



Article

Phenotypic and MALDI-TOF MS Characterization of Presumptive Antimicrobial-Resistant Bacteria from Hungarian Aquatic Environments

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Abstract

Antimicrobial resistance (AMR) in aquaculture environments has become an increasing concern due to the dissemination of antibiotic-resistant bacteria in aquatic ecosystems. This study investigated the occurrence of presumptive antimicrobial-resistant bacteria in commercial fish farms in Hajdú-Bihar county, Hungary. Between 2023 and 2024, water samples were collected from fishponds from Hajdúszoboszló, Biharugra, and Debrecen, followed by membrane filtration with 0.45 µm pore size mixed cellulose ester membrane filters, bacterial isolation using selective media including vancomycin-resistant enterococci (VRE) agar, Eosin Methylene Blue (EMB) agar, and Endo's medium. The phenotypic characterization revealed the presence of diverse bacterial populations that could be associated with potential antimicrobial resistance. Bacterial identification using Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) showed several bacterial genera, predominantly *Acinetobacter*, *Pseudomonas*, *Enterococcus*, *Bacillus*, and *Escherichia*. A total of 16 presumptive antimicrobial-resistant bacterial isolates recovered from selective media were identified using MALDI-TOF MS. Several detected species, including *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus faecium*, and *Enterococcus faecalis*, are recognized as opportunistic bacteria associated with antimicrobial resistance dissemination. The findings demonstrate that aquaculture waters in Hajdú-Bihar county may serve as reservoirs of antimicrobial-resistant bacteria.

Keywords: aquaculture; antimicrobial-resistant bacteria; fishponds; vancomycin-resistant bacteria; MALDI-TOF MS; *Enterococcus*; *Acinetobacter*

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1. Introduction

Aquaculture is one of the fastest-growing food production sectors worldwide and plays an essential role in global food security and increasing fish production [1–3]. However, the rapid expansion and intensification of aquaculture practices, characterized by accelerated production cycles, high stocking densities, and broadened species diversification, systematically increase exposure to pathogens and opportunities for disease transmission. Across bacterial, viral, and fungal threats, the literature converges on mechanisms driven by high crowding, stress, water quality deterioration, and movement of animals and products, which together elevate incidence, severity, and economic losses from infectious diseases and bacterial outbreaks in cultured fish species [4–9]. To reduce the losses caused by bacterial infections, antibiotics have frequently been applied in aquaculture systems for both therapeutic and prophylactic purposes [10–12]. The use of antibiotics in aquaculture has been reported by several studies from different countries. For example, in 2019, a review by Lulijwa et al. [11] reported that 11 of the top 15 fish-producing countries used 67 antibiotic compounds, 73% of which used sulphadiazine, oxytetracycline, and florfenicol. The extensive, and sometimes uncontrolled, use of antimicrobial agents in fish farming has contributed to the emergence and dissemination of antimicrobial-resistant bacteria and facilitated horizontal gene transfer (HGT) of resistance determinants within and between aquatic and terrestrial ecosystems [13–17]. As a result, aquaculture systems are increasingly recognized as important reservoirs and vectors for antimicrobial resistance (AMR) dissemination, raising concerns regarding fish health, environmental sustainability, and public health [16].

In the European Union (EU), the use of antibiotics is strictly regulated under the Veterinary Medicinal Products Regulation (EU 2019/6) [18]. Although therapeutic use under veterinary prescription remains permitted, the use of antibiotics for growth promotion is prohibited. These measures have contributed to a remarkable reduction in veterinary antimicrobial sales, by approximately 43% between 2019 and 2023 [19,20]. Despite strict European regulations on antibiotic use, antimicrobial resistance remains a challenge in aquatic environments. Although Europe shows relatively low antibiotic contamination of water (11.36 ng/L) compared with Asia (193.10 ng/L) and Africa (381.87 ng/L), the persistence of antibiotic resistance determinants continues to cause ecological and public health concerns [21]. The aquatic environments still provide favorable conditions for the persistence and horizontal transfer of antibiotic resistance genes (ARGs) among bacterial communities, even when the selective pressure from environmental antibiotic residues disappears [22,23]. Antimicrobial-resistant infections remain a major public health concern in Europe. According to recent estimates from the European Centre for Disease Prevention and Control (ECDC), antimicrobial resistance is associated with more than 35,000 deaths annually in the EU/EEA, highlighting the urgent need for coordinated surveillance and stewardship efforts across human, animal, and environmental sectors [23]. Additionally, modeling studies have projected that, by 2050, antimicrobial resistance could increase the annual death from 700,000 to 10 million and result in losses of up to USD 100 trillion [24–26]. According to Murray et al. [27], resistance involving fluoroquinolones and β -lactam antibiotics (including carbapenems, cephalosporins, and penicillins) accounted for more than 70% of deaths attributable to antimicrobial resistance globally. ARB in aquatic systems has gained increasing scientific attention due to their potential role in environmental dissemination of resistance determinants and possible transmission to humans and animals [14].

Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) has emerged as a rapid, accurate, and cost-effective method for bacterial identification in aquatic environments and aquaculture systems, enabling genus- and species-level identification based on unique protein spectral fingerprints generated from microbial cells [28,29]. In aquaculture, MALDI-TOF MS has been successfully applied for the rapid identification of bacterial pathogens, particularly *Vibrio* spp. and other members of Vibrionaceae isolated from fish, thereby facilitating disease diagnosis and epidemiological investigations

[30–32]. In routine microbiological workflows, bacterial colonies obtained through culture-based methods can be rapidly analyzed using MALDI-TOF MS, providing reliable species-level identification and demonstrating high concordance with conventional biochemical and molecular method [28,29,31]. Despite its advantages, this technique may exhibit limited discriminatory power when distinguishing closely related bacterial species, particularly among environmental isolates that are underrepresented in reference databases.

The objective of this study was to isolate presumptive antimicrobial-resistant bacteria from selected Hungarian aquaculture environments using VRE, EMB, and Endo media, and to identify the recovered isolates by MALDI-TOF MS analysis. The findings of this study contribute to the understanding of presumptive antimicrobial-resistant bacterial communities in Hungarian aquaculture environments and emphasize the importance of continuous microbiological monitoring and responsible antimicrobial management in sustainable aquaculture production systems.

2. Materials and Methods

2.1. Sample Collection

Water samples were collected as grab samples from each sampling site during the respective campaigns. One bottle (500 mL) sample was collected from each pond under aseptic conditions and transported to the University of Debrecen's microbiology laboratory in Hungary for microbiological analysis. Where possible, samples were collected from areas that were accessible and representative of the wider water body. The aim of this preliminary survey was to determine the presence of presumptive antimicrobial-resistant bacteria in different aquaculture environments in Hajdú-Bihar County, Hungary (Figure 1).

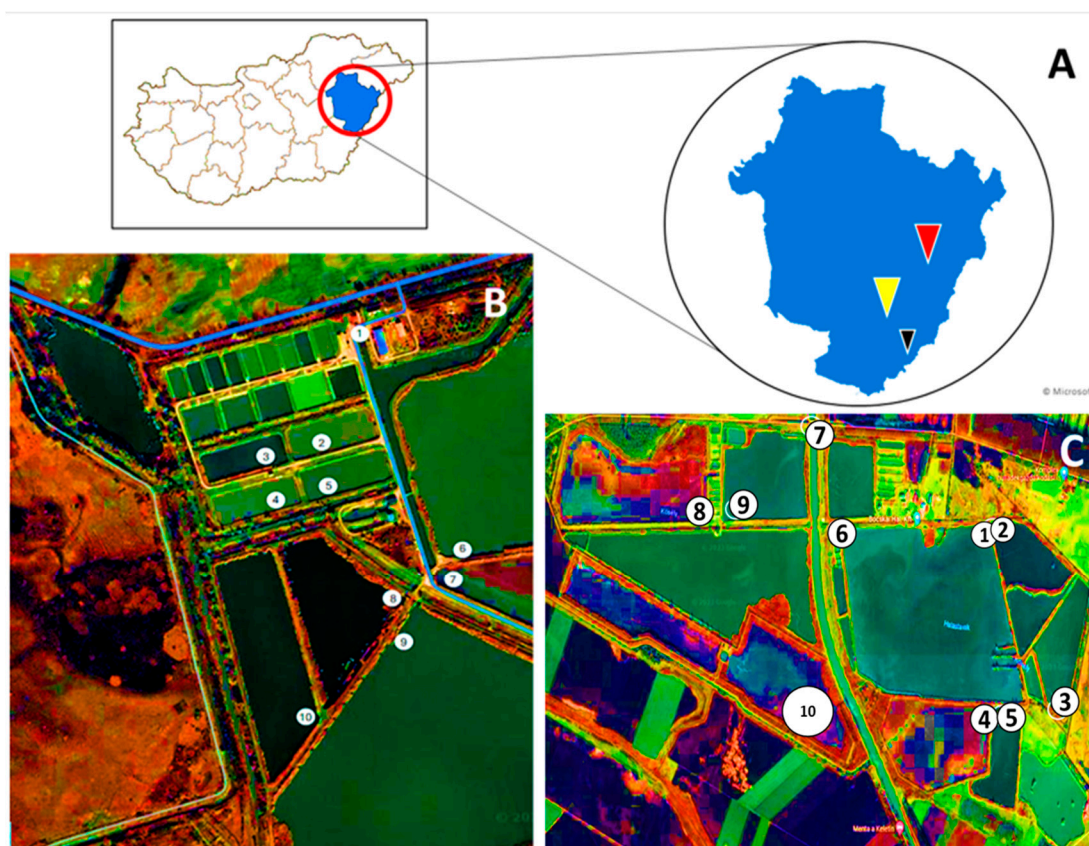


Figure 1. Geographic location of the sampling sites in eastern Hungary. (A) Map of Hungary highlighting Hajdú-Bihar County and the distribution of sampling locations within the county. (B) Detailed view of sampling sites at commercial fishponds in Hajdúszoboszló. (C) Aquaculture pond sampling sites in Biharugra.

A total of 23 water samples were collected during the study from different locations; ten of these samples were obtained from 10 fishponds in Hajdúszoboszló (~47.45° N, 21.40° E) (April 2023), including R1, R2, R6, R7, RA, R1T, U1, U2, U5 and the Eastern Main Canal. A further ten samples were collected from aquaculture ponds in Biharugra (~46.97° N, 21.60° E) (October, 2023). In September 2024, supplementary samples (3 samples) were collected from various fish sites within Hajdú-Bihar county, Debrecen (~47.53° N, 21.63° E). The investigated sites include Vezér lakes, the Great Park/Nagyerdő, and fish tanks of RAS at the Fish Laboratory, University of Debrecen. Water samples were collected independently from each pond, with one sample obtained per fishpond. All samples were aseptically collected in sterile containers and transported to the laboratory for microbiological analysis, in order to determine the possibility of occurrence of antibiotic-resistant bacteria.

Sampling was conducted during two consecutive years to facilitate access to multiple aquaculture sites and to expand the geographical coverage of the survey. The study was designed as a qualitative assessment of bacterial occurrence, rather than a temporal comparison; therefore, environmental parameters were not statistically compared between sampling periods

2.2. Filtration and Culture Conditions

The samples were transported to the laboratory of microbiology, University of Debrecen. The samples were kept under refrigerated conditions at 4 °C for a maximum of 24 h prior to analysis. Water samples were processed using the membrane filtration technique, whereby each sample was mixed thoroughly, and a measured volume of 100 mL was filtered through sterile 0.45 µm pore size mixed cellulose ester membrane filters (Millipore, Merck KGaA, Darmstadt, Germany) using a vacuum filtration apparatus. Following filtration, the membranes were aseptically placed onto selective and differential culture media for the isolation of presumptive antibiotic-resistant bacteria. Three types of selective media were used for bacterium isolation, including Vancomycin-Resistant Enterococci (VRE) medium (chromID™ VRE, bioMérieux, Marcy-l'Étoile, France) and Eosin Methylene Blue (EMB) agar (Merck KGaA, Darmstadt, Germany), supplemented with 2 mg/L cefotaxim. In addition, Endo agar (Merck KGaA, Darmstadt, Germany) was used for the isolation and characterization of coliform and other Gram-negative bacteria present in the water samples. All inoculated media were incubated aerobically for 48 to 72 h at 37 °C; the colonies were noticed and phenotypically characterized. The selected bacterial colonies were cultured on blood agar plates for 24 h at 37 °C and then submitted to MALDI-TOF analysis.

2.3. MALDI-TOF Mass Spectrometry Analysis of Cultured Bacteria

The MALDI-TOF MS (Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry) Biotyper (Bruker Daltonics, Bremen, Germany) identification method (Figure 2) was used to identify 23 bacterial isolates. The isolates were individually sub-cultured on blood agar plates (prepared in the laboratory) and, after a 24 h incubation at 37 °C, pure bacterial colonies were used for identification. Small amounts of bacteria were transferred to single spots on the target plate by plastic inoculating loops, then topped with 1 µL 70% formic acid. After drying, 1 µL matrix solution was pipetted onto the samples. Generation of spectra and analysis were conducted as defined by Kačániová et al. [33]. MALDI-TOF MS score values were interpreted according to the modified criteria proposed by Smith et al. [34] for environmental bacterial isolates. Scores between 2.00 and 3.00 were considered high-confidence identifications, scores between 1.70 and 1.99 indicated low-confidence identifications, and scores below 1.70 were considered unreliable.

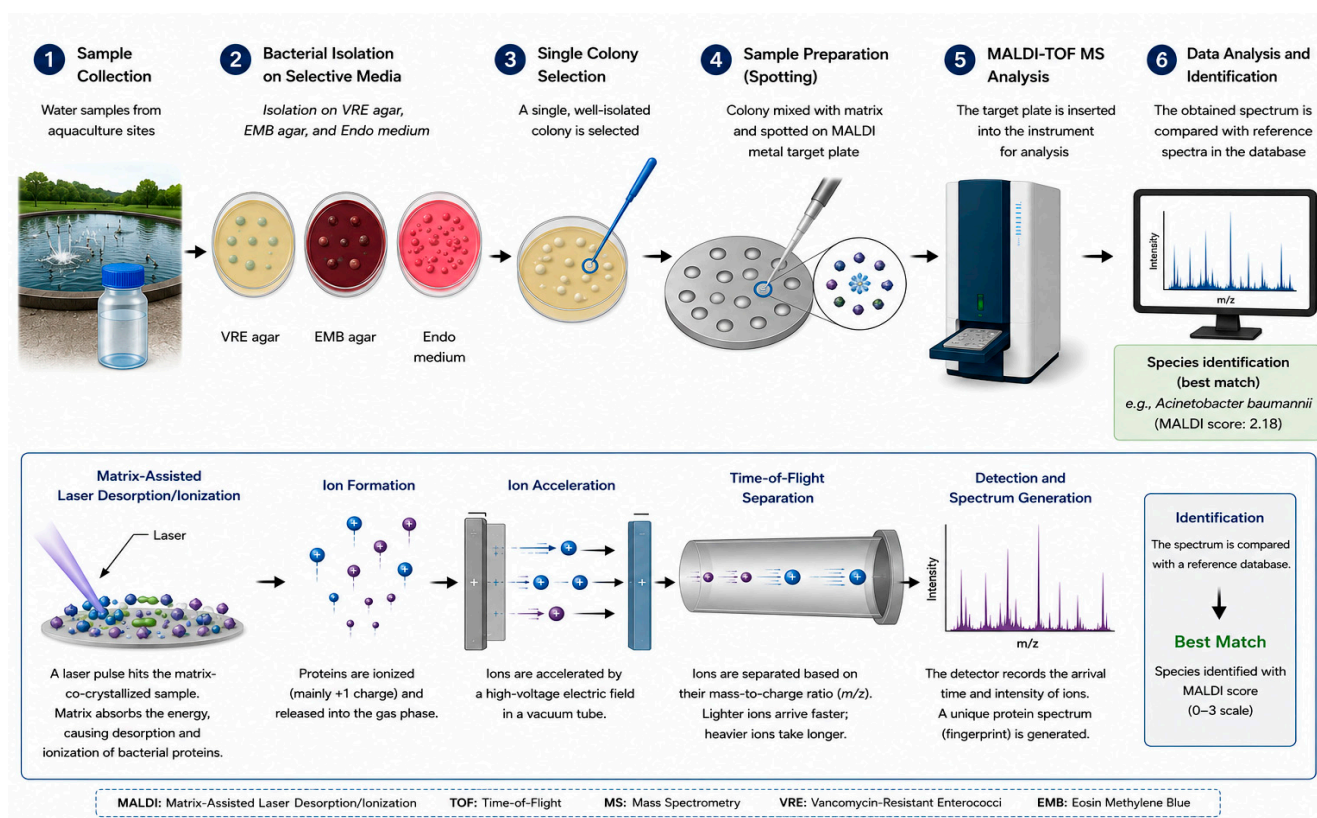


Figure 2. Schematic overview of the MALDI-TOF MS workflow for the isolation, preparation, and identification of presumptive antibiotic-resistant bacteria from aquaculture water samples. The procedure includes sample collection, bacterial isolation on selective media, colony selection, MALDI target plate preparation, mass spectrometry analysis, and bacterial identification based on protein spectral fingerprinting.

3. Results

3.1. Phenotypic Characteristics

The bacterial isolates obtained from the water samples, Hajdú-Bihar County, Hungary, exhibited phenotypic characteristics on selective media that could reflect the presence of presumptive antimicrobial-resistant, and potentially pathogenic, strains. Colonies on vancomycin-resistant agar (Figures 3 and 4) showed growth patterns that are typical of *Enterococcus* spp. This was suggestive of presumptive vancomycin tolerance or resistance, which is a significant indicator of multidrug resistance among enterococci. Growth on EMB (Figure 3A) and Endo agar (Figure 3C) indicated the presence of Gram-negative enteric bacteria, including lactose- and non-lactose-fermenting isolates and coliform bacteria. These phenotypic traits suggest the presence of diverse presumptive antibiotic-resistant bacterial populations in the sampled aquatic environment, which highlight the relevance of these results for further antimicrobial resistance testing in aquaculture-associated environments.

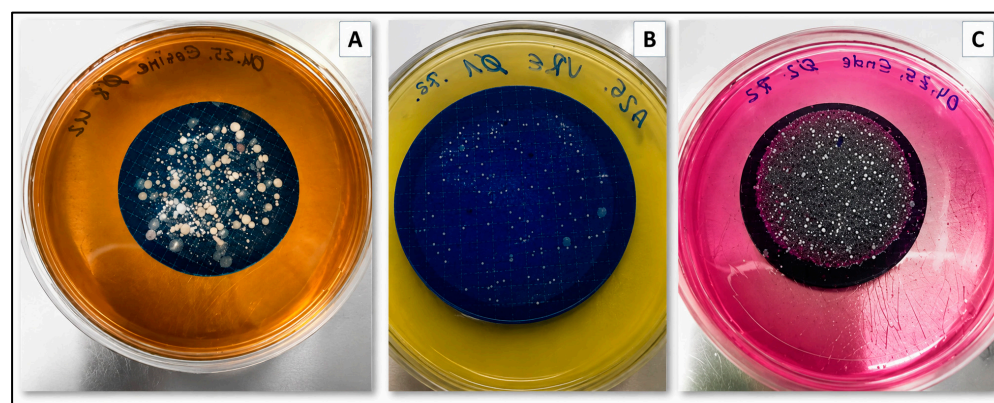


Figure 3. Bacterial colonies recovered from water samples collected in Hajdú-Bihar County, Hungary, and cultivated on selective media: (A) Eosin methylene blue (EMB) agar, showing characteristic colony growth of Gram-negative enteric bacteria; (B) vancomycin-resistant agar (VRA), showing bacterial growth under selective conditions for the presumptive recovery of vancomycin-resistant enterococci; and (C) Endo agar, showing characteristic colony growth of Gram-negative enteric bacteria.

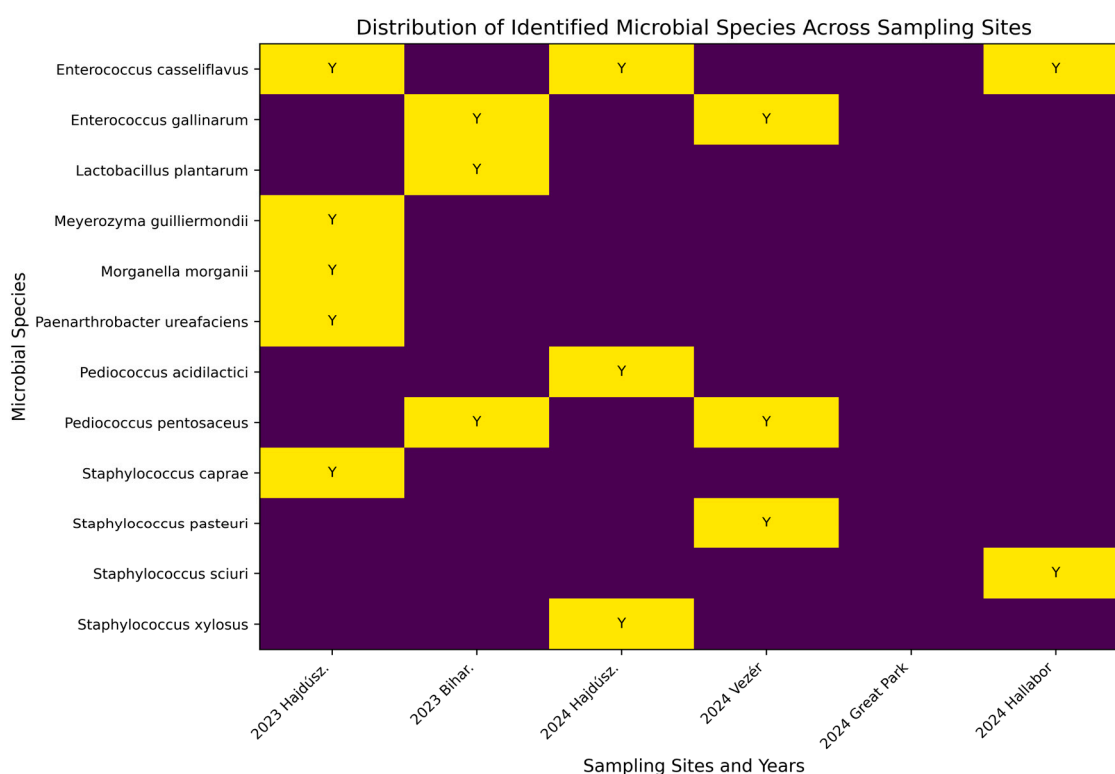


Figure 4. Detection matrix of presumptive vancomycin-resistant bacterial isolates recovered from water samples on VRE medium and subsequently identified by MALDI-TOF MS. Yellow boxes indicate the occurrence of bacterial isolates recovered under the selective conditions of VRE medium.

3.2. MALDI-TOF MS Analysis

MALDI-TOF MS analysis identified 23 bacterial isolates from fish pond samples collected from two sites in Hajdú-Bihar County, Hungary, between April and October 2023 (Table 1). The isolates belonged to several genera, including *Acinetobacter*, *Pseudomonas*, *Enterococcus*, *Bacillus*, *Escherichia*, *Lactobacillus*, *Pediococcus*, *Stenotrophomonas*, *Ochrobactrum*, and *Comamonas*. Members of the genus *Acinetobacter* were the most prevalent (eight isolates), followed by *Pseudomonas* spp. (five isolates). Several of the identified bacterial

species are known to be associated with antimicrobial resistance, including *E. coli*, *Enterococcus casseliflavus*, *Enterococcus gallinarum*, and *Acinetobacter baumannii*.

Table 1. Bacterial isolates identified from two different sampling sites in Hajdú-Bihar county (Hajdúszoboszló and Biharugra, April and October 2023, respectively), Hungary, using MALDI-TOF MS analysis.

No.	Location	Organism (Best Match)	Medium	MALDI Score Value
1	H + B	<i>Acinetobacter baumannii</i>	N/A	2.18
2	H + B	<i>Enterococcus casseliflavus</i>	VRE	2.04
3	B	<i>Enterococcus gallinarum</i>	VRE	2.30
4	H + B	<i>Stenotrophomonas maltophilia</i>	N/A	2.08
5	B	<i>Lactobacillus plantarum</i>	VRE	2.40
6	B	<i>Acinetobacter junii</i>	N/A	2.24
7	B	<i>Pseudomonas alcaligenes</i>	N/A	2.32
8	B	<i>Pediococcus pentosaceus</i>	VRE	2.15
9	H + B	<i>Escherichia coli</i>	N/A	2.03
10	B	<i>Pseudomonas mendocina</i>	N/A	2.15
11	B	<i>Pseudomonas otitidis</i>	N/A	2.42
12	B	<i>Acinetobacter junii</i>	N/A	2.27
13	B	<i>Pseudomonas aeruginosa</i>	N/A	2.24
14	B	<i>Ochrobactrum intermedium</i>	N/A	2.45
15	H + B	<i>Acinetobacter pittii</i>	N/A	2.03
16	B	<i>Acinetobacter tandoii</i>	N/A	2.22
17	B	<i>Bacillus pumilus</i>	N/A	1.82
18	B	<i>Pseudomonas putida</i>	N/A	1.99
19	B	<i>Comamonas aquatica</i>	N/A	1.96
20	H + B	<i>Acinetobacter baumannii</i>	N/A	1.87
21	B	<i>Bacillus pumilus</i>	N/A	1.75
22	B	<i>Acinetobacter tandoii</i>	N/A	1.76
23	H + B	<i>Acinetobacter pittii</i>	N/A	1.75

Scores of ≥ 2.0 indicate a high-confidence identification, while scores ranging from 1.7 to 1.99 indicate a low-confidence identification. H: Hajdúszoboszló, B: Biharugra, VRE: vancomycin-resistant enterococci, N/A: not available.

MALDI score values ranged from 1.75 to 2.45, with 16 bacterial isolates producing scores ≥ 2.0 . However, the highest identification scores were recorded for *Ochrobactrum intermedium* (2.45), *Pseudomonas otitidis* (2.42), and *Lactobacillus plantarum* (2.40). In contrast, a total of 30 bacterial isolates were identified from four sampling sites in Hajdú-Bihar County, Hungary, in 2024 (Table 2). The isolates comprised 10 genera, including *Acinetobacter*, *Pseudomonas*, *Enterococcus*, *Pediococcus*, *Staphylococcus*, *Bacillus*, *Enterobacter*, *Comamonas*, *Achromobacter*, and *Chryseobacterium*. The most frequently detected genera were *Acinetobacter* (seven isolates), *Pseudomonas* (nine isolates), and *Enterococcus* (five isolates). *Acinetobacter pittii* was the most common species, occurring at three sampling sites with scores ≥ 2.05 .

Table 2. Bacterial isolates identified from 4 sampling sites from Hajdú-Bihar, Hungary (2024).

Sample ID	No.	Organism (Best Match)	MALDI Score Value
Debrecen (Vezér lake)	1	<i>Acinetobacter junii</i>	2.17
	2	<i>Acinetobacter soli</i>	2.10
	3	<i>Pseudomonas otitidis</i>	2.17
	4	<i>Pseudomonas aeruginosa</i>	2.25

	5	<i>Pseudomonas mendocina</i>	2.11
	6	<i>Staphylococcus pasteurii</i>	1.96
Debrecen (The Great Park, Nagyerdő)	7	<i>Enterococcus casseliflavus</i>	1.81
	8	<i>Pseudomonas mendocina</i>	2.03
	9	<i>Acinetobacter pittii</i>	2.05
	10	<i>Pseudomonas alcaliphila</i>	1.85
Debrecen (Fish laboratory)	11	<i>Staphylococcus sciuri</i>	2.04
	12	<i>Enterococcus casseliflavus</i>	2.35
	13	<i>Acinetobacter pittii</i>	2.16
	14	<i>Acinetobacter baylyi</i>	2.34
	15	<i>Pseudomonas aeruginosa</i>	2.28
	16	<i>Bacillus cereus</i>	1.94
Hajdúszoboszló	17	<i>Pediococcus acidilactici</i>	2.07
	18	<i>Pediococcus pentosaceus</i>	2.20
	19	<i>Enterococcus gallinarum</i>	2.12
	20	<i>Pseudomonas otitidis</i>	2.22
	21	<i>Enterobacter bugandensis</i>	2.27
	22	<i>Acinetobacter soli</i>	2.24
	23	<i>Enterococcus faecium</i>	2.27
	24	<i>Enterococcus faecalis</i>	2.28
	25	<i>Acinetobacter pittii</i>	2.33
	26	<i>Achromobacter insulitus</i>	2.00
	27	<i>Comamonas aquatica</i>	2.21
	28	<i>Chryseobacterium arthrosphaerae</i>	2.11
	29	<i>Pseudomonas alcaliphila</i>	2.28
	30	<i>Pseudomonas monteilii</i>	1.85

Scores of ≥ 2.0 indicate a high-confidence identification, while scores ranging from 1.7 to 1.99 indicate a low-confidence identification.

Several species of clinical and environmental importance were identified, including *Pseudomonas aeruginosa*, *Enterobacter bugandensis*, *Enterococcus faecium*, and *Acinetobacter* spp. MALDI score values ranged from 1.81 to 2.35, with 25 isolates yielding ≥ 2.0 , indicating high-confidence species identification. The highest score was obtained for *Enterococcus casseliflavus* isolated from the fish laboratory (2.35), followed by *Acinetobacter baylyi* (2.34) and *Acinetobacter pittii* (2.33).

4. Discussion

Aquaculture has been recognized as a potential source for the dissemination of antimicrobial-resistant bacteria and ARGs in aquatic environments [14,35–37]. Although the use of antibiotics as growth promoters in aquaculture has been banned or increasingly restricted in many countries, and alternative strategies, including probiotics [38–40] and prebiotics [41–43], have been adopted to reduce dependence on antimicrobial treatments to improve growth performance and the intestinal architectures of different fish species, the occurrence of antibiotic-resistant bacteria remains a persistent concern. Previous studies have demonstrated that aquatic ecosystems can act as reservoirs of resistant bacteria and ARGs, allowing resistance determinants to persist and circulate within microbial communities, even when direct antimicrobial selection pressure is reduced.

The present study demonstrates that aquaculture environments in Hajdú-Bihar County, Hungary, harbor diverse bacterial communities that include several bacterial species associated with AMR or ARGs. The growth of bacterial colonies on selective media, particularly VRE agar, may indicate the presence of bacterial populations that are capable of surviving under the presence of antibiotics. These findings are somewhat consistent

with a previous report from Hungary suggesting that aquatic environments can act as reservoirs and dissemination pathways for antibiotic-resistant bacteria or ARGs, even in regions where antimicrobial use is strictly regulated [44]. The detection of presumptive antimicrobial-resistant bacteria in both commercial fishponds and aquaculture-associated waters highlights the ecological importance of monitoring aquaculture systems as part of a One Health approach to AMR surveillance.

MALDI-TOF MS analysis revealed a predominance of *Acinetobacter* and *Pseudomonas* species across the investigated sites. Members of these genera are frequently reported in aquatic environments and are recognized for their remarkable adaptability and capacity to acquire resistance determinants. The repeated isolation of *Acinetobacter baumannii* [45–47], *Acinetobacter pittii* [48], and *Pseudomonas aeruginosa* [49] is of particular interest because these species are considered opportunistic pathogens with documented roles in the dissemination of antimicrobial resistance genes. Their occurrence in aquaculture waters suggests that environmental reservoirs may contribute to the persistence and circulation of resistant bacterial populations. An unexpected finding of the present study was the isolation of *Meyerozyma guilliermondii* from VRE medium. Although this medium is formulated for the selective recovery of vancomycin-resistant enterococci, selective media are not entirely specific and may permit the growth of non-target microorganisms, particularly in environmental samples. *M. guilliermondii* is a ubiquitous environmental yeast that has been reported from aquatic habitats and may exhibit tolerance to the selective conditions of the medium. Its recovery in the present study should therefore be interpreted as an incidental finding, rather than evidence of vancomycin resistance.

More importantly, the presence of *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterococcus gallinarum* in Hajdúszoboszló and Biharugra, 2023 with MALDI scores ≥ 2.0 is relevant to antimicrobial resistance surveillance because these taxa have previously been associated with antimicrobial resistance and resistance determinants. For example, a recent study by Libisch et al. [44] detected multiple acquired ARGs in the intestinal microbiome of common carp in Békés County, Hungary using metagenomic sequencing, including genes conferring resistance to β -lactams, tetracyclines, quinolones, sulfonamides, and trimethoprim. The occurrence of bacterial taxa commonly associated with antimicrobial resistance in the present study, together with the detection of acquired ARGs in Hungarian carp populations reported by Libisch et al. [44], highlights the importance of aquaculture environments as potential reservoirs of resistance determinants and underscores the need for molecular investigations and genetic analysis to determine whether the detected isolates in our study harbor intrinsic or acquired resistance mechanisms.

Interestingly, *A. baumannii*, found in the current study, is considered as a major cause of concern for causing significant problems due to its ability to acquire antimicrobial drug resistance [50]. Similarly, *Pseudomonas aeruginosa* is naturally resistant to a broad range of antimicrobial agents [51,52]. On the other hand, *E. coli* is an increasingly worrying problem to humans and animals. Although *E. coli* is intrinsically susceptible, this species has a high ability to acquire resistance genes through HGT [53].

The results also demonstrate the effectiveness of MALDI-TOF MS as a rapid and reliable tool for bacterial identification in environmental microbiology studies. Most isolates yielded score values above 2.0, indicating high-confidence species-level identification. The ability of MALDI-TOF MS to rapidly characterize diverse bacterial communities represents a valuable advantage for routine surveillance programs and epidemiological investigations in aquaculture. Culture-based isolation combined with MALDI-TOF MS can provide a useful preliminary approach for taxonomic screening of bacterial populations in aquaculture environments; however, antimicrobial susceptibility testing and/or molecular characterization of resistance determinants are required for subsequent assessment of antimicrobial resistance.

A major limitation of the present study is that the study was designed as a qualitative survey to identify representative bacterial isolates recovered from selective and differential culture media, rather than to quantify bacterial abundance in the investigated aquatic environments. Therefore, quantitative ecological parameters, such as bacterial counts (CFU), isolation frequencies, and prevalence at individual sampling sites, were not determined. Although the identification of bacterial species provides valuable baseline information on the occurrence of microorganisms of potential relevance to antimicrobial resistance surveillance, future studies should integrate quantitative microbiological analyses with antimicrobial susceptibility testing, PCR-based screening of resistance genes to obtain a more comprehensive understanding of the distribution, and ecological significance of these bacteria in aquaculture environments in Hungary.

5. Conclusions

The current study investigated bacterial communities associated with commercial fish farms and aquaculture-related aquatic environments in Hajdú-Bihar County, Hungary. Using selective and differential culture media in combination with MALDI-TOF MS, this work provides a preliminary characterization of bacterial species recovered from these environments. Several of the identified taxa have previously been reported in the literature as being associated with antimicrobial resistance, underscoring their potential relevance for environmental surveillance within a One Health framework. However, as antimicrobial susceptibility testing and molecular characterization were beyond the scope of the present study, the recovered isolates should not be interpreted as confirmed antimicrobial-resistant bacteria. Future studies integrating phenotypic susceptibility testing, molecular detection of resistance determinants, and genomic approaches are warranted to provide a more comprehensive understanding of antimicrobial resistance dynamics in aquaculture ecosystems.

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Conflicts of Interest: The authors declare no conflicts of interest.

References

1. FAO. *The State of World Fisheries and Aquaculture 2024: Blue Transformation in Action*; FAO: Rome, Italy, 2024.
2. Mohammed, E.A.H.; Ahmed, A.E.M.; Kovács, B.; Pál, K. The Significance of Probiotics in Aquaculture: A Review of Research Trend and Latest Scientific Findings. *Antibiotics* **2025**, *14*, 242. <https://doi.org/10.3390/antibiotics14030242>.
3. Magondu, E.; Njogu, L.; Ouko, K.; Mboya, J.B.; Muthoka, M.; Yossa, R.; Munguti, J.; Opiyo, M.A.; Meenakshisundaram, M.; Tanga, C.M.; et al. A PESTEL—SWOT Matrix Analysis of Sustainable Aquafeed Sector: A Case of Kenya's Fish Feed Sector. *Rev. Aquac.* **2025**, *17*, e70081. <https://doi.org/10.1111/raq.70081>.

4. Martínez-Lara, P.; Martínez-Porchas, M.; Gollas-Galván, T.; Hernández-López, J.; Robles-Porchas, G.R. Granulomatosis in Fish Aquaculture: A Mini Review. *Rev. Aquac.* **2021**, *13*, 259–268. <https://doi.org/10.1111/raq.12472>.
5. Owatari, M.S.; Mouriño, J.L.P.; Martins, M.L. Antimicrobial Resistance: A Concern Related to Streptococcosis in Tilapia Farming. *Mar. Fish. Sci.* **2024**, *37*, 337–352. <https://doi.org/10.47193/mafis.3722024010508>.
6. Laltlanmawia, C.; Saha, H.; Ghosh, L.; Saha, R.K.; Malla, S. Identification and Analysis of Pathogenic Bacteria Causing Outbreaks in Indian Major Carp Aquaculture of Tripura. *J. Aquat. Anim. Health* **2023**, *35*, 263–279. <https://doi.org/10.1002/aah.10198>.
7. Bayliss, S.C.; Verner-Jeffreys, D.W.; Bartie, K.L.; Aanensen, D.M.; Sheppard, S.K.; Adams, A.; Feil, E.J. The Promise of Whole Genome Pathogen Sequencing for the Molecular Epidemiology of Emerging Aquaculture Pathogens. *Front. Microbiol.* **2017**, *8*, 121. <https://doi.org/10.3389/fmicb.2017.00121>.
8. Crane, M.; Hyatt, A. Viruses of Fish: An Overview of Significant Pathogens. *Viruses* **2011**, *3*, 2025–2046. <https://doi.org/10.3390/v3112025>.
9. Abarike, E.D.; Jian, J.; Tang, J.; Cai, J.; Yu, H.; Lihua, C.; Jun, L. Influence of Traditional Chinese Medicine and Bacillus Species (TCMBS) on Growth, Immune Response and Disease Resistance in Nile Tilapia, *Oreochromis Niloticus*. *Aquac. Res.* **2018**, *49*, 2366–2375. <https://doi.org/10.1111/are.13691>.
10. Chowdhury, S.; Rheman, S.; Debnath, N.; Delamare-Deboutteville, J.; Akhtar, Z.; Ghosh, S.; Parveen, S.; Islam, K.; Islam, M.A.; Rashid, M.M.; et al. Antibiotics Usage Practices in Aquaculture in Bangladesh and Their Associated Factors. *One Health* **2022**, *15*, 100445. <https://doi.org/10.1016/j.onehlt.2022.100445>.
11. Lulijwa, R.; Rupia, E.J.; Alfaro, A.C. Antibiotic Use in Aquaculture, Policies and Regulation, Health and Environmental Risks: A Review of the Top 15 Major Producers. *Rev. Aquac.* **2020**, *12*, 640–663. <https://doi.org/10.1111/raq.12344>.
12. Karmakar, S.; Das, R.; Das, S.; Bera, T.; Das, S.; Mondal, S. Assessment of Antimicrobial Usage and Associated Factors among Aquaculture Farmers in a District of Eastern India. *Clin. Epidemiol. Glob. Health* **2026**, *40*, 102410. <https://doi.org/10.1016/j.cegh.2026.102410>.
13. Gao, P.; Mao, D.; Luo, Y.; Wang, L.; Xu, B.; Xu, L. Occurrence of Sulfonamide and Tetracycline-Resistant Bacteria and Resistance Genes in Aquaculture Environment. *Water Res.* **2012**, *46*, 2355–2364. <https://doi.org/10.1016/j.watres.2012.02.004>.
14. Mohammed, E.A.H.; Kovács, B.; Kuunya, R.; Mustafa, E.O.A.; Abbo, A.S.H.; Pál, K. Antibiotic Resistance in Aquaculture: Challenges, Trends Analysis, and Alternative Approaches. *Antibiotics* **2025**, *14*, 598. <https://doi.org/10.3390/antibiotics14060598>.
15. Pepi, M.; Focardi, S. Antibiotic-Resistant Bacteria in Aquaculture and Climate Change: A Challenge for Health in the Mediterranean Area. *IJERPH* **2021**, *18*, 5723. <https://doi.org/10.3390/ijerph18115723>.
16. Mourão, A.V.; Fernandes, D.; De Sousa, T.; Calouro, R.; Saraiva, S.; Igrejas, G.; Poeta, P. Aquatic Resistome in Freshwater and Marine Environments: Interactions Between Commensal and Pathogenic in the Context of Aquaculture and One Health. *Microorganisms* **2025**, *13*, 1591. <https://doi.org/10.3390/microorganisms13071591>.
17. D’Costa, V.M.; Griffiths, E.; Wright, G.D. Expanding the Soil Antibiotic Resistome: Exploring Environmental Diversity. *Curr. Opin. Microbiol.* **2007**, *10*, 481–489. <https://doi.org/10.1016/j.mib.2007.08.009>.
18. SGS—Global Services. Managing Antibiotic Residues in Aquaculture Products for EU Compliance. 2025. Available online: <https://www.sgs.com/en/news/2025/11/managing-antibiotic-residues-in-aquaculture-products-for-eu-compliance> (accessed on 5 August 2026).
19. Sassi, A.; Basher, N.S.; Kirat, H.; Meradji, S.; Ibrahim, N.A.; Idres, T.; Touati, A. The Role of the Environment (Water, Air, Soil) in the Emergence and Dissemination of Antimicrobial Resistance: A One Health Perspective. *Antibiotics* **2025**, *14*, 764. <https://doi.org/10.3390/antibiotics14080764>.
20. Veterinary Medicinal Products Regulation. European Medicines Agency (EMA). Available online: <https://www.ema.europa.eu/en/veterinary-regulatory-overview/veterinary-medicinal-products-regulation> (accessed on 24 July 2026).
21. Rosato, R.; Castellani, F.; Ricci, M.; Manucci, A.; Di Simone, V.; Scortichini, G.; Saluti, G. Antibiotics in the Aquatic Environment. A Deep Voyage across Chemical Occurrence Data, Socio-Economic Relationships and International Policies on Antimicrobial Consumption and Resistance within 2014–2024. *Sci. Total Environ.* **2025**, *1002*, 180543. <https://doi.org/10.1016/j.scitotenv.2025.180543>.
22. Niegowska, M.; Sanseverino, I.; Navarro, A.; Lettieri, T. Knowledge Gaps in the Assessment of Antimicrobial Resistance in Surface Waters. *FEMS Microbiol. Ecol.* **2021**, *97*, fiab140. <https://doi.org/10.1093/femsec/fiab140>.
23. European Centre for Disease Prevention and Control. *Antimicrobial Resistance in the EU/EEA (EARS-Net)—Annual Epidemiological Report for 2024*; European Centre for Disease Prevention and Control (ECDC): Stockholm, Sweden, 2025.
24. Iwu, C.D.; Korsten, L.; Okoh, A.I. The Incidence of Antibiotic Resistance within and beyond the Agricultural Ecosystem: A Concern for Public Health. *MicrobiologyOpen* **2020**, *9*, e1035. <https://doi.org/10.1002/mbo3.1035>.

25. Jasovský, D.; Littmann, J.; Zorzet, A.; Cars, O. Antimicrobial Resistance—A Threat to the World's Sustainable Development. *Upsala J. Med. Sci.* **2016**, *121*, 159–164. <https://doi.org/10.1080/03009734.2016.1195900>.
26. Alhaji, N.B.; Maikai, B.-V.; Kwaga, J.K.P. Antimicrobial Use, Residue and Resistance Dissemination in Freshwater Fish Farms of North-Central Nigeria: One Health Implications. *Food Control* **2021**, *130*, 108238. <https://doi.org/10.1016/j.foodcont.2021.108238>.
27. Murray, C.J.L.; Ikuta, K.S.; Sharara, F.; Swetschinski, L.; Robles Aguilar, G.; Gray, A.; Han, C.; Bisignano, C.; Rao, P.; Wool, E.; et al. Global Burden of Bacterial Antimicrobial Resistance in 2019: A Systematic Analysis. *Lancet* **2022**, *399*, 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0).
28. Bizzini, A.; Greub, G. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry, a Revolution in Clinical Microbial Identification. *Clin. Microbiol. Infect.* **2010**, *16*, 1614–1619. <https://doi.org/10.1111/j.1469-0691.2010.03311.x>.
29. Tsuchida, S.; Umemura, H.; Nakayama, T. Current Status of Matrix-Assisted Laser Desorption/Ionization–Time-of-Flight Mass Spectrometry (MALDI-TOF MS) in Clinical Diagnostic Microbiology. *Molecules* **2020**, *25*, 4775. <https://doi.org/10.3390/molecules25204775>.
30. Kapetanović, D.; Vardić Smrzlić, I.; Gavrilović, A.; Jug-Dujaković, J.; Perić, L.; Kazazić, S.; Mišić Radić, T.; Kolda, A.; Čanković, M.; Žunić, J.; et al. Characterization of *Vibrio* Populations from Cultured European Seabass and the Surrounding Marine Environment with Emphasis on *V. Anguillarum*. *Microorganisms* **2022**, *10*, 2159. <https://doi.org/10.3390/microorganisms10112159>.
31. Pastuszka, A.; Guz, L.; Michalak, K.; Pietras-Ożga, D.; Puk, K. *Vibrio* infection in freshwater fish in Poland. *Pol. J. Vet. Sci.* **2024**, *27*, 117–125. <https://doi.org/10.24425/pjvs.2024.149341>.
32. Lai, O.; Tinelli, A.; Soloperto, S.; Crescenzo, G.; Galante, D.; Calarco, A.; Tribuzio, M.; Manzulli, V.; Caioni, G.; Zizzadoro, C.; et al. Observed Prevalence and Characterization of Fluoroquinolone-Resistant and Multidrug-Resistant Bacteria in Loggerhead Sea Turtles (*Caretta caretta*) from the Adriatic Sea. *Antibiotics* **2025**, *14*, 252. <https://doi.org/10.3390/antibiotics14030252>.
33. Kačániová, M.; Kunová, S.; Sabo, J.; Ivanišová, E.; Žiarovská, J.; Felšöciiová, S.; Fatrcová-Šramková, K.; Terentjeva, M. Isolation and Identification of Lactic Acid Bacteria in Wine Production by MALDI-TOF MS Biotyper. *Acta Hort. Regiotect.* **2020**, *23*, 21–24. <https://doi.org/10.2478/ahr-2020-0006>.
34. Smith, E.M.; Willis, Z.N.; Blakeley, M.; Lovatt, F.; Purdy, K.J.; Green, L.E. Bacterial Species and Their Associations with Acute and Chronic Mastitis in Suckler Ewes. *J. Dairy Sci.* **2015**, *98*, 7025–7033. <https://doi.org/10.3168/jds.2015-9702>.
35. Milijasevic, M.; Veskovic-Moracanin, S.; Babic Milijasevic, J.; Petrovic, J.; Nastasijevic, I. Antimicrobial Resistance in Aquaculture: Risk Mitigation within the One Health Context. *Foods* **2024**, *13*, 2448. <https://doi.org/10.3390/foods13152448>.
36. Wu, J.; Ye, F.; Qu, J.; Dai, Z. Insight into the Antibiotic Resistance of Bacteria Isolated from Popular Aquatic Products Collected in Zhejiang, China. *Pol. J. Microbiol.* **2023**, *72*, 61–67. <https://doi.org/10.33073/pjm-2023-010>.
37. Miranda, C.D.; Godoy, F.A.; Lee, M.R. Current Status of the Use of Antibiotics and the Antimicrobial Resistance in the Chilean Salmon Farms. *Front. Microbiol.* **2018**, *9*, 1284. <https://doi.org/10.3389/fmicb.2018.01284>.
38. Torres-Maravilla, E.; Parra, M.; Maisey, K.; Vargas, R.A.; Cabezas-Cruz, A.; Gonzalez, A.; Tello, M.; Bermúdez-Humarán, L.G. Importance of Probiotics in Fish Aquaculture: Towards the Identification and Design of Novel Probiotics. *Microorganisms* **2024**, *12*, 626. <https://doi.org/10.3390/microorganisms12030626>.
39. Nayak, S. Probiotics and Immunity: A Fish Perspective. *Fish. Shellfish Immunol.* **2010**, *29*, 2–14.
40. Mohammed, E.A.H.; Fehér, M.; Bársony, P.; Teye-Gaga, C.; Czeglédi, L.; Freytag, C.; Váradi, A.; Ahmed, A.E.M.; Pál, K. Growth Performance, Gut Integrity and Intestinal Microbiome Responses of Juvenile Common Carp (*Cyprinus carpio* L.) to Probiotic and Prebiotic Supplementation. *Animals* **2026**, *16*, 433. <https://doi.org/10.3390/ani16030433>.
41. Song, S.K.; Beck, B.R.; Kim, D.; Park, J.; Kim, J.; Kim, H.D.; Ringø, E. Prebiotics as Immunostimulants in Aquaculture: A Review. *Fish. Shellfish Immunol.* **2014**, *40*, 40–48. <https://doi.org/10.1016/j.fsi.2014.06.016>.
42. Ringø, E.; Olsen, R.E.; Gifstad, T.Ø.; Dalmo, R.A.; Amlund, H.; Hemre, G.-I.; Bakke, A.M. Prebiotics in Aquaculture: A Review. *Aquat. Nutr.* **2010**, *16*, 117–136. <https://doi.org/10.1111/j.1365-2095.2009.00731.x>.
43. Xu, W.; Lutz, C.G.; Taylor, C.M.; Ortega, M.C. Improvement of Fish Growth and Metabolism by Oligosaccharide Prebiotic Supplement. *Aquat. Nutr.* **2022**, *2022*, 5715649. <https://doi.org/10.1155/2022/5715649>.
44. Libisch, B.; Abdulkadir, S.; Keresztény, T.; Papp, P.P.; Olasz, F.; Fébel, H.; Sándor, Z.J.; Rasschaert, G.; Lambrecht, E.; Heyndrickx, M.; et al. Detection of Acquired Antibiotic Resistance Genes in Domestic Pig (*Sus scrofa*) and Common Carp (*Cyprinus carpio*) Intestinal Samples by Metagenomics Analyses in Hungary. *Antibiotics* **2022**, *11*, 1441. <https://doi.org/10.3390/antibiotics11101441>.
45. Kyriakidis, I.; Vasileiou, E.; Pana, Z.D.; Tragiannidis, A. *Acinetobacter Baumannii* Antibiotic Resistance Mechanisms. *Pathogens* **2021**, *10*, 373. <https://doi.org/10.3390/pathogens10030373>.

46. Ayoub Moubareck, C.; Hammoudi Halat, D. Insights into *Acinetobacter Baumannii*: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. *Antibiotics* **2020**, *9*, 119. <https://doi.org/10.3390/antibiotics9030119>.
47. Vrancianu, C.O.; Gheorghe, I.; Czobor, I.B.; Chifiriuc, M.C. Antibiotic Resistance Profiles, Molecular Mechanisms and Innovative Treatment Strategies of *Acinetobacter Baumannii*. *Microorganisms* **2020**, *8*, 935. <https://doi.org/10.3390/microorganisms8060935>.
48. Liu, W.; Wang, H.; Zhu, W.; Li, X.; He, Y.; Su, W. Genomic Characterization of a Carbapenem-Resistant *Acinetobacter Pittii* Strain Harboring Chromosome-Borne blaNDM-1 from China. *Pathogens* **2025**, *14*, 1037. <https://doi.org/10.3390/pathogens14101037>.
49. Tu, Y.-Y.; Lu, Q.; Zhang, N.; Leng, J.; Yang, Q.; Yu, J.; Zhu, C.-K.; He, T.; Hu, J.-Y.; Lv, M.-J.; et al. A Comprehensive Investigation of Potential Bacterial Pathogens in Largemouth Bass (*Micropterus salmoides*). *Microorganisms* **2025**, *13*, 1413. <https://doi.org/10.3390/microorganisms13061413>.
50. Peleg, A.Y.; Seifert, H.; Paterson, D.L. *Acinetobacter baumannii*: Emergence of a Successful Pathogen. *Clin. Microbiol. Rev.* **2008**, *21*, 538–582. <https://doi.org/10.1128/CMR.00058-07>.
51. Poole, K. *Pseudomonas aeruginosa*: Resistance to the Max. *Front. Microbio.* **2011**, *2*, 65. <https://doi.org/10.3389/fmicb.2011.00065>.
52. Poole, K. Outer Membranes and Efflux: The Path to Multidrug Resistance in Gram- Negative Bacteria. *CPB* **2002**, *3*, 77–98. <https://doi.org/10.2174/1389201023378454>.
53. Poirel, L.; Madec, J.-Y.; Lupo, A.; Schink, A.-K.; Kieffer, N.; Nordmann, P.; Schwarz, S. Antimicrobial Resistance in *Escherichia coli*. *Microbiol. Spectr.* **2018**, *6*, 10.1128/microbiolspec.arba-0026-2017. <https://doi.org/10.1128/microbiolspec.ARBA-0026-2017>

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