoglycoside: neomycin (Ne, 30µg); o ne glycopeptide: vancomycin (Va,

Table 1. Information on the *F. oreochromis* isolates retrieved from diseased tilapia used in this study

No.	Isolates	Provinces	Collection years	No.	Isolates	Provinces	Collection years
1	Fl.Til-HD01	Hai Duong	2023	27	Fl.Til-BN04	Bac Ninh	2023
2	Fl.Til-HD02	Hai Duong	2023	28	Fl.Til-BN05	Bac Ninh	2023
3	Fl.Til-HD03	Hai Duong	2023	29	Fl.Til-BN06	Bac Ninh	2023
4	Fl.Til-HD04	Hai Duong	2023	30	Fl.Til-BN07	Bac Ninh	2023
5	Fl.Til-HD05	Hai Duong	2023	31	Fl.Til-BN08	Bac Ninh	2023
6	Fl.Til-HD06	Hai Duong	2023	32	Fl.Til-BN09	Bac Ninh	2023
7	Fl.Til-HD07	Hai Duong	2023	33	Fl.Til-BN10	Bac Ninh	2024
8	Fl.Til-HD08	Hai Duong	2023	34	Fl.Til-BN11	Bac Ninh	2024
9	Fl.Til-HD09	Hai Duong	2023	35	Fl.Til-BN12	Bac Ninh	2024
10	Fl.Til-HD10	Hai Duong	2023	36	Fl.Til-BN13	Bac Ninh	2024
11	Fl.Til-HD11	Hai Duong	2023	37	Fl.Til-BN14	Bac Ninh	2024
12	Fl.Til-HD12	Hai Duong	2023	38	Fl.Til-BN15	Bac Ninh	2024
13	Fl.Til-HD13	Hai Duong	2023	39	Fl.Til-BN16	Bac Ninh	2024
14	Fl.Til-HD14	Hai Duong	2023	40	Fl.Til-HB01	Hoa Binh	2023
15	Fl.Til-HD15	Hai Duong	2023	41	Fl.Til-HB02	Hoa Binh	2023
16	Fl.Til-HD16	Hai Duong	2024	42	Fl.Til-HB03	Hoa Binh	2023
17	Fl.Til-HD17	Hai Duong	2024	43	Fl.Til-HB04	Hoa Binh	2023
18	Fl.Til-HD18	Hai Duong	2024	44	Fl.Til-HB05	Hoa Binh	2023
19	Fl.Til-HD19	Hai Duong	2024	45	Fl.Til-HB06	Hoa Binh	2023
20	Fl.Til-HD20	Hai Duong	2024	46	Fl.Til-HB07	Hoa Binh	2023
21	Fl.Til-HD21	Hai Duong	2024	47	Fl.Til-HB08	Hoa Binh	2023
22	Fl.Til-HD22	Hai Duong	2024	48	Fl.Til-HB09	Hoa Binh	2023
23	Fl.Til-HD23	Hai Duong	2024	49	Fl.Til-HB10	Hoa Binh	2024
24	Fl.Til-BN01	Bac Ninh	2023	50	Fl.Til-HB11	Hoa Binh	2024
25	Fl.Til-BN02	Bac Ninh	2023	51	Fl.Til-HB12	Hoa Binh	2024
26	Fl.Til-BN03	Bac Ninh	2023				

30µg); one fluoroquinolones: ofloxacin (Of, 5µg); two tetracyclines: doxycycline (Dx, 30µg) and oxytetracycline (OTC, 30µg); and one amphenicol: florfenicol (Fl, 30µg).

The selected antibiotics included those commonly used in Vietnamese aquaculture as well as agents frequently detected as residues in aquaculture water samples (Hoa *et al*., 2011; Binh *et al*., 2018; Hedberg *et al*., 2018). Based on the diameter of the inhibition zones, bacterial isolates were categorized as "sensitive," "intermediate," or "resistant," using interpretive criteria from CLSI M100 (2015) and CLSI VET04 (2020).

Determination of the minimum inhibitory concentration (MIC)

Six antimicrobial agents (Merck, Darmstadt, Germany), namely amoxicillin, erythromycin, florfenicol, oxytetracycline, sulfamethoxazole/trimethoprim (ST, 19:1), and doxycycline, were used for the MIC determination of the *F. oreochromis* isolates. These agents are currently approved for use in aquaculture in Vietnam.

MIC testing was conducted following the broth microdilution protocol recommended for *F. columnare* in the CLSI guideline VET04 (CLSI, 2020). Antimicrobial stock solutions

were prepared according to CLSI VET04 specifications. Each microdilution plate included a positive control (bacterial inoculum without an antimicrobial), a negative control (broth only), and two-fold serial dilutions of each antimicrobial agent across 16 concentrations ranging from 0.008 to 256 mg L^{-1}, using diluted cation-adjusted Mueller-Hinton broth (DCAMHB, 4 g L^{-1}; Merck, Darmstadt, Germany). Bacterial inhibition at different antibiotic concentrations was also assessed in 10mL glass test tubes to allow visual observation of growth suppression.

Escherichia coli ATCC 25922 (ATCC, USA) was used as the quality control strain, and acceptable quality control ranges were referenced from CLSI VET04 (CLSI, 2020). The MIC was defined as the lowest concentration of the antimicrobial that completely inhibited visible bacterial growth (CLSI, 2020). MIC_{50} and MIC_{90} values were calculated for each antimicrobial agent.

Data analysis

Descriptive statistics were used to analyze the resistance and susceptibility rates of the bacterial isolates to the 15 antibiotics using Excel software.

Results and Discussion

Bacterial recovery and identification

All 51 bacterial isolates were successfully recultured on TYES medium for use in this study (**Table 1**). After 36 hours of incubation, colonies displayed yellow pigmentation with characteristic rhizoid morphology, ranging in size from 0.5 to 3mm. The bacteria were Gram-negative, filamentous, and measured 2 to 10µm in length. Additional biochemical features included no growth on TSA medium, gliding motility, and positive reactions for oxidase, catalase, flexirubin pigment, and Congo red absorption (**Figure 1**, **Table 2**).

The phenotypic characteristics of the *F. oreochromis* isolates obtained from diseased tilapia were consistent with those of the reference strain *Flavobacterium columnare* ATCC 23463, and aligned with previous descriptions of *F. oreochromis* infecting tilapia by Bernardet & Bowman (2006) and Dong *et al*. (2015a). According to Dong *et al*. (2016), *F. columnare* with a rhizoid colony type is highly virulent to fish host. The pathogenicity of rhizoid colonies has also been demonstrated in tilapia cultured in Vietnam (Nhinh *et al*., 2023), as well as in black carp (*Mylopharyngodon piceus*) (Hoai *et al*., 2025) and striped catfish (*Ictalurus punctatus*) (Dong *et al*., 2015b). PCR analysis confirmed that all 51 isolates were positive for *F. oreochromis*, with amplification products appearing at approximately 470bp, consistent with the band position of the positive control strain *F. columnare* ATCC 23463 (**Figure 2**).

Antibiotic resistance profiles determined by the disk diffusion method

The antibiotic susceptibility testing of the 51 *F. oreochromis* isolates against the 15 antibiotics revealed considerable variability in resistance and sensitivity across the different agents (**Table 3**, **Figure 3**). All isolates were resistant to oxacillin, and high resistance levels were also.

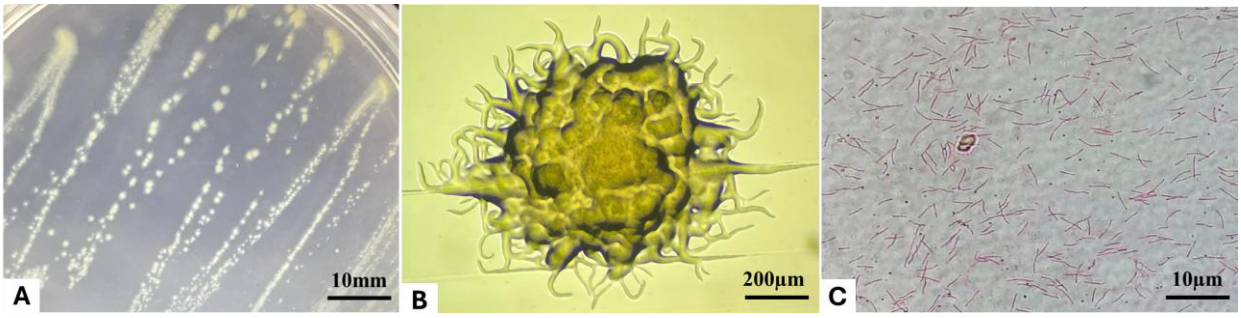
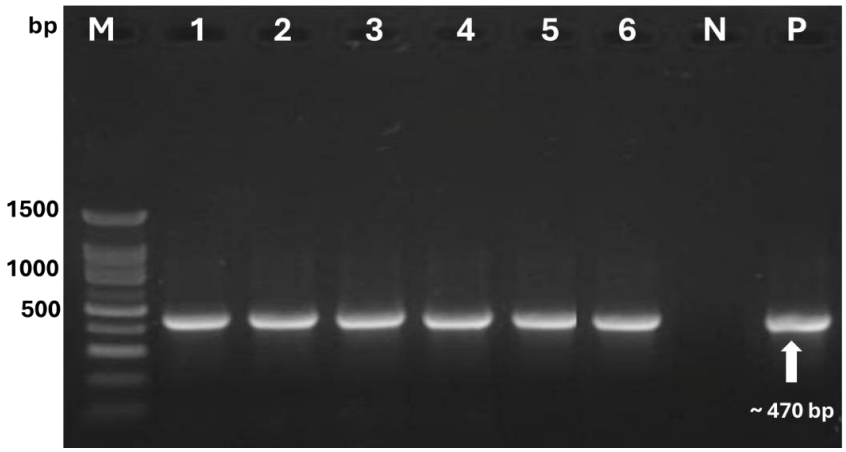


Figure 1. Colony morphology on TYES agar (A, B) and cell morphology (C) of a *Flavobacterium oreochromis* isolate used in this study

Table 2. Morphological and biochemical characteristics of the *Flavobacterium oreochromis* isolates in this study

Characteristics	Isolates from this study (n = 51)	*F. columnare* ATCC 23463
Gram staining	-	-
Bacterial morphology	long, slender rod	long, slender rod
Colony morphology	rhizoid	rhizoid
Gliding motility	+	+
Growth on TSA	-	-
Flexirubin pigments	+	+
Congo red	+	+
Cytochrome Oxidase	+	+
Gram staining	-	-



Note: M: molecular marker; lanes 1-6: six representative isolates of F. oreochromis retrieved from tilapia in this study; N, P: negative and positive controls.

Figure 2. Representative gel electrophoresis of PCR products of species-specific primers for *F. oreochromis* identification

observed for neomycin (72.1%) and vancomycin (58.9%). Among the six antibiotics approved for use in aquaculture in Vietnam, three exhibited resistance rates exceeding 30%, namely sulfamethoxazole-trimethoprim (37.3%), doxycycline (35.3%), and oxytetracycline (41.2%). In contrast, resistance to florfenicol and amoxicillin were lower, at 11.8% and 5.9%, respectively, and no resistance was detected to the combination antibiotic amoxicillin-clavulanic acid. Isolates exhibited resistance rates below 10% for antibiotics in the cephalosporin and fluoroquinolone classes.

Multidrug resistance (MDR) was observed in all the isolates, with resistance to between 2 and 10 antibiotics (**Table 4**). Specifically, 43.1% of the isolates were resistant to 3-4 antibiotics, while 27.5% showed resistance to 7-10 antibiotics simultaneously. Notably, the isolates exhibited a wide diversity in resistance profiles, with 14 distinct resistance phenotypes identified.

The use of antibiotics remains one of the most common strategies for managing bacterial infections in aquaculture. However, improper or abuse of antibiotics has been identified as a major driver of the rapid emergence of antibiotic resistance and multidrug resistance (MDR) in aquatic pathogens (Watts *et al*., 2017; Santos & Ramos, 2018). In this study, all the *F. columnare* isolates obtained from diseased tilapia exhibited multidrug resistance, with resistance recorded against 2 to 10 different antibiotics. Notably,

Table 3. Antibiotic resistance and susceptibility frequencies of the *Flavobacterium oreochromis* isolates (n = 51) observed in this study

Antimicrobial agents	No. of isolates (%)		
	Sensitive	Intermediate	Resistance
Penicillins (PNs)			
Oxacillin (Ox)	0 (0)	0 (0)	51 (100)
Amoxicillin (Ax)	45 (88.3)	3 (5.9)	3 (5.9)
B-Lactam/β-Lactamase inhibitor combination (BL/BLI)			
Amoxicillin-Clavulanic acid (AC)	47 (92.2)	4 (7.9)	0 (0)
Cephalosporin (CPs)			
Ceftriaxone (Ct)	44 (86.3)	7 (13.8)	0 (0)
Cefuroxime (Cu)	33 (64.8)	15 (29.5)	3 (5.9)
Cefotaxime (Cx)	28 (55)	23 (45.1)	0 (0)
Macrolides (MCs)			
Erythromycin (Er)	37 (72.6)	4 (7.9)	10 (19.7)
Quinolones (QLs)			
Nalidixic (Na)	31 (60.8)	3 (5.9)	17 (33.4)
Flouroquinolones (FQNs)			
Ofloxacin (Of)	47 (92.2)	0 (0)	4 (7.9)
Sulfonamides (SULs) (Folate pathway inhibitors)			
Sulfamethoxzole-Trimethoprim (ST)	32 (62.8)	0 (0)	19 (37.3)
Aminoglycosides (AMGs)			
Neomycin (Ne)	4 (7.9)	8 (15.7)	39 (76.5)
Glycopeptide (GLs)			
Vancomycin (Va)	16 (31.4)	6 (11.8)	29 (56.9)
Tetracyclines (TCs)			
Doxycycline (Dx)	30 (58.9)	3 (5.9)	18 (35.3)
Oxytetracycline (OTC)	23 (45.1)	7 (13.8)	21 (41.2)
Amphenicols (AMPs)			
Florfenicol (Fl)	41 (80.4)	4 (7.9)	6 (11.8)

27.5% of the isolates were resistant to between 7 and 10 antibiotics. Similar MDR observations have been reported in *F. columnare* isolated from infected tilapia in Egypt (El-Tawab *et al*., 2020) and other freshwater fish (Declercq *et al*., 2013a). Furthermore, the detection of 14 distinct resistance phenotypes among the isolates highlights the diversity in resistance profiles, which may be attributed to variability in antibiotic use across farming regions or the presence of antibiotic residues in water sources, potentially introduced through runoff from livestock farming or urban wastewater (Preena *et al*., 2020).

Importantly, this study also revealed a notable reduction in susceptibility to several antibiotics currently approved for use in aquaculture in Vietnam. Specifically, the *F.*

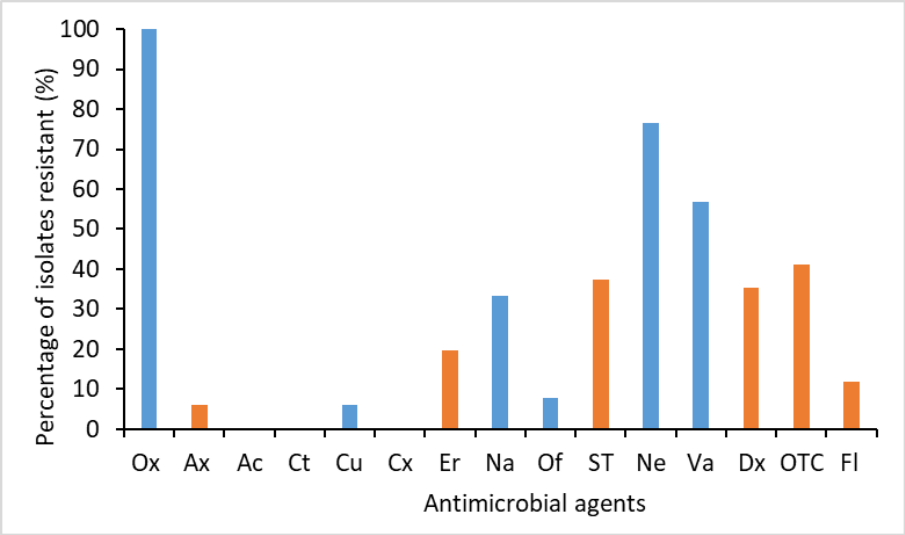


Figure 3. Antibiotic resistance frequencies of the *F. oreochromis* isolates obtained from diseased tilapia. The orange columns indicate antibiotics approved for use in aquaculture in Vietnam.

Table 4. Multidrug resistance phenotypes of the *F. oreochromis* isolates in infected tilapia observed in this study

No. of resistant antibiotics	Resistance phenotypes	No. of resistant isolates	Percentage of isolates resistant (%)
2	Ox + OTC	5	9.8
	Ox+Ne	7	13.7
	Ox+Na	3	5.9
3	Ox + Ne + Va	11	21.6
	Ox+Ax+Ne	3	5.9
4	Ox+ST+Ne+Va	4	7.8
	Ox+ST+Dx+OTC	4	7.8
7	Ox+Na+Of+ST+Ne+Va+Dx	2	3.9
8	Ox+Na+Of+ST+Ne+Va+Dx+OTC	2	3.9
	Ox+Er+Na+ST+Ne+Va+Dx+OTC	4	7.8
	Ox+Er+Na+Ne+Va+Dx+OTC+Fl	3	5.9
10	Ox+Cu+Er+Na+ST+Ne+Va+Dx+OTC+FL	3	5.9

oreochromis isolates exhibited resistance rates exceeding 30% to oxytetracycline, doxycycline, and sulfamethoxazole-trimethoprim. Continued misuse of these antibiotics, including inappropriate dosing or treatment duration, could further exacerbate resistance, ultimately reducing treatment efficacy. This trend could contribute to an increased risk of horizontal gene transfer of resistance determinants between bacterial species (Watts *et al*., 2017). In northern Vietnam, antibiotic resistance has also been reported in other bacterial pathogens affecting tilapia, such as *Aeromonus hydrophila* (Nhinh *et al*., 2021), *Edwardsiella ictaluri* (Nhinh *et al*., 2022), and *Streptocuoccus agalactiae* (Truong Thi My Hanh *et al*., 2024), highlighting the need for systematic monitoring and stricter antibiotic management across the aquaculture sector.

MIC results

The MIC results of *E. coli* (QC strain) fell into acceptable ranges based on the CLSI

guidelines (2020). The results of the MIC testing for the six antibiotics approved for use in aquaculture revealed substantial variation in the MIC distributions of the *F. oreochromis* isolates retrieved from diseased tilapia (**Table 5**; **Figure 4**). For amoxicillin, the MIC values ranged from 0.063 to 1 µg mL^{-1}, with MIC_{50} and MIC_{90} values of 0.25 and 0.5 µg mL^{-1}, respectively. Erythromycin showed a broader MIC distribution, ranging from 0.125 to 8 µg mL^{-1}, with MIC_{50} at 0.5 µg mL^{-1} and MIC_{90} at 2 µg mL^{-1}. Florfenicol exhibited MIC values between 0.25 and 8 µg mL^{-1}, with MIC_{50} and MIC_{90} values of 1 and 4 µg mL^{-1}, respectively.

Oxytetracycline showed a MIC range of 0.016 to 1 µg mL^{-1}, with MIC_{50} and MIC_{90} values of 0.125 and 0.25 µg mL^{-1}, respectively. Doxycycline had the narrowest MIC distribution, from 0.125 to 1 µg mL^{-1}, with corresponding MIC_{50} and MIC_{90} values of 0.25 and 0.5 µg mL^{-1}, respectively. In contrast, sulfamethoxazole-trimethoprim had a wider MIC range, from 0.25 to 8 µg mL^{-1}, with MIC_{50} and MIC_{90} values of 2 and 4 µg mL^{-1}, respectively.

Studies on the antibiotic resistance and minimum inhibitory concentration (MIC) of *F. oreochromis* remain limited. The MIC results obtained in this study revealed considerable variations in MIC ranges across different antibiotics, indicating differences in antimicrobial susceptibility among the *F. oreochromis* isolates. Compared with the MIC

Table 5. Distribution of the MIC values of the *F. oreochromis* isolates for six antimicrobial agents

Antimicrobials	Number of isolates with MIC (µg mL^{-1})												MIC_{50}	MIC_{90}
	0.008	0.016	0.032	0.063	0.125	0.25	0.5	1	2	4	8	16		
Amoxicillin				5	16	21	6	3					0.25	0.5
Erythromycin					7	13	12	9	5	3	2		0.5	2
Florfenicol						4	8	17	9	11	2		1	4
Oxytetracycline		6	9	7	11	13	3	2					0.125	0.25
Doxycycline					18	21	9	3					0.25	0.5
Sul/Trim						11	7	7	9	12	5		2	4

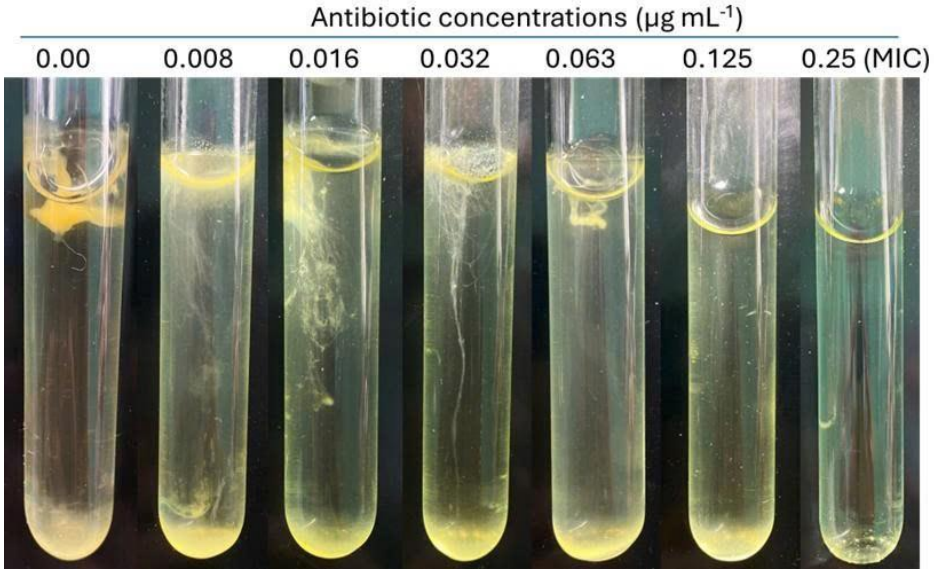


Figure 4. Visualization of the growth inhibition of *F. oreochromis* at different concentrations of amoxicillin. Bacterial growth decreased progressively with increasing antibiotic concentrations (0-0.125 µg mL^{-1}), and no visible growth was observed at concentrations ≥0.25 µg mL^{-1}, indicating the MIC at this level.

data reported by Declercq *et al*. (2013) for *F. columnare* isolated from 17 freshwater fish species collected from various regions worldwide, our findings show both notable differences and a tendency toward increased MIC thresholds. For example, the MIC range for erythromycin in the previous study was 0.06-4 μg mL^{-1}, while in the present study, it was 0.125-8 μg mL^{-1}. Similarly, sulfamethoxazole-trimethoprim exhibited a broader MIC range in our study (0.25-8 μg mL^{-1}) compared to the 0.064-4 μg mL^{-1} previously reported. Interestingly, the MIC range for oxytetracycline in this study (0.016-1 μg mL^{-1}) was lower than the range of 0.032-4 μg mL^{-1} reported by Declercq *et al*. (2013a). Within the same antibiotic, considerable variation in the MIC values was observed among the bacterial isolates.

In Vietnam, MIC data for bacterial pathogens affecting tilapia remain limited. Dung *et al*. (2008) reported MIC values for *E. ictaluri* isolated from pangasius, in which the MIC_{50} and MIC_{90} for amoxicillin were both 0.5 μg mL^{-1}, comparable to 0.25 and 0.5 μg mL^{-1} observed for *F. oreochromis* in this study. For florfenicol, the MIC value for *E. ictaluri* was 0.25 μg mL^{-1}, whereas in our study, the *F. oreochromis* isolates had MIC_{50} and MIC_{90} values of 1 and 4 μg mL^{-1}, respectively. The most pronounced contrast was observed with oxytetracycline: MIC_{50} and MIC_{90} values for *E. ictaluri* were 32 and 64 μg mL^{-1}, respectively, while for *F. oreochromis* in this study, the values were only 0.125 and 0.25 μg mL^{-1}.

The variations in MIC values of the isolates observed in this study, whether within the same antibiotic or compared with other reports, may result from differences in antibiotic usage practices across geographical regions, fish species, culture systems, and bacterial isolates. The findings underscore that antibiotic dosages for treating bacterial infections in aquaculture should not be generalized across pathogens or host species. Determining the MIC values is crucial not only for monitoring antimicrobial resistance trends but also for designing effective treatment protocols and optimizing dosage. The use of subtherapeutic doses may result in treatment failure and promote the development of antibiotic resistance.

Conclusions

Flavobacterium oreochromis isolated from diseased tilapia exhibited considerable variability in susceptibility and resistance across different antibiotics. The relatively high resistance rates to several commonly used antimicrobial agents in aquaculture highlight the potential decline in treatment efficacy and a narrowing of antibiotic options for managing infections. MIC values vary among bacterial isolates, species, and host fish species, indicating that using a uniform antibiotic dosage across different bacterial infections may compromise treatment outcomes and increase the risk of antimicrobial resistance.

Acknowledgements

This work was supported by the Vietnam-Belgium project under grant number T2025VB-13-8.

References

Barony G., Tavares G., Assis G., Luz R., Figueiredo H. & Leal C. (2015). New hosts and genetic diversity of *Flavobacterium columnare* isolated from Brazilian native species and Nile tilapia. Diseases of aquatic organisms. 117(1): 1-11.

Bernardet J.-F. & Bowman J. P. (2006). The genus *Flavobacterium*. Prokaryotes. 7: 481-531.

Bernardet J.-F. & Grimont P. A. (1989). Deoxyribonucleic acid relatedness and phenotypic characterization of *Flexibacter columnaris* sp. nov., nom. rev., *Flexibacter psychrophilus* sp. nov., nom. rev., and *Flexibacter maritimus* Wakabayashi, Hikida, and Masumura 1986. International Journal of Systematic and Evolutionary Microbiology. 39(3): 346-354.

Binh V. N., Dang N., Anh N. T. K. & Thai P. K. (2018). Antibiotics in the aquatic environment of Vietnam: sources, concentrations, risk and control strategy. Chemosphere. 197: 438-450.

Cain K. D. & LaFrentz B. R. (2007). Laboratory maintenance of *Flavobacterium psychrophilum* and *Flavobacterium columnare*. Current Protocols in Microbiology. 6(1): 13B. 11.11-13B. 11.12.

Chen J., Sun R., Pan C., Sun Y., Mai B., & Li Q. X. (2020). Antibiotics and food safety in aquaculture.

Journal of Agricultural and Food Chemistry. 68(43): 11908-11919.

CLSI (2015). Performance Standards For Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI document M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute. ISBN 1-56238-990-4.

CLSI (2020). Performance Standards for Antimicrobial Testing of Bacteria Isolated from Aquatic Animals. 3rd ed. CLSI supplement VET04. Wayne, PA: Clinical and Laboratory Standards Institute. ISBN 1-56238-990-4.

Declercq A., Boyen F., Van Den Broeck W., Bossier P., Karsi A., Haesebrouck F. & Decostere A. (2013a). Antimicrobial susceptibility pattern of *Flavobacterium columnare* isolates collected worldwide from 17 fish species. Journal of Fish Diseases. 36(1): 45-55.

Declercq A. M., Haesebrouck F., Van den Broeck W., Bossier P. & Decostere A. (2013b). Columnaris disease in fish: a review with emphasis on bacterium-host interactions. Veterinary Research. 44(1): 1-17.

Dong H., LaFrentz B., Pirarat N. & Rodkhum C. (2015a). Phenotypic characterization and genetic diversity of *Flavobacterium columnare* isolated from red tilapia, *Oreochromis* sp., in Thailand. Journal of Fish Diseases. 38(10): 901-913.

Dong H. T., Nguyen V. V., Phiwsaiya K., Gangnonngiw W., Withyachumnarnkul B., Rodkhum C. & Senapin S. (2015b). Concurrent infections of *Flavobacterium columnare* and *Edwardsiella ictalur*i in striped catfish, *Pangasianodon hypophthalmus* in Thailand. Aquaculture. 448: 142-150.

Dong H., Senapin S., LaFrentz B. & Rodkhum C. (2016). Virulence assay of rhizoid and non-rhizoid morphotypes of *Flavobacterium columnare* in red tilapia, *Oreochromis* sp., fry. Journal of Fish Diseases. 39(6): 649-655.

Dung T. T., Haesebrouck F., Tuan N. A., Sorgeloos P., Baele M. & Decostere A. (2008). Antimicrobial susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. Microbial Drug Resistance. 14(4): 311-316.

El-Tawab A., El-Hofy F., EL-Gamal R., Awad S., El-Mougy E. & Mohamed S. (2020). Phenotypic and genotypic characterization of Antibiotic resistant strains of *Flavobacterium columnare* isolated from *Oreochromis niloticus* (Nile tilapia). Benha Veterinary Medical Journal. 38(2): 141-145.

Figueiredo H., Klesius P., Arias C., Evans J., Shoemaker C., Pereira Jr. D. & Peixoto M. (2005). Isolation and characterization of strains of *Flavobacterium columnare* from Brazil. Journal of Fish Diseases. 28(4): 199-204.

Haenen O. L., Dong H. T., Hoai T. D., Crumlish M., Karunasagar I., Barkham T., Chen S. L., Zadoks R., Kiermeier A. & Wang B. (2023). Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Reviews in Aquaculture. 15: 154-185.

Truong Thi My Hanh, Nguyen Thi Hanh, Le Thi May, Truong Thi Thanh Vinh & Dang Thi Lua (2024). Antimicrobial resistance in *Streptococcus agalactiae* in tilapia (*Oreochromis* sp.) farming in northern Vietnam. Vinh University Journal of Science. 53 (2A): 15-23 (in Vietnamese).

Hedberg N., Stenson I., Pettersson M. N., Warshan D., Nguyen-Kim H., Tedengren M. & Kautsky N. (2018). Antibiotic use in Vietnamese fish and lobster sea cage farms; implications for coral reefs and human health. Aquaculture. 495: 366-375.

Hoa P. T. P., Managaki S., Nakada N., Takada H., Shimizu, A., Anh D. H., Viet P. H. & Suzuki S. (2011). Antibiotic contamination and occurrence of antibiotic-resistant bacteria in aquatic environments of northern Vietnam. Science of the Total Environment. 409(15): 2894-2901.

Hoai T. D., Nhinh D. T., Giang N. T. H., Van Van K. & Dong H. T. (2025). First report on *Flavobacterium columnare* causing disease outbreaks in cultured black carp, *Mylopharyngodon piceus*, in Northern Vietnam. Microbial Pathogenesis. 202: 107413.

LaFrentz B. R., Králová S., Burbick C. R., Alexander T. L., Phillips C. W., Griffin M. J., Waldbieser G. C., García J. C., de Alexandre Sebastião F. & Soto E. (2022). The fish pathogen *Flavobacterium columnare* represents four distinct species: *Flavobacterium columnare*, *Flavobacterium covae* sp. nov., *Flavobacterium davisi*i sp. nov. and *Flavobacterium oreochromis* sp. nov., and emended description of *Flavobacterium columnare*. Systematic and Applied Microbiology. 45(2): 126293.

MARD (2019). Decision to approve the plan of tilapia farming development by 2020, driven by 2030 issued on May 6, 2016 by the Ministry of Agriculture and Rural Development, Vietnam (MARD) (in Vietnamese).

Nhinh D. T., Giang N. T. H., Van Van K., Dang L. T., Dong H. T. & Hoai T. D. (2022). Widespread presence of a highly virulent *Edwardsiella ictaluri* strain in farmed tilapia, *Oreochromis* spp. Transboundary and Emerging Diseases. 69(5): e2276-e2290.

Nhinh D. T., Le D. V., Van K. V., Huong Giang N. T., Dang L. T. & Hoai T. D. (2021). Prevalence, virulence gene distribution and alarming the multidrug resistance of *Aeromonas hydrophila* associated with disease outbreaks in freshwater aquaculture. Antibiotics. 10(5): 532.

Nhinh D. T., Trinh T. T., Hoa D. T., Tuan N. N., Giang N. T. H., Van K. V., Lua D. T. & Hoai T. D. (2023). Study on isolation and some pathological characteristics of *Flavobacterium columnare* causing infection in tilapia (*Oreochromis* spp.) cultured in Northern Vietnam.

Vietnam Journal of Science and Technology (B). 65(2): 41-47 (in Vietnamese).

Prabu E., Rajagopalsamy C., Ahilan B., Jeevagan I. & Renuhadevi M. (2019). Tilapia–an excellent candidate species for world aquaculture: a review. Annual Research & Review in Biology. 31(3): 1-14.

Preena P., Arathi D., Raj N. S., Arun Kumar T., Arun R., S., Reshma R. & Raja Swaminathan T. (2020). Diversity of antimicrobial-resistant pathogens from a freshwater ornamental fish farm. Letters in Applied Microbiology. 71(1): 108-116.

Romero J., Feijoó C. G. & Navarrete P. (2012). Antibiotics in aquaculture–use, abuse and alternatives. Health and Environment in Aquaculture. 159(1): 159-198.

Santos L. & Ramos F. (2018). Antimicrobial resistance in aquaculture: Current knowledge and alternatives to tackle the problem. International Journal of Antimicrobial Agents. 52(2): 135-143.

Soto E., Mauel M. J., Karsi A. & Lawrence M. (2008). Genetic and virulence characterization of *Flavobacterium columnare* from channel catfish (*Ictalurus punctatus*). Journal Of Applied Microbiology. 104(5): 1302-1310.

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

Wang M. & Lu M. (2016). Tilapia polyculture: a global review. Aquaculture Research. 47(8): 2363-2374.

Watts J. E., Schreier H. J., Lanska L. & Hale M. S. (2017). The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. Marine Drugs. 15(6): 158.

Welker T. L., Shoemaker C. A., Arias C. R. & Klesius P. H. (2005). Transmission and detection of *Flavobacterium columnare* in channel catfish *Ictalurus punctatus*. Diseases of Aquatic Organisms. 63(2-3): 129-138.