Polish Journal of Veterinary Sciences Vol. 29, No. 2 (2026), 179–189

DOI

Original article

Tilapia aquaculture as a reservoir of antimicrobial resistance and zoonotic bacteria: evidence from Makassar, Indonesia

M.F. Mursalim[1,2], H.M. Raharjo[5], Z.A. Kholilulloh[1,2], H.Y. Azis[3,4], M.A. Nurdin[1,2], and N. Mar'ah[6], A.P.N. Hartanti[1], U. Amalia[1], M.J.S. Risal[1], M. Mabrok[7], S.F. Aljasir[8]

[1] Veterinary Study Program, Faculty of Medicine, Hasanuddin University, Makassar 90245, Sulawesi Selatan, Indonesia
[2] Veterinary Paramedic Study Program, Faculty of Vocational Study, Hasanuddin University, Makassar 90245, Sulawesi Selatan, Indonesia
[3] Faculty of Marine and Fisheries Sciences, Hasanuddin University, Makassar 90245, Sulawesi Selatan, Indonesia
[4] Aquaculture and Post-Harvest Fisheries Technology Study Program, Faculty of Vocational Studies, Hasanuddin University, Makassar, 90245, Indonesia
[5] Division of Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, 60115, Indonesia
[6] South Sulawesi Animal, Fish and Plant Quarantine Center, Makassar, 90245, Indonesia
[7] Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt
[8] Department of Veterinary Preventive Medicine, College of Veterinary Medicine, Qassim University, Buraydah 52571, Saudi Arabia

Correspondence to: M.F. Mursalim, e-mail: m.fadhlullah@vet.unhas.ac.id,
S.F. Aljasir, e-mail: s.aljasir@qu.edu.sa

Abstract

A total of 69 bacterial isolates representing 13 species were recovered from 60 tilapia and 14 water samples. Isolates were identified via phenotypic characterization, biochemical assays and VITEK MS, and antimicrobial susceptibility was assessed using the Kirby-Bauer disk diffusion method, followed by calculation of the multiple antibiotic resistance (MAR) index. Dominant taxa included *Bacillus cereus* (21.7%), *Plesiomonas shigelloides* (17.4%) and *Enterobacter* spp. (15.9%), while 11.6% remained unclassified, likely reflecting limitations of the VITEK MS reference database or insufficient spectral matching. Zoonotic species such as *Klebsiella pneumoniae* and *Acinetobacter johnsonii* were also detected. Species distribution did not differ significantly between fish and water, indicating uniform microbial dissemination. Gram-negative isolates showed higher resistance than Gram-positive isolates, with multidrug resistance, particularly against erythromycin–penicillin combinations, most prevalent in *Aeromonas* spp. MAR indices ≥ 0.2 suggest possible exposure to environments associated with antimicrobial contamination. While water quality was generally suitable for tilapia, localized hypoxia and acidic pH may promote pathogen persistence. The coexistence of multidrug-resistant and zoonotic bacteria highlights the need for integrated health management and responsible antimicrobial use in urban aquaculture.

Keywords: antimicrobial resistance, tilapia aquaculture, urban freshwater systems, zoonotic bacteria.

Introduction

In Indonesia, freshwater aquaculture has rapidly expanded through extensification and intensification, with tilapia (*Oreochromis niloticus*) being a major farmed species due to its resilience, market demand, and nutritional value (Food and Agriculture Organization 2024). In Makassar, South Sulawesi, aquaculture is vital for meeting rising fish demand. However, intensive practices, such as high stocking densities and large-scale operations, have increased the risk of infectious disease outbreaks, causing significant economic losses. Tilapia are particularly susceptible to bacterial infections, with pathogens including *Aeromonas* spp., *Plesiomonas shigelloides*, *Streptococcus* spp., *Edwardsiella* spp., *Vibrio* spp., and *Mycobacterium* spp., many of which also pose zoonotic risks (Wamala et al. 2018, Sakala et al. 2022, Chitambo et al. 2023, Haenen et al. 2023). This risk is further exacerbated by the widespread misuse of antibiotics, promoting the development and spread of antimicrobial resistance (AMR). Resistant bacteria and their resistance genes can be transmitted to humans through direct handling of fish, consumption of contaminated products, or contact with contaminated water (Cabello et al. 2013, Reverter et al. 2020).

The global rise in multidrug resistance (MDR) poses a major public health threat, with increasing reports of MDR bacterial pathogens from diverse sources underscoring the need for prudent antibiotic use (Raharjo et al. 2023, Ibrahim et al. 2024). Despite the global threat of AMR, surveillance efforts in Indonesia have been limited, particularly in urban freshwater systems. Although several studies have reported the presence of resistant bacteria in marine and freshwater environments (Mursalim et al. 2022, Raharjo et al. 2022), urban aquaculture ecosystems remain understudied. These ecosystems, characterized by restricted water exchange, waste accumulation and proximity to residential areas, create conditions that increase bacterial persistence and human exposure risk. Specifically, the microbial risks associated with AMR in Tanjung Bunga Lake, an intensively used urban aquaculture site in Makassar for tilapia farming, have not been thoroughly assessed (Sulfikar 2013). The lack of local data hampers risk assessment, weakens regulatory oversight, and limits the ability to develop targeted biosecurity and antibiotic stewardship strategies (Preena et al. 2020, Zaky et al. 2021). Previous aquaculture studies have largely relied on culture-based methods with limited resolution, often underestimating microbial diversity and overlooking fastidious pathogens, while focusing mainly on phenotypic resistance without considering environmental drivers or host–pathogen–environment interactions. This study provides the first systematic assessment of potentially zoonotic and antimicrobial-resistant bacteria in tilapia and water from Tanjung Bunga Lake. Using VITEK MS for identification and standardized Kirby-Bauer disc diffusion for susceptibility testing, we characterized bacterial diversity and resistance patterns and evaluated environmental factors that support pathogen persistence. By establishing baseline data for Indonesian urban aquaculture, these findings support evidence-based management within a One Health framework and highlight the need for strengthened surveillance, practical antibiotic use, and stricter regulatory oversight to protect aquaculture and public health.

Materials and Methods

Sample collection

From March to April 2024, 60 clinically healthy tilapia (*Oreochromis niloticus*; 14-21 cm, ~90 g) were collected from 7 cages (8-9 fish per cage) at Tanjung Bunga Lake, Makassar city, Indonesia, along with 14 water samples collected in sterile 100 ml from seven cages across the lake to capture spatial variability (Table 1, Fig. 1). Seven sampling stations were strategically established to capture the spatial heterogeneity of the tilapia cage aquaculture system in Tanjung Bunga Lake. The stations were positioned across distinct sectors of the lake characterized by variations in proximity to the shoreline and residential effluent inputs, differences in water circulation dynamics, gradients in stocking density and feeding intensity, and contrasting levels of hydrodynamic exposure, ranging from relatively open-water conditions to more enclosed areas. The fish were caught by dip-netting and placed in a plastic bag containing water and air. The fish were subsequently transported under controlled conditions to the Integrated Laboratory at the Faculty of Veterinary Medicine, Hasanuddin University, for bacteriological and antimicrobial resistance analyses.

Bacterial isolation and identification

Before necropsy, all the fish were euthanized via the immersion method with clove oil at a concentration of 60 mg/L. Internal organs (liver, spleen, kidney and brain) were aseptically sampled from each fish using sterile inoculating loops inserted directly into the tissues. Samples were streaked onto tryptic soy agar (TSA; Bacto™) with 5% sheep blood and incubated at 37°C for 24 h to isolate dominant bacteria. After incubation, three to five morphologically distinct colonies per sample were selected based on differences in size, pigmentation, hemolysis and morphology to avoid duplicate isolates.

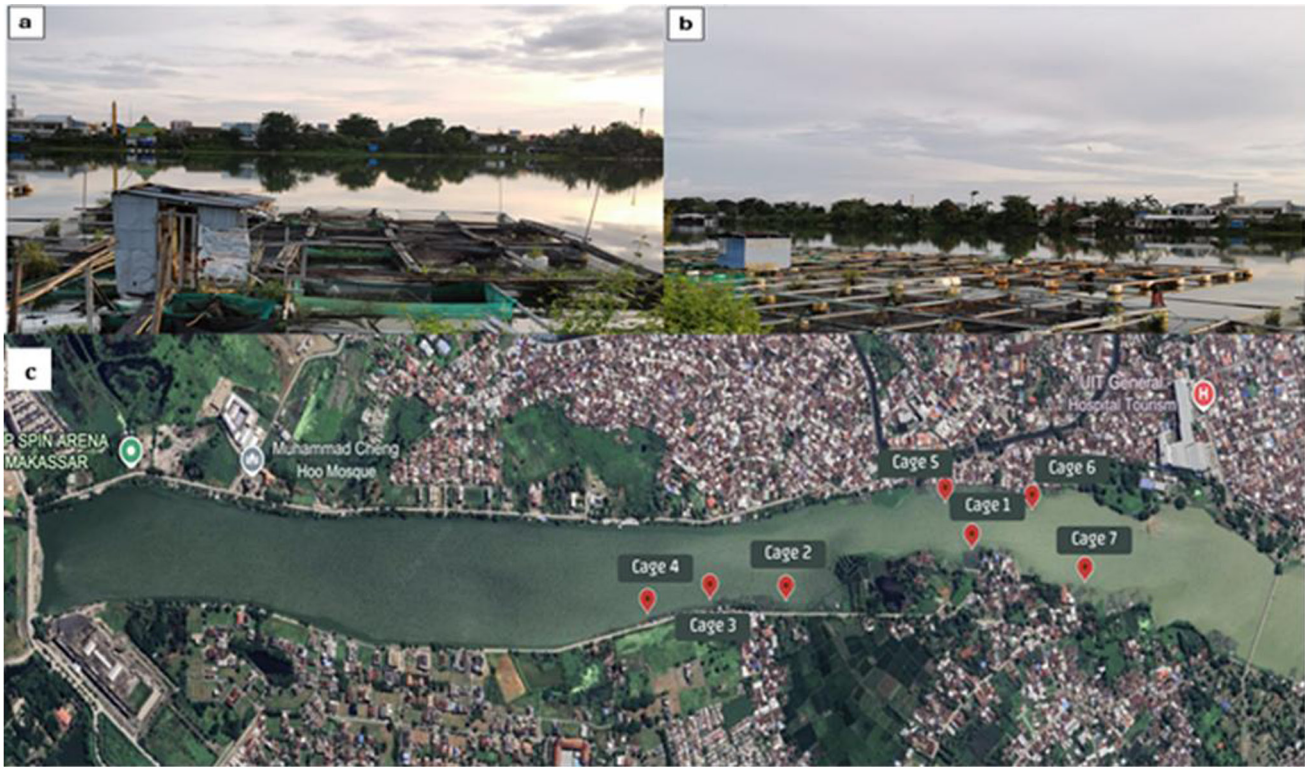

Fig. 1. Sampling sites of tilapia cage aquaculture in Tanjung Bunga Lake, Makassar City: (a, b) Photographic views of floating cages; (c) Spatial distribution of sampling stations on satellite map.

Table 1. Geographic coordinates of the tilapia cage sampling stations in Tanjung Bunga Lake, Makassar city.

No	Sample station	Coordinates
1	Cage 1	S: 5°11′00/E: 119°24’28
2	Cage 2	S: 5°10′50/E: 119°24’12
3	Cage 3	S: 5°10′46/E: 119°24’09
4	Cage 4	S: 5°10′43/E: 119°24’04
5	Cage 5	S: 5°10′58/E: 119°24’32
6	Cage 6	S: 5°11′01/E: 119°24’34
7	Cage 7	S: 5°11′04/E: 119°24’31

Water samples were aseptically collected in sterile glass bottles in the morning and transported on ice for immediate analysis. Inside-cage samples were collected at a depth of 30-50 cm within the cage perimeter, representing water directly influenced by stocking density, feeding, and organic waste. Outside-cage samples were collected at the same depth, 2–3 m from the cage boundary, reflecting adjacent lake water with minimal confinement effects. This paired design allowed assessment of cage-related impacts while accounting for background lake conditions. The water samples were filtered through cellulose ester membranes (0.45 µm pore size) and centrifuged at 2,500 rpm for 5 min before swabbing. Pure colonies from internal organs and water were preliminarily identified based on colony morphology, Gram staining, oxidase and catalase activity, motility, indole production, and esculin hydrolysis (Cowan 1993). For preservation, isolates were grown in tryptic soy broth (TSB; Bacto™) with 20% glycerol and stored at -80°C until further analysis

VITEK MS assay

Bacterial identification was performed via VITEK MS (bioMérieux, France), an automated MALDI-TOF MS platform. A single colony from each isolate was spotted onto a target slide, overlaid with VITEK MS-CHCA matrix solution, air-dried, and inserted into the instrument for spectral acquisition. The generated spectra were compared with the VITEK MS database, and identification was reported via percentage-scaled confidence values (Fang et al. 2012). The identification results were classified based on confidence scores as follows: good identification (confidence level ≥60.0%

and <99.9%), excellent identification (confidence level ≥99.9%), and no reliable identification (confidence level <60.0%), according to the criteria described by Luo et al. (2022).

Water quality analysis

Water quality was assessed simultaneously with tilapia sampling at Tanjung Bunga Lake in Makassar city. At each sampling site, 100 mL of surface water was collected in sterile glass bottles, which were immediately sealed and transported on ice to the laboratory for analysis. The in-situ measurements included water temperature (digital thermometer), pH (portable pH meter), and salinity (refractometer). Laboratory analyses of dissolved oxygen (DO), ammonia, nitrate and nitrite concentrations were performed at the Water Quality Laboratory of the Faculty of Marine Science and Fisheries, Hasanuddin University. Standard methods were applied in accordance with the American Public Health Association guidelines, using spectrophotometric and titrimetric techniques (Eaton).

Antimicrobial susceptibility testing

Antimicrobial susceptibility was assessed using the disk diffusion method following CLSI VET04 guidelines (CLSI, 2020), with *Escherichia coli* ATCC 25922 as the quality control. Six commonly used antibiotics were tested: ciprofloxacin (CIP, 5 µg), chloramphenicol (CN, 10 µg), erythromycin (E, 15 µg), gentamicin (G, 10 µg), penicillin (P, 10 µg), and tetracycline (TE, 30 µg) (Oxoid, UK). Isolates were cultured in TSB at 28°C for 24 h with shaking (160 rpm), adjusted to 0.5 McFarland (1.5×10^8 CFU/mL), and spread onto Mueller-Hinton agar. Disks were applied, and plates incubated at 37 °C for 24 h. Zone diameters were interpreted as susceptible (S), intermediate (I), or resistant (R) per CLSI standards. For species without established breakpoints, values from closely related species were used with justification. Multidrug resistance (MDR) was defined as nonsusceptibility to ≥1 agent in three or more antimicrobial classes. The multiple antibiotic resistance (MAR) index was calculated as MAR = a / b, where a is the number of antibiotics to which an isolate was non-susceptible and b is the total tested (n=6); MAR ≥ 0.2 indicates exposure to high-risk contamination sources (Krumperman 1983).

Statistical analyses

Statistical analyses were conducted using SAS software (v. 9.4; SAS Institute Inc., Cary, NC, USA) with significance set at $p<0.05$. Associations between categorical variables were assessed using Fisher's exact test, and the Wilcoxon signed-rank test compared water physicochemical parameters between inside- and outside-cage samples. Antimicrobial resistance patterns were visualized with a heatmap in OriginPro 9.7 (OriginLab, Northampton, MA, USA). VITEK MS identification and water quality analyses were performed in triplicate to ensure reliability and reproducibility.

Results

Isolation and identification

A total of 69 bacterial isolates were recovered from 60 fish and 14 water samples, representing 13 distinct taxa, as shown in Figure 2 and Table 2. The dominant species were *Bacillus cereus* (21.7%), *Plesiomonas shigelloides* (17.4%), and *Enterobacter asburiae/ Enterobacter cloacae* (15.9%), which collectively accounted for more than half of all isolates. Notably, 11.6% of the isolates could not be classified, indicating uncharacterized strains or limitations in the VITEK MS reference database. Other identified species included *Bacillus megaterium* (7.2%), *Aeromonas* spp. (5.8%) and *Bacillus subtilis* (5.8%), reflecting significant microbial diversity. Opportunistic pathogens, such as *Acinetobacter johnsonii* and *Klebsiella pneumoniae* (2.9% each), are noteworthy because of their zoonotic potential and association with antimicrobial resistance. Additionally, *Staphylococcus* species (*S. sciuri*, *S. gallinarum*, and *S. arlettae*) were detected at low prevalence rates (1.5% each). Statistical analysis revealed no significant associations between bacterial species and sample origin (fish vs. water) ($p>0.05$), indicating a relatively uniform distribution of bacterial taxa across both sources. The biochemical characterization of 12 bacterial isolates obtained from tilapia and associated water samples categorized them into Gram-negative taxa (*Enterobacteriaceae* and related genera) and Gram-positive taxa (notably *Bacillus* and *Staphylococcus* species) (Table 3). The most discriminative traits among these isolates were observed in the oxidase and motility tests. Non-enteric genera such as *Plesiomonas*, *Aeromonas*, and *Acinetobacter* exhibited positive oxidase activity, thereby clearly distinguishing them from *Enterobacter* and *Klebsiella*, which are oxidase-negative. Furthermore, high motility was characteristic of *Bacillus*, *Plesiomonas*, and *Aeromonas*, in contrast to the non-motile behavior of *Staphylococcus*, *Klebsiella*, and *Acinetobacter*. While all the isolates were catalase-positive, additional phenotypic variation, particularly differences in hemolytic patterns (β versus γ hemolysis), provided further taxonomic resolution.

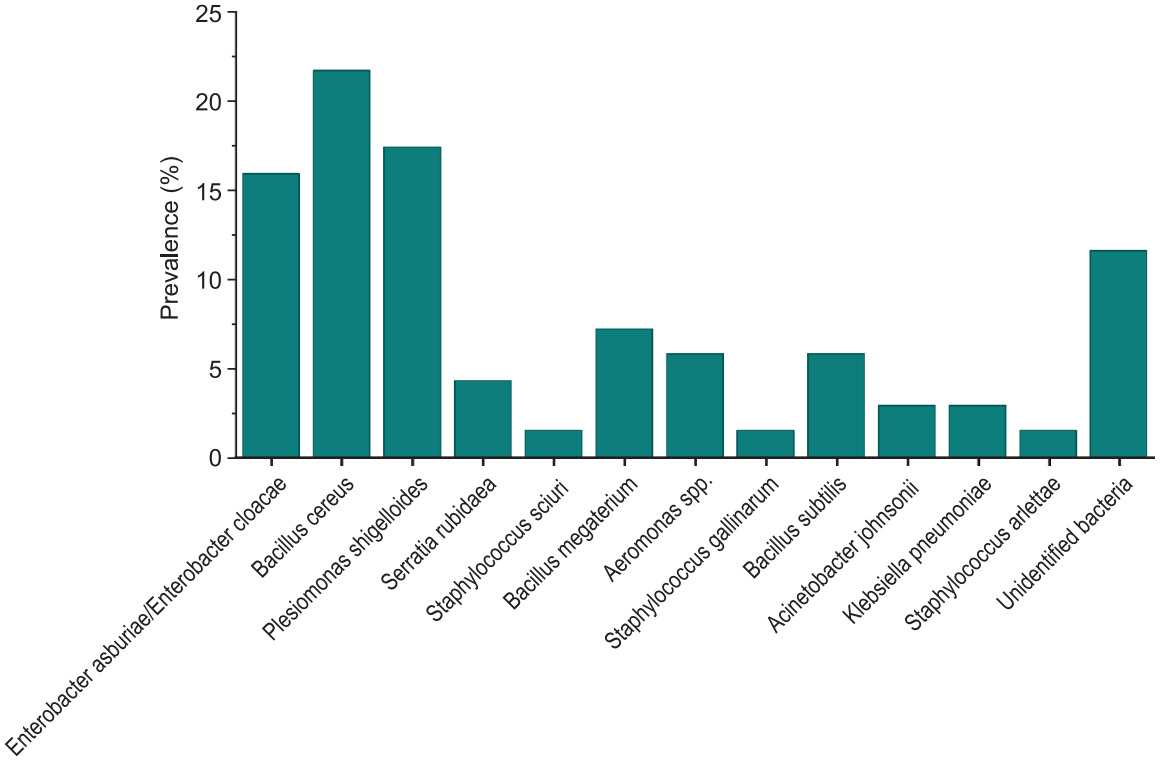

Fig. 2. Prevalence of bacterial species isolated from tilapia and water samples in Tanjung Bunga Lake.

Table 2. Distribution and prevalence of bacterial species isolated from tilapia and water samples in Tanjung Bunga Lake.

Total	Cage 1		Cage 2		Cage 3		Cage 4		Cage 5		Cage 6		Cage 7		Total	
	Fish	Water	Fish	Water	Fish	Water	Fish	Water	Fish	Water	Fish	Water	Fish	Water	No	%
Enterobacter asburiae/ Enterobacter cloacae	2.0	1.0	0.0	1.0	0.0	0.0	2.0	1.0	1.0	0.0	2.0	0.0	0.0	1.0	11.0	15.9
Bacillus cereus	1.0	2.0	1.0	0.0	0.0	0.0	0.0	1.0	2.0	3.0	1.0	1.0	2.0	1.0	15.0	21.7
Plesiomonas shigelloides	2.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	2.0	3.0	1.0	2.0	1.0	12.0	17.4
Serratia rubidaea	2.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	4.3
Staphylococcus sciuri	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.5
Bacillus megaterium	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	2.0	5.0	7.2
Aeromonas spp.	1.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	4.0	5.8
Staphylococcus gallinarum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0	1.5
Bacillus subtilis	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	1.0	4.0	5.8
Acinetobacter johnsonii	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	2.0	2.9
Klebsiella pneumoniae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	2.0	2.9
Staphylococcus arlettae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	1.0	1.5
Unidentified bacteria	0.0	2.0	1.0	0.0	1.0	1.0	2.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	8.0	11.6
Total															69.0	100.0

Antimicrobial susceptibility profile

The antimicrobial resistance profiles exhibited marked interspecific variability (Table 4) and were further visualized in a heatmap generated with OriginPro software (Figure 3). Clustering analysis revealed increased resistance to erythromycin and penicillin among Gram-negative taxa, particularly *Enterobacter* spp., *Plesiomonas shigelloides*, and *Klebsiella pneumoniae*. In contrast, *Bacillus* spp. and *Staphylococcus sciuri* clustered within predominantly susceptible profiles, underscoring clear taxonomic divergence in resistance burden. Overall, erythromycin and penicillin showed the highest non-susceptibility frequencies, particularly among Gram-negative isolates. *Enterobacter* spp., *P. shigelloides*, *Serratia rubidaea*, and *K. pneumoniae* exhibited complete resistance to erythromycin and high levels of resistance to penicillin.

Table 3. Biochemical characteristics of bacterial isolates from tilapia and water samples.

Bacterial Isolates	Number of Isolates	Gram Staining	Biochemical Characteristics Isolates (%)						
			Morphology	Hemolysis	Oxidase	Catalase	Motility	Indole Production	Esculin Hydrolysis
Enterobacter spp.	11.0	-ve	Rods	variable	0.0	100.0	variable	0.0	100.0
Bacillus cereus	15.0	+ve	Rods	β	0.0	100.0	100.0	0.0	100.0
Plesiomonas shigelloides	12.0	-ve	Rods	β	100.0	100.0	100.0	100.0	0.0
Serratia rubidaea	3.0	-ve	Coccobacillus	γ	0.0	100.0	100.0	0.0	100.0
Staphylococcus sciuri	1.0	+ve	Cocci	γ	100.0	100.0	0.0	0.0	100.0
Bacillus megaterium	5.0	+ve	Rods	γ	Variable	100.0	100.0	0.0	100.0
Aeromonas spp.	4.0	-ve	Rods	β	100.0	100.0	100.0	50.0	40.0
Staphylococcus gallinarum	1.0	+ve	Cocci	β	0.0	100.0	0.0	0.0	100.0
Bacillus subtilis	4.0	+ve	Rods	β	Variable	100.0	100.0	0.0	100.0
Acinetobacter johnsonii	2.0	-ve	Rods	β	100.0	100.0	0.0	0.0	0.0
Klebsiella pneumoniae	2.0	-ve	Rods	γ	0.0	100.0	0.0	0.0	100.0
Staphylococcus arlettae	1.0	+ve	Cocci	γ	0.0	100.0	0.0	0.0	100.0

Table 4. Antimicrobial susceptibility test (%) of bacterial isolates from tilapia and water samples from Tanjung Bunga Lake.

Bacterial Species	Total Retrieved Isolates	E (15 µg)	G (10 µg)	CN (10 µg)	CIP (5 µg)	P (10 µg)	TE (30 µg)
		S/I/R	S/I/R	S/I/R	S/I/R	S/I/R	S/I/R
Enterobacter spp.	11.0	0.0/0.0/100.0	100.0/0.0/0.0	72.7/0.0/27.3	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0
Bacillus cereus	15.0	80.0/20.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	93.3/0.0/6.7	0.0/0.0/100.0	93.3/0.0/6.7
Plesiomonas shigelloides	12.0	0.0/25.0/75.0	50.0/25.0/25.0	100.0/0.0/0.0	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0
Serratia rubidaea	3.0	0.0/0.0/100.0	100.0/0.0/0.0	67.7/33.3/0.0	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0
Staphylococcus sciuri	1.0	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0
Bacillus megaterium	5.0	80.0/20.0/0.0	60.0/20.0/20.0	100.0/0.0/0.0	60.0/20.0/20.0	0.0/0.0/100.0	100.0/0.0/0.0
Aeromonas spp.	4.0	50.0/0.0/50.0	50.0/0.0/50.0	50.0/0.0/50.0	50.0/0.0/50.0	50.0/0.0/50.0	50.0/0.0/50.0
Staphylococcus gallinarum	1.0	0.0/0.0/100.0	100.0/0.0/0.0	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0	0.0/100.0/0.0
Bacillus subtilis	4.0	75.0/25.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	75.0/0.0/25.0	100.0/0.0/0.0	75.0/0.0/25.0
Acinetobacter johnsonii	2.0	100.0/0.0/0.0	0.0/50.0/50.0	0.0/0.0/100.0	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0
Klebsiella pneumoniae	2.0	0.0/0.0/100.0	0.0/0.0/100.0	0.0/100.0/0.0	0.0/100.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0
Staphylococcus arlettae	1.0	0.0/0.0/100.0	100.0/0.0/0.0	100.0/0.0/0.0	100.0/0.0/0.0	0.0/0.0/100.0	100.0/0.0/0.0

S = susceptible, I = intermediate, R = resistant, E = erythromycin, G = gentamicin, CN = chloramphenicol, CIP = ciprofloxacin, P = penicillin; TE = tetracycline.

Aeromonas spp. displayed a distinct pattern of 50% resistance and 50% susceptibility across all tested antibiotics, reflecting either strain-level diversity or inconsistent exposure to antimicrobial agents. In contrast, Gram-positive bacteria presented relatively high susceptibility to the tested antimicrobial agents. *Bacillus cereus* was fully susceptible to gentamicin and chloramphenicol, with low resistance rates to ciprofloxacin (6.7%) and tetracycline (6.7%). *Staphylococcus sciuri* demonstrated complete susceptibility to all the tested agents. Similarly, *Bacillus megaterium* and *Bacillus subtilis* remained largely susceptible, underscoring the comparatively lower resistance burden among these species. An exception was *Acinetobacter johnsonii*, which was resistant to penicillin and chloramphenicol but remained fully susceptible to ciprofloxacin, erythromycin, and tetracycline. Statistical analysis confirmed that resistance patterns differed significantly among antibiotics ($p<0.05$), indicating that the choice of antimicrobial agent is a critical determinant of bacterial susceptibility profiles. The analysis of resistance patterns (Table 5) revealed that the most common resistance profile was a combination of erythromycin and penicillin (E+P) in *Enterobacter, P. shigelloides,* and *Serratia*. Triple-drug resistance combinations, including E+CN+P, E+G+P, and CIP+P+TE, were detected in *Enterobacter*, *P. shigelloides*, *B. cereus*, *Serratia,* and *Klebsiella*, indicating the emergence of multidrug resistance (MDR) across species. *Aeromonas* spp. demonstrated the most concerning antimicrobial

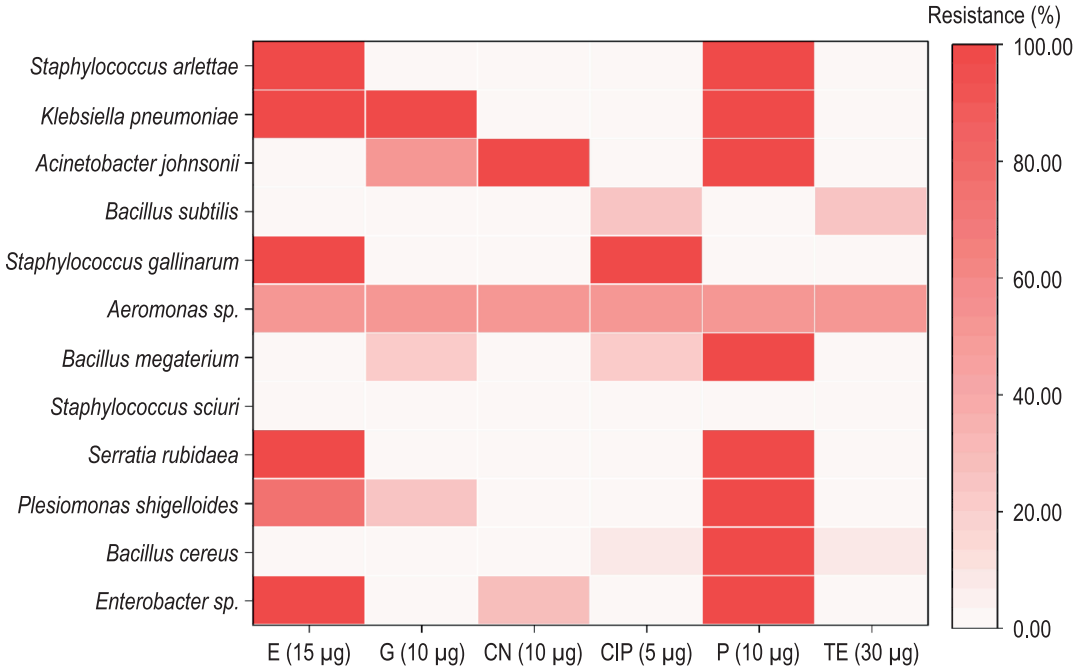

Fig. 3. Heatmap illustrating interspecies variation in antimicrobial resistance profiles among bacterial isolates. The color gradient represents the degree of resistance, with darker shades indicating greater resistance. E = erythromycin, G = gentamicin, CN = chloramphenicol, CIP = ciprofloxacin, P = penicillin; TE = tetracycline.

Table 5. Distribution of antimicrobial resistance patterns among bacterial isolates from tilapia and water samples from Tanjung Bunga Lake.

Resistance Pattern	*Entero-bacter* spp. (*n*=11)	*Bacillus cereus* (*n*=15)	*Plesiomonas shigelloides* (*n*=12)	*Serratia rubidaea* (*n*=3)	*Staphylococ-cus sciuri* (*n*=1)	*Bacillus megaterium* (*n*=5)	*Aeromonas* spp. (*n*=4)	*Staphy-lococcus gallinarum* (*n*=1)	*Bacillus subtilis* (*n*=4)	*Acine-tobacter johnsonii* (*n*=2)	*Klebsiella pneumoniae* (*n*=2)	*Staphylococ-cus arlettae* (*n*=1)
P	-	13.0	-	-	-	-	-	-	-	-	-	-
CN, P	-	-	-	-	-	-	-	-	-	1.0	-	-
CIP, E	-	-	-	-	-	-	-	1.0	-	-	-	-
E, P	8.0	-	9.0	2.0	-	-	-	-	-	-	-	1.0
G, P	-	-	-	-	-	2.0	-	-	-	-	-	-
CIP, P	-	1.0	-	-	-	-	-	-	-	-	-	-
CIP, TE	-	-	-	-	-	-	-	-	2.0	-	-	-
CN, G, P	-	-	-	-	-	-	-	-	-	1.0	-	-
CIP, G, P	-	-	-	-	-	3.0	-	-	-	-	-	-
CIP, P, TE	-	1.0	-	-	-	-	-	-	-	-	-	-
E, CN, P	3.0	-	-	1.0	-	-	-	-	-	-	-	-
E, G, P	-	-	3.0	-	-	-	-	-	-	-	2.0	-
CIP, E, T, P	-	-	-	-	-	-	-	-	-	-	-	-
CN, CIP, E, G, P	-	-	-	-	-	-	-	-	-	-	-	-
CN, CIP, E, G, P, TE	-	-	-	-	-	-	2.0	-	-	-	-	-

E = erythromycin, G = gentamicin, CN = chloramphenicol, CIP = ciprofloxacin, P = penicillin; TE = tetracycline.

resistance profile, with 50% of the isolates exhibiting resistance to all the tested antibiotics and displaying a broad resistance pattern encompassing six different antimicrobial agents.

Multiple antibiotic resistance (MAR) index

The majority of the isolates presented multiple antibiotic resistance (MAR) index values ranging from 0.33 to 0.50. Notably, 50% of the *Aeromonas* spp. isolates had a MAR index of 1.0, indicating resistance to all the tested antimicrobial classes. *K. pneumoniae*, *A. johnsonii*, and *B. megaterium* also showed elevated MAR values of 0.50. MAR indices of 0.2 or higher indicate exposure to significant antibiotic use, suggesting that most bacteria in this study originated from environments affected by antibiotics. In contrast, *S. arlettae* exhibited a low MAR index (0.16), consis-

Table 6. Distribution of multiple antibiotic resistance (MAR) indices among bacterial isolates.

No.	Bacterial Species	No. of Isolates	% MAR 0.16	% MAR 0.33	% MAR 0.50	% MAR 0.66	% MAR 0.83	% MAR 1.00
1	*Enterobacter* spp.	11.0	0.0	72.7	**27.3**	0.0	0.0	0.0
2	*Bacillus cereus*	15.0	86.6	6.7	6.7	0.0	0.0	0.0
3	*Plesiomonas shigelloides*	12.0	0.0	75.0	**25.0**	0.0	0.0	0.0
4	*Serratia rubidaea*	3.0	0.0	66.7	**33.3**	0.0	0.0	0.0
5	*Staphylococcus sciuri*	1.0	0.0	0.0	0.0	0.0	0.0	0.0
6	*Bacillus megaterium*	5.0	0.0	40.0	**60.0**	0.0	0.0	0.0
7	*Aeromonas* spp.	4.0	0.0	0.0	0.0	0.0	0.0	**50.0**
8	*Staphylococcus gallinarum*	1.0	0.0	100.0	0.0	0.0	0.0	0.0
9	*Bacillus subtilis*	4.0	0.0	50.0	0.0	0.0	0.0	0.0
10	*Acinetobacter johnsonii*	2.0	0.0	50.0	**50.0**	0.0	0.0	0.0
11	*Klebsiella pneumoniae*	2.0	0.0	0.0	**50.0**	0.0	0.0	0.0
12	*Staphylococcus arlettae*	1.0	100.0	0.0	0.0	0.0	0.0	0.0

MAR index ≥0.2 indicates that the isolate originated from an environment with high antibiotic exposure. Bold values highlight isolates with elevated MAR indices (≥0.5), indicating multidrug resistance. The percentages represent the proportion of isolates within each species corresponding to the respective MAR index

Table 7. Spatial variation in the physicochemical water quality parameters of Tanjung Bunga Lake.

Water Quality Parameters		Sampling Site							Reference Standard Range
		Cage 1	Cage 2	Cage 3	Cage 4	Cage 5	Cage 6	Cage 7	
Temperature (°C)	Outside	30.00	30.00	29.00	30.00	31.00	31.00	31.00	28.00-32.00°C
	Inside								
Salinity (mg/L)	Outside	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00-30.00 mg/L
	Inside								
pH	Outside	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00-9.00
	Inside								
Dissolve Oxygen (mg/L)	Outside	7.36	2.88	10.24	5.76	3.48	4.48	6.08	>3.00 mg/L
	Inside	5.12	2.24	6.08	5.44	4.16	5.44	8.31	
Nitrate (mg/L)	Outside	0.15	0.14	0.02	0.19	0.13	0.12	0.02	<0.20 mg/L
	Inside	0.14	0.16	0.02	0.13	0.13	0.12	0.01	
Nitrite (mg/L)	Outside	0.09	0.56	0.08	0.06	0.06	0.05	0.05	<0.20 mg/L
	Inside	0.09	0.06	0.05	0.06	0.05	0.06	0.05	
Ammonia (mg/L)	Outside	0.01	0.01	0.003	0.003	0.006	0.004	0.003	<0.02 mg/L
	Inside	0.01	0.01	0.004	0.003	0.006	0.004	0.003	

Salinity values of 0.00 mg/L indicate measurements below the instrument detection limit.

tent with its isolation from an environment under minimal antibiotic pressure. These findings demonstrate considerable variation in resistance intensity among species, with some taxa showing a strong tendency toward multidrug resistance. The MAR indices (%) of the retrieved isolates are presented in Table 6.

Water quality analysis

Most physicochemical parameters were within acceptable ranges for tilapia culture, as shown in Table 7. The water temperature in the outer areas ranged from 29 to 31°C, within the optimal range of 28 to 32°C. Salinity was consistently 0 mg/L across all the sites, confirming the lake's freshwater status in accordance with its geographic and ecological setting. The pH was slightly acidic (pH = 6 at all outer points) but within the tolerable range for tilapia (6–9). The dissolved oxygen (DO) exhibited substantial spatial variation (2.24-10.24 mg/L). The low DO values at outer point 2 (2.88 mg/L) and inner point 2 (2.24 mg/L) fell below the recommended minimum (>3 mg/L), poten-

tially stressing the fish, suppressing immune responses, and favoring anaerobic microbial growth. In contrast, the elevated DO at point 3 (10.24 mg/L) was likely associated with intensive microalgal photosynthesis. Nitrate ranged from 0.0141 to 0.1875 mg/L, remaining within safe limits (<0.2 mg/L). The nitrite concentrations were generally low (0.0451–0.556 mg/L) but exceeded the threshold at outer point 2, suggesting localized pollution at this point. Ammonia remained within safe levels (<0.02 mg/L) at all the sites, with the highest concentration recorded at Point 1 (0.0095 mg/L). Although nontoxic in its current ionic form, ammonia can shift to a more toxic free form (NH_3) under elevated pH and temperature, posing potential ecological risks. Wilcoxon signed-rank tests indicated no statistically significant differences between inside- and outside-cage measurements for temperature ($p=0.842$), pH ($p=1.000$), dissolved oxygen (DO; $p=0.317$), nitrate ($p=0.528$), nitrite ($p=0.463$), and ammonia ($p=0.779$).

Discussion

This study hypothesized that tilapia and their aquatic environment in Tanjung Bunga Lake harbor potentially zoonotic bacteria exhibiting antimicrobial resistance (AMR). Our findings support this hypothesis, demonstrating the presence of several pathogenic species, including *Bacillus cereus*, *Plesiomonas shigelloides*, *Enterobacter* spp., and *Klebsiella pneumoniae*, with diverse multidrug-resistant (MDR) profiles. The predominance of *B. cereus*, *P. shigelloides*, and *Enterobacter* spp. reflects a microbial community shaped by urban environmental pressures and is consistent with reports from aquaculture systems in Asia and Africa, where these taxa are commonly associated with subclinical infections in fish and zoonotic transmission to humans (Chitambo et al. 2023). In contrast, investigations of clinically infected tilapia in Africa revealed a relatively high prevalence of *Aeromonas hydrophila* (43.8%), *Aeromonas sobria* (20.8%), *Edwardsiella tarda* (8.3%), *Streptococcus* spp. (6.3%), and *Flavobacterium* spp. (4.2%) (Wamala et al. 2018), suggesting that bacterial communities differ substantially between healthy and diseased hosts. Although *Aeromonas* spp. were detected at a relatively low prevalence in this study, their presence remains significant, given their documented virulence and resistance potential in aquaculture environments (Igbinosa et al. 2017, Fernández-Bravo and Figueras 2020).

Interestingly, *B. cereus* is also widely used as a probiotic in Nile tilapia farming, where it enhances nonspecific immunity and beneficially modulates the gut microbiota, thereby improving host resistance to pathogens (Wang et al. 2017). Similarly, *P. shigelloides* is a frequent commensal of freshwater fish species, including tilapia, carp, trout, and eels, but can act as an opportunistic pathogen under stress or immunocompromised conditions (Janda et al. 2016). In Egypt, *Enterobacter* spp. isolated from tilapia have demonstrated substantial diversity, with several strains displaying zoonotic potential and distinct antibiotic resistance profiles (Hamza et al. 2020). The AMR profiles observed in this study highlight the critical risks to aquaculture and public health. The most notable finding was widespread resistance to erythromycin and penicillin across several genera, including *Enterobacter* spp., *Plesiomonas shigelloides*, *Serratia rubidaea* and *Klebsiella pneumoniae*. These patterns are consistent with previous studies showing the poor efficacy of β-lactams and macrolides against Gram-negative bacteria owing to intrinsic outer membrane permeability barriers and efflux pump systems (Blair et al. 2015, Poirel et al. 2018, Reverter et al. 2020, Phu et al. 2021). *Aeromonas* spp., a key pathogen known to cause septicemia in fish and wound infections in humans, displayed a concerning profile of 50% resistance and 50% susceptibility across all tested antibiotics, including ciprofloxacin and gentamicin, which are agents frequently used in human and veterinary medicine. This heterogeneous resistance likely reflects the coexistence of multiple strains harboring distinct resistance determinants, potentially mediated by plasmids, integrons or horizontal gene transfer in antibiotic-rich aquatic environments (Sun et al. 2019).

Compared with Gram-positive isolates, Gram-negative isolates generally presented greater resistance burdens. *B. cereus*, *B. subtilis*, and *S. sciuri* remained mostly susceptible, particularly to chloramphenicol, ciprofloxacin and tetracycline. For example, *B. cereus* is sensitive to gentamicin and chloramphenicol, with minimal resistance to ciprofloxacin and tetracycline. These findings align with earlier studies reporting comparatively lower resistance in Gram-positive aquatic bacteria (Kesarcodi-Watson et al. 2008). Additionally, antimicrobial susceptibility testing relies on a single quality-control strain, which may limit comprehensive validation of both Gram-negative and Gram-positive isolates. The high non-susceptibility to penicillin and erythromycin observed among Gram-negative bacteria is consistent with well-established intrinsic resistance mechanisms, including outer membrane permeability barriers and active efflux systems characteristic of these taxa. While antimicrobial selection pressure associated with aquaculture activities cannot be excluded, the absence of direct data on antibiotic usage in the present study limits causal inference. Therefore, the observed resistance to these antibiotics should be interpreted with

caution, as it may primarily reflect intrinsic rather than acquired resistance. Nonetheless, the presence of resistance to commonly used antimicrobial agents may indicate broader environmental exposure to antimicrobials, warranting further investigation. Environmental conditions may further influence the dynamics of pathogens and resistance expression. Low dissolved oxygen (DO < 3 mg/L) observed at some sites is known to impair fish immune responses while favoring facultative anaerobic bacterial growth (Bulbul Ali and Mishra 2022). Although ammonia concentrations were low, increases in pH and temperature could shift ammonium toward the more toxic unionized ammonia (NH_3) form, thereby compromising fish health and increasing the risk of infection (Kleinhappel et al. 2019, Parvathy et al. 2023). Although most physicochemical parameters fell within acceptable ranges, nitrite levels at one site exceeded the recommended threshold, presenting an additional stressor with the potential to disrupt fish physiology and increase susceptibility to opportunistic pathogens (Kroupova et al. 2005).

In conclusion, this study identified bacteria of public health significance in Nile tilapia from Tanjung Bunga Lake and characterized their antibiotic resistance profiles, revealing notable food safety and public health risks. The findings underscore the urgent need for improved aquaculture management to curb the emergence and spread of antibiotic-resistant bacteria. Future research should focus on longitudinal monitoring of resistance dynamics and the development of sustainable, eco-friendly interventions to reduce antibiotic reliance in aquaculture. This study has limitations. Culture-based methods capture only the culturable fraction of the microbial community, with incubation conditions potentially favoring some taxa while underrepresenting fastidious, anaerobic, or viable but non-culturable organisms. Additionally, 12% of isolates remained unidentified due to VITEK MS limitations. The results reflect culturable bacteria rather than the full microbiome, and integrating molecular approaches could enhance ecological resolution and generalizability. The absence of molecular confirmation and resistance gene profiling further restricts interpretation, while the cross-sectional, single-site design prevents assessment of seasonal or spatial variations.

Acknowledgements

We gratefully acknowledge the Veterinary Study Program microbiology laboratory for providing the facilities necessary for this research. This study was supported by the Institute for Research and Community Service, Hasanuddin University, under grant number 00309/UN4.22/PT.01.03/2024. The Researchers would like to thank the Deanship of Graduate Studies and Scientific Research at Qassim University for financial support (QU-APC-2026).

Author Declarations

Ethics approval

Fish sampling and handling were performed in accordance with protocols approved by the Hasanuddin University Health Research Ethics Commission (UH24050348).

Use of generative artificial intelligence

The authors used ChatGPT to assist with grammar correction and minor language editing. The AI tool did not contribute to the scientific content, interpretation, or conclusions. All content was reviewed and approved by the authors.

Conflict of interest

The authors declare no competing interests.

References

Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ (**2015**) Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol 13: 42-51.

Bulbul AA, Mishra A (**2022**) Effects of dissolved oxygen concentration on freshwater fish: a review. Int J Fish Aquat Stud 10: 113-127.

Cabello FC, Godfrey HP, Tomova A, Ivanova L, Dölz H, Millanao A, Buschmann AH (**2013**) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. Environ Microbiol 15: 1917-1942.

Chitambo B, Munyeme M, Hang'ombe B (**2023**) Identifying bacteria with public health significance from farmed Nile Tilapia (Oreochromis niloticus), Zambia. Int J Microbiol 2023: 6650378.

CLSI (The Clinical and Laboratory Standards Institute) (**2020**) Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals. CLSI guideline VET04 (3rd ed.). Wayne, Pennsylvania: Clinical Laboratory Standards Institute.

Cowan ST (**1993**) Manual for the identification of medical bacteria. J Clin Pathol 46: 975.

Eaton A, APHA (American Public Health Association), AWWA (American Water Works Association), and WEF (Water Environment Federation) (**2005**) Standard methods for the examination of water and wastewater. 21st ed. Method.

Fang H, Ohlsson AK, Ullberg M, Özenci V (**2012**) Evaluation of species-specific PCR, Bruker MS, VITEK MS, and the VITEK 2 system for the identification of clinical Enterococcus isolates. Eur J Clin Microbiol Infect Dis 31: 3073-3077.

Food and Agriculture Organization (**2024**) The State of World Fisheries and Aquaculture 2024 – Blue Transformation in action. FAO, Rome.

Fernández-Bravo A, Figueras M J (**2020**) An update on the genus Aeromonas: taxonomy, epidemiology, and pathogenicity. Microorganisms 8: 129.

Haenen OLM, Dong HT, Hoai TD, Crumlish M, Karunasagar I, Barkham T, Chen SL, Zadoks R, Kiermeier A, Wang B, Gamarro EG, Takeuchi M, Azmai MNA, Fouz B, Pakingking Jr R, Wei ZW, Bondad-Reantaso MG (**2023**) Bacterial diseases of tilapia, their zoonotic potential and risk of antimicrobial resistance. Rev Aquac 15: 154-185.

Hamza D, Dorgham S, Ismael E, El-Moez SI, Elhariri M, Elhelw R, Hamza E (**2020**) Emergence of β-lactamase and carbapenemase-producing Enterobacteriaceae at integrated fish farms. Antimicrob Resist Infect Control 9: 67.

Ibrahim GA, Mabrok M, Alfifi KJ, Alatawy M, Al-Otaibi AS, Alenzi AM, Rahman AN, El-Malt RM, Ibrahim SA, El-Tarabili RM, Algammal A (**2024**) Pathogenicity, resistance patterns, virulence traits, and resistance genes of re-emerging extensively drug-resistant (XDR) Aeromonas veronii in Oreochromis niloticus. Aquac Int 32: 6987-7006.

Igbinosa IH, Beshiru A, Odjadjare EE, Ateba CN, Igbinosa EO (**2017**) Pathogenic potentials of *Aeromonas* species isolated from aquaculture and abattoir environments. Microb Pathog 107: 185-192.

Janda JM, Abbott SL, Mciver CJ (**2016**) Plesiomonas shigelloides revisited. Clin Microbiol Rev 29: 349-374.

Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (**2008**) Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274: 1-14.

Kleinhappel TK, Burman, OH, John, EA, Wilkinson A, Pike TW (**2019**) The impact of water pH on association preferences in fish. Ethology 125: 195-202.

Kroupova H, Machova J, Svobodova Z (**2005**) Nitrite influence on fish: a review. Vet Med Czech 50: 461-471.

Krumperman PH (**1983**) Multiple antibiotic resistance indexing of Escherichia coli to identify high-risk sources of fecal contamination of foods. Appl Environ Microbiol 46: 165-170.

Luo L, Liang L, Zhang R, Chen W, Yu F, Zhao Y, Yue J (**2022**) Using Vitek MS v3.0 to identify nontuberculous Mycobacteria in liquid media in a clinical microbiology laboratory. Microbiol Spectr 10: e0201822.

Mursalim MF, Budiyansah H, Raharjo HM, Debnath PP, Sakulworakan R, Chokmangmeepisarn P, Yindee J, Piasomboon P, Elayaraja S, Rodkhum C (**2022**) Diversity and antimicrobial susceptibility profiles of Aeromonas spp. isolated from diseased freshwater fishes in Thailand. J Fish Dis 45: 1149-1163.

Parvathy AJ, Das, BC, Jifiriya MJ, Varghese T, Pillai D, Kumar VJ (**2023**) Ammonia-induced toxico-physiological responses in fish and management interventions. Rev Aquac 15: 452-479.

Phu DH, Cuong NV, Truong DB, Kiet BT, Hien VB, Thu HT, Yen LK, Minh, NT, Padungtod P, Setyawan E, Thwaites G, Rushton J, Carrique-Mas J (**2021**) Reducing antimicrobial usage in small-scale chicken farms in Vietnam: a 3-year intervention study. Front Vet Sci 7: 612993.

Poirel L, Madec, JY, Lupo A, Schink A-K, Kieffer N, Nordmann P, Schwarz S (**2018**) Antimicrobial resistance in Escherichia coli. Microbiol Spectr 6: 10.1128.

Preena PG, Swaminathan, TR, Kumar VJ., Singh IS (**2020**) Antimicrobial resistance in aquaculture: a crisis for concern. Biologia 75: 1497-1517.

Raharjo HM, Budiyansah H, Mursalim MF, Chokmangmeepisarn P, Sakulworakan R, Debnath PP, Sivaramasamy E, Intan ST, Chuanchuen R, Dong HT, Mabrok M, Rodkhum C (**2023**) The first evidence of $bla_{CTX-M-55}$, QnrVC5, and novel insight into the genome of MDR Vibrio vulnificus isolated from Asian sea bass (Lates calcarifer) identified by resistome analysis. Aquaculture 571: 739500.

Raharjo HM, Budiyansah H, Mursalim MF, Chokmangmeepisarn P, Sakulworakan R, Madyod S, Sewaka M, Sonthi M, Debnath PP, Elayaraja S, Rung-Ruangkijkrai T, Dong HT, Rodkhum C (**2022**) Distribution of Vibrionaceae in farmed Asian sea bass, Lates calcarifer in Thailand and their high prevalence of antimicrobial resistance. J Fish Dis 45: 1355-1371.

Reverter M, Sarter S, Caruso D, Avarre JC, Combe M, Pepey E, Pouyaud L, Vega-Heredía S, de Verdal H, Gozlan RE (**2020**) Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat Commun 11: 1870.

Sakala T, Mdegela RH, Hangombe BM (**2022**) Identifying bacteria associated with diseased Oreochromis niloticus in Lake Kariba, Zambia. Asian J Trop Biotechnol 19.

Sulfikar S (**2013**) Water quality survey of Tanjung Bunga Rowing Lake, Makassar. J Nat Sci 2: 32-51.

Sun D, Jeannot K, Xiao Y, Knapp CW (**2019**) Editorial: Horizontal gene transfer mediated bacterial antibiotic resistance. Front Microbiol 10: 1933.

Wamala S, Mugimba K, Mutoloki S, Evensen Ø, Mdegela R, Byarugaba D, Sørum H (**2018**) Occurrence and antibiotic susceptibility of fish bacteria isolated from Oreochromis niloticus (Nile tilapia) and Clarias gariepinus (African catfish) in Uganda. Fish Aquat Sci 21: 6.

Zaky MM, Toubar SM, El-Shafey AS (**2021**) Antibiotic-resistant bacteria in water and fish: a risk to human health. Int J Celiac Dis 9: 77-81.