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Diversity, Virulence, and Antimicrobial Profiles of *Aeromonas* spp. in Aquaculture Products: A Decade-Long Study in Eastern China (2012–2022)

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ABSTRACT

Aeromonas, a foodborne zoonotic pathogen found in aquatic products, causes severe diarrhea in humans. We analyzed antibiotic susceptibility patterns to identify changes in the pathogenicity and resistance of *Aeromonas* spp. We assessed their prevalence in aquaculture products from 2012 to 2022 and characterized their virulence and antibiotic-resistance genes. Virulence and antibiotic-resistance genes were identified using PCR assays, and whole-genome sequencing was utilized to investigate antimicrobial resistance profiles and virulence factors. Antibiotic susceptibility testing was performed using the microdilution method. We identified eight *Aeromonas* species (68 strains) in *Litopenaeus vannamei* and eight fish species. Four types, encompassing 10 known virulence genes, were identified: Type I (exotoxins/enzymes/adhesion factors), Type II (exotoxins/enzymes), Type III (exotoxins/adhesion factors), and Type IV (enzymes). Types I and II were most common. From 2020 to 2022, nearly all strains contained three major classes of virulence genes: exotoxins, enzymes, and adhesion factors. Twelve resistance genes from five major classes were detected. Resistance phenotypes were evaluated using approved aquaculture antibiotics; resistance frequencies to chloramphenicol, sulfamonomethoxine, and sulfamethoxazole/trimethoprim were higher in 2020–2022 than in 2012–2018. This study highlights the evolving pathogenicity and resistance of *Aeromonas* spp. in aquaculture products over the past decade, emphasizing its importance for food safety.

1. INTRODUCTION

The global aquaculture industry has experienced rapid growth in response to the increasing demand for high-protein aquatic products, presenting both opportunities and challenges [1]. Aquatic species, including fish and shrimp, are essential for food security [2, 3]. However, this expansion has raised concerns about the overuse of antibiotics and the spread of pathogenic bacteria [4, 5]. *Aeromonas spp.*, a group of opportunistic pathogens, pose significant threats to farmed aquatic species and human health [6-8]. In fish, *Aeromonas* can cause infections such as septicemia and ulcers, while in humans, infections often arise from ingesting contaminated aquatic products or direct contact with infected aquatic environments, leading to conditions such as gastroenteritis, wound infections, and sepsis [6, 7]. Virulence genes are important indicators of pathogenicity, and the synergistic action of multiple virulence genes can enhance pathogenicity [8, 9].

Although awareness of antimicrobial resistance in agriculture is increasing, the One Health approach—which encompasses the interconnections between human, animal, and environmental health—has predominantly focused on terrestrial livestock, neglecting the substantial impact of aquaculture on antibiotic use and bacterial transmission [4, 5]. Given the extensive scale of aquaculture, this industry serves as a fertile environment for the adaptation and dissemination of bacterial pathogens [10].

In China, the incidence of infections related to *Aeromonas* has risen, underscoring the critical need for enhanced surveillance in aquaculture [11]. However, current efforts to monitor antibiotic resistance in aquaculture are inadequate, highlighting the need for more focused attention on these emerging risks [12]. A significant challenge in this area is the fragmented nature of available data. Most studies have concentrated on ready-to-eat aquatic products, whereas long-term datasets or those covering a broad spectrum of aquaculture species are notably lacking. Additionally, global data on freshwater aquaculture species are insufficient, particularly in developed countries such as the United States and European nations [13]. Among Asian countries, China notably lacks comprehensive datasets on this issue, despite its significant role in global aquaculture production. In contrast, countries such as Korea and Thailand have made more progress in this area [14, 15]. Shanghai, as a representative city in Eastern China, is characterized by its high population density and significant demand for high-protein aquatic products, making it an ideal location for investigating antimicrobial resistance in aquaculture products. Multilocus sequence typing (MLST) is a crucial tool in bacterial epidemiology, allowing for the tracking of genetic diversity in pathogens such as *Aeromonas spp.* by analyzing several

housekeeping genes [16-18]. This technique facilitates tracing bacterial infections, identifying high-risk antimicrobial resistance genes, and monitoring emerging resistant strains [16, 19]. In this study, MLST was used to explore the distribution, variation, and resistance patterns of *Aeromonas* strains, thereby enhancing our understanding of their epidemiology.

This study focused on *Aeromonas spp.* isolated from commonly farmed aquatic species in Shanghai, characterizing their antimicrobial resistance profiles and assessing their public health implications. The findings are expected to inform and enhance monitoring and control strategies for pathogens in aquaculture systems. Ultimately, this study aimed to establish a comprehensive database to manage pathogenic and resistant *Aeromonas* strains in aquatic products, thereby ensuring the safety and sustainability of these resources.

2. MATERIALS AND METHODS

2.1 Bacterial strains and culture conditions

A total of 68 *Aeromonas* strains were isolated from nine aquatic species: mandarin fish (*Siniperca chuatsi*), yellow catfish (*Pelteobagrus fulvidraco*), crucian carp (*Carassius auratus*), grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), black carp (*Mylopharyngodon piceus*), perch (*Perca fluviatilis*), catfish (*Ictalurus punctatus*), and shrimp (*Litopenaeus vannamei*) in Shanghai from 2012 to 2022. These isolates were preserved in 20% glycerol at -80℃ for long-term storage. Working cultures were propagated in Luria-Bertani agar and broth. Details regarding the bacterial isolates used in this study are provided in Table S1. The animal study protocol was approved by the Institutional Review Board of the Shanghai Fisheries Research Institute (protocol code SFRI-202303).

2.2 Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted using the microdilution method, following the standards of the Clinical and Laboratory Standards Institute (CLSI). The susceptibility of the isolated strains to the drugs was assessed, with *Escherichia coli* ATCC25922 used as the quality control strain. Eight antibacterial agents from five classes were used: sulfamonomethoxine (SMM) and sulfamethoxazole/trimethoprim (SXT) from the sulfonamide class; doxycycline (DOX) from the tetracycline class; enrofloxacin (ENR) from the quinolone class; neomycin sulfate (NEO) from the aminoglycoside class; and florfenicol (FFC) and thiamphenicol (THI) from the chloramphenicol (CHL) class.

For quality control, a drug-free sterile negative control and a drug-free bacterial positive control were established. Each well was inoculated with 100 μL of bacterial suspension diluted to 1×10^6 colony-forming units/mL, followed by incubation at 30℃ for 24 h. Results were then observed, and strains were categorized as resistant (R), intermediate (I), or susceptible (S) based on the minimum inhibitory concentration breakpoints, according to CLSI guidelines.

2.3 Polymerase chain reaction analysis

2.3.1 Species identification

Bacterial genomic DNA extraction and purification were performed using a bacterial genomic DNA kit (Takara Bio Inc., Shiga, Japan), according to the manufacturer's instructions. PCR amplification was performed using the primers gyrB3F (5′-TCCGGCGGTCTGCACGGCGT-3′) and gyrB14R (5′-TTGTCCGGGTTGTACTCGTC-3′), targeting an approximately 1,100 bp *gyrB* gene fragment [20]. The PCR reaction mixture included 12.5 μL of PCR mix, 1 μL each of 10 μM gyrB3F and 10 μM gyrB14R, and 50 ng of total DNA template, with a final volume of 25 μL of sterilized double-distilled water. PCR amplification was as follows: initial denaturation at 94℃ for 3 min, 30 cycles at 94℃ for 30 s, annealing at 52℃ for 30 s, and extension at 72℃ for 1 min, followed by a final extension at 72℃ for 5 min. PCR products were visualized using electrophoresis on a 1.5% agarose gel (Sangon Biotech, Shanghai, China) in 1 × TAE buffer.

2.3.2 Determination of virulence genes

To assess potential pathogenicity, PCR analysis of virulence genes, including the Aer (*aer*), heat-labile enterotoxin (*alt*), heat-stable enterotoxin (*ast*), cytotoxic enterotoxin (*act*), lipase (*lip*), deoxyribonuclease (*exu*), glycerophospholipid cholesterol acyltransferase (*gcaT*), elastase (*ahyB*), temperature-sensitive protease (*eprCAI*), and flagellin (*fla*) genes, was performed according to the methods described by Nawaz et al. [21]. The presence of virulence gene combinations was categorized into four types: Type I (exotoxins + enzymes + adhesion factors), Type II (exotoxins + enzymes), Type III (exotoxins + adhesion factors), and Type IV (enzymes).

2.3.3 Determination of antibiotic-resistance genes (ARGs)

PCR analysis was conducted to determine the presence or absence of 12 ARGs classified into five categories (Table S1). Previously published primer sequences were used for each target ARG. PCR fragments were sequenced by Sangon Biotech (Shanghai, China) and analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST).

2.4 Whole-genome sequencing (WGS) and comparative genomic analysis

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, USA) as per the manufacturer's protocol. Sequencing of qualified DNA was performed by Shanghai Winnerbio Technology Co., Ltd. (Shanghai, China) using the Illumina NovaSeq 6000 platform. To determine the presence of plasmids, the filtered Illumina reads were mapped using SOAP (https://soap.genomics.org.cn/) to the bacterial plasmid database based on GenBank. The rRNA and tRNA genes were identified using Barrnap (version 0.9, https://www.cbs.dtu.dk/services/RNAmmer/) and tRNAscan-SE (version 2.0.8, http://trna.ucsc.edu/software/) with default settings, respectively. Prodigal (version 2.6.3) was used to predict the open reading frame using default parameters. Predicted gene sequences were translated and searched against the National Center for Biotechnology Information (NCBI) non-redundant, UniProt/Swiss-Prot, Pfam, Clusters of Orthologous Groups, and Kyoto Encyclopedia of Genes and Genomes databases for annotation. Additional annotation was conducted using the Virulence Factors of Pathogenic Bacteria and the Comprehensive Antibiotic Resistance Database databases.

2.5 Phylogenetic analysis and average nucleotide identity analysis

Using the existing *Aeromonas* MLST scheme available at http://pubmlst.org/, six housekeeping genes (*gyrB*, *groL*, *gltA*, *metG*, *ppsA*, and *recA)* were retrieved from the pubMLST database (https://pubmlst.org/organisms/Aeromonas-spp) for MLST analysis. The genomes of *Aeromonas* strains were then subjected to average nucleotide identity (ANI) analysis to elucidate their phylogenetic relationships. The ANI values were computed using the ANI calculator (Rodriguez, Kostas Lab, Riverside, CA, USA) with default parameters, considering genomes with ANI values ≥ 95% as belonging to the same species, as defined by Colston et al. [16]. A total of 45 reference genomes used for ANI analysis are listed in Table S2. To examine the genetic relatedness among isolates, a phylogenetic tree was constructed using the ParSNP tool (https://github.com/marbl/parsnp) from the Harvest suite [19] based on all identified single-nucleotide polymorphisms in each isolate [22].

2.6 Prediction of virulence factor genes (VFGs) and ARGs

Candidate VFGs and ARGs were analyzed and selected using the Virulence Factors of Pathogenic Bacteria and the Comprehensive Antibiotic Resistance Database databases. All genes were filtered using a 70% similarity threshold. Heatmap visualization was used to present the evolutionary and predicted results of VFGs (Tables S3–S9) and ARGs (Tables S10–S16).

2.7 Data submission to NCBI

The genomic data of *Aeromonas* strains analyzed in this study were submitted to NCBI under the BioProject accession number PRJNA1219774. Sample data are accessible under SUBID SUB15067125. The NCBI accession numbers and corresponding organisms are provided in Table 1.

2.8 Data analysis

MS Excel 2021 (Microsoft, Redmond, WA, USA) was used to analyze the data and generate graphics. These graphics included the classification of VFGs and the number of VFGs carried by isolates in the periods 2012–2014, 2015–2018, and 2020–2022 (Figure 1); the ARGs; the number of ARGs in *Aeromonas* isolates during 2012–2014, 2015–2018, and 2020–2022 (Figure 2); information on the source, quantity, and name of the isolates (Table 2); prevalence of resistance to different antibiotics (Table 3); and prevalence of intermediate resistance to different antibiotics (Table 4). Data were considered significantly different when $P < 0.05$.

Table 1. NCBI accession numbers and corresponding organisms

BioSample	Accession	Organism
SAMN46657247	JBLNHL000000000000	*Aeromonas veronii* A15
SAMN46657246	JBLNHM000000000000	*A. veronii* A17
SAMN46657245	JBLNHN000000000000	*A. veronii* A16
SAMN46657244	JBLNH000000000000	*A. hydrophila* A12
SAMN46657243	JBLNHP000000000	*Aeromonas* sp. A04
SAMN46657242	JBLNHQ000000000000	*A. veronii* A03
SAMN46657241	JBLNHR000000000000	*A. hydrophila* A01

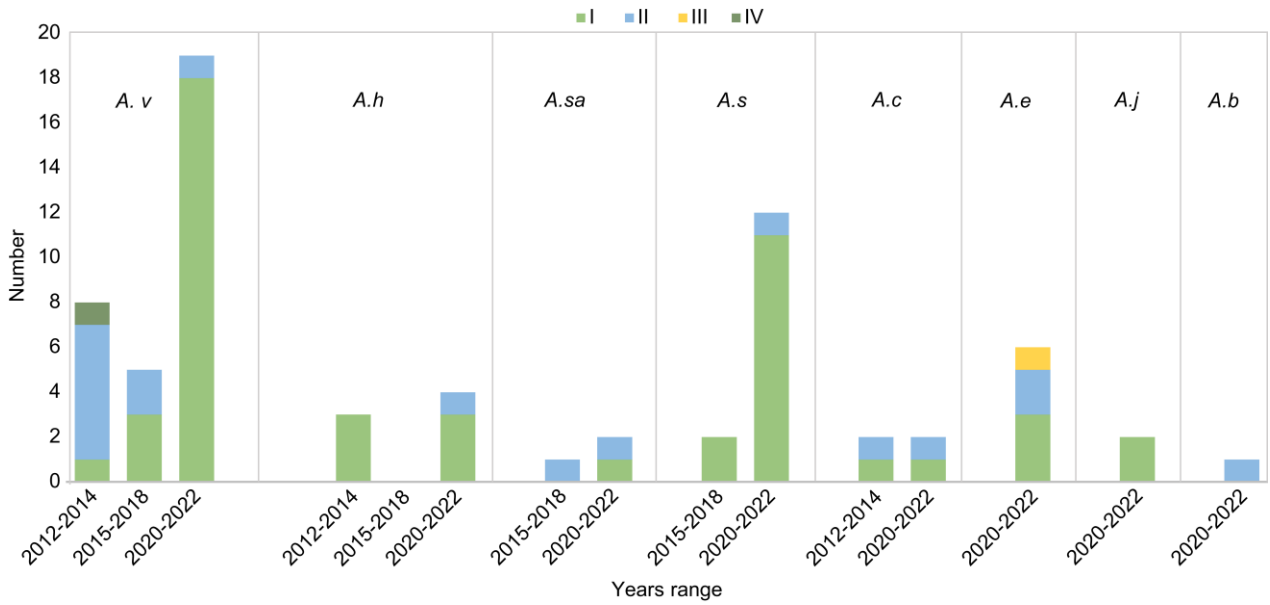

Figure 1. Classification of virulence genes. Number of VFGs carried by isolates in 2012–2014, 2015–2018, and 2020–2022
A.v = Aeromonas veronii, *A.h = A. hydrophila*, *A.c = A. caviae*, *A.s = A. sobria*, *A.sa = A. salmonicida*, *A.e = A. enteropelogenes*, *A.j = A. jandaei*

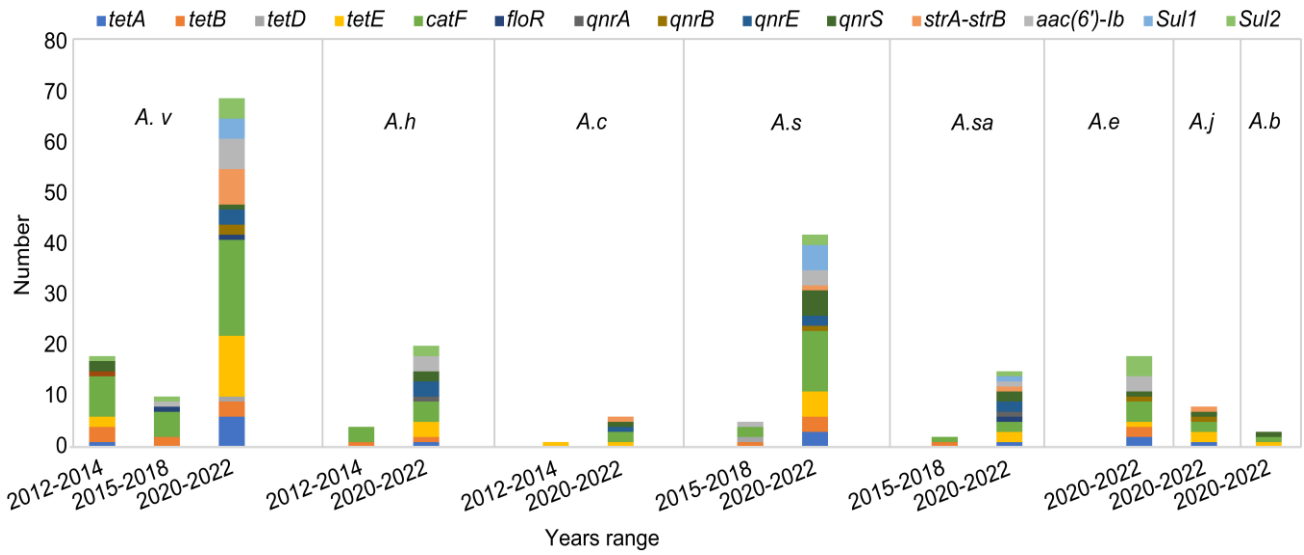

Figure 2. Antibiotic-resistance genes (ARGs). Number of ARGs in Aeromonas isolates in 2012–2014, 2015–2018, and 2020–2022

A.v = Aeromonas veronii, A.h = A. hydrophila, A.c = A. caviae, A.s = A. sobria, A.sa = A. salmonicida, A.e = A. enteropelogenes, A.j = A. jandaei

Table 2. Information on the source, quantity, and name of the isolates

Source	Number and Name of Isolates							
	Aeromonas veronii (n = 32)	*A. sobria* (n = 14)	*A. hydrophila* (n = 7)	*A. enteropelogenes* (n = 6)	*A. salmonicida* (n = 3)	*A. caviae* (n = 3)	*A. jandaei* (n = 2)	*A. bestiarum* (n = 1)
Mandarin fish	0	1	2	0	0	0	0	0
Yellow catfish	1	0	0	0	0	0	0	0
Crucian carp	9	6	2	3	1	2	2	0
Grass carp	2	5	1	1	0	0	0	1
Silver carp	2	0	0	0	0	0	0	0
Black carp	3	0	1	1	1	0	0	0
Perch	3	0	0	0	0	1	0	0
Catfish	1	0	0	0	0	0	0	0
Litopenaeus vannamei	11	2	1	1	1	0	0	0

Table 3. Prevalence of resistance to different antibiotics

Year Range	Strain	Number of Isolates with Antibiotic Resistance (n, %)						
		Tetracycline	Chloramphenicol		Quinolone	Aminoglycoside	Sulfonamide	
		DOX	FFC	THI	ENR	NEO	SMM	SXT
2012–2014	*Aeromonas veronii* (n = 8)	1 (12.5)		1 (12.5)	1 (12.5)			1 (12.5)
	A. hydrophila (n = 3)							
	A. caviae (n = 1)							
2015–2018	*A. veronii* (n = 5)	2 (40.0)						
	A. sobria (n = 2)						1 (50.0)	1 (50.0)
	A. salmonicida (n = 1)							
2020–2022	*A. veronii* (n = 19)			2 (10.5)	2 (10.5)	1 (5.3)	1 (5.3)	2 (10.5)
	A. hydrophila (n = 4)			2 (50.0)			1 (25.0)	2 (50.0)
	A. sobria (n = 12)						1 (8.3)	1 (8.3)
	A. enteropelogenes (n = 6)			2 (33.3)				
	A. salmonicida (n = 2)							
	A. caviae (n = 2)			1 (50.0)			1 (50.0)	1 (50.0)
	A. jandaei (n = 2)						1 (50.0)	1 (50.0)
	A. bestiarum (n = 1)							

DOX, doxycycline; FFC, florfenicol; THI, thiamphenicol; ENR, enrofloxacin; NEO, neomycin sulfate; SMM, sulfamonomethoxine; SXT, sulfamethoxazole/trimethoprim.

Table 4. Prevalence of intermediate resistance to different antibiotics

Year Range	Strain	Number of Isolates with Antibiotic Resistance (n, %)						
		Tetracycline	Chloramphenicol		Quinolone	Aminoglycoside	Sulfonamide	
		DOX	FFC	THI	ENR	NEO	SMM	SXT
2012–2014	*Aeromonas veronii* (n = 8)	1 (12.5)	1 (12.5)					
	A. hydrophila (n = 3)	1 (33.3)	1 (33.3)					
	A. caviae (n = 1)				1 (100)			
2015–2018	*A. veronii* (n = 5)		2 (40.0)		1 (20.0)			
	A. sobria (n = 2)							
	A. salmonicida (n = 1)		1 (100)					
2020–2022	*A. veronii* (n = 19)	1 (5.3)	1 (5.3)		5 (26.3)			
	A. hydrophila (n = 4)	1 (25.0)	1 (25.0)					
	A. sobria (n = 12)		3 (25.0)					
	A. enteropelogenes (n = 6)				2 (33.3)	1 (16.7)		
	A. salmonicida (n = 2)				1 (50)			
	A. caviae (n = 2)					1 (50)		
	A. jandaei (n = 2)				1 (50)			
	A. bestiarum (n = 1)							

DOX, doxycycline; FFC, florfenicol; THI, thiamphenicol; ENR, enrofloxacin; NEO, neomycin sulfate; SMM, sulfamonomethoxine; SXT, sulfamethoxazole/trimethoprim

3. RESULTS

3.1 Strains and genotype diversity

From 2012 to 2022, we isolated and purified 68 strains of *Aeromonas* from aquaculture products originating from nine sources (Table 2). Molecular identification revealed the presence of eight distinct species: *A. veronii*, *A. sobria*, *A. hydrophila*, *A. enteropelogenes*, *A. salmonicida*, *A. caviae*, *A. jandaei*, and *A. bestiarum*. To assess temporal changes, the 68 strains were classified into three time periods. From 2012 to 2014, aquaculture products predominantly carried three species: *A. veronii*, *A. hydrophila*, and *A. caviae*. Between 2015 and 2018, the dominant species were *A. veronii*, *A. sobria*, and *A. salmonicida*. Between 2020 and 2022, all eight species mentioned above were present, indicating the highest level of diversity.

Aeromonas exhibited wide distribution across various host species, with *L. vannamei* primarily hosting five types of bacteria, predominantly *A. veronii*, whereas fish species primarily hosted eight types of bacteria, dominated by *A. veronii*, *A. sobria*, and *A. hydrophila*.

3.2 Temporal variation in virulence gene distribution

The distribution of virulence genes in the 68 *Aeromonas* strains across different time intervals revealed dynamic changes in their genetic makeup (Figure 1). Between 2012 and 2014, all ten known VFGs were detected, with *aer* (11/12, 91.67%), *lip* (11/12, 91.67%), and *exu* (12/12, 100%) exhibiting relatively high detection rates. In 2015–2018, the *ast* gene was not detected. The occurrence of *alt* (12/12, 100%) was relatively high among the detected genes. Other genes with high detection rates were *aer* (8/8, 100%), *lip* (7/8, 87.50%), and *exu* (8/8, 100%). Similarly, in 2020–2022, nine known virulence genes, not including *gcaT*, were identified. The detection rates of *aer* (46/48, 95.83%), *lip* (44/48, 91.67%), and *exu* (36/48, 75%) remained high, while that of *alt* (34/48, 70.83%), which encodes an exotoxin, increased.

The analysis of virulence gene combinations revealed dynamic patterns in *Aeromonas* strains over the 10-year period. The *aer*, *lip*, and *exu* genes showed consistently high detection rates across all three intervals, indicating their importance in the virulence profile of *Aeromonas*. Virulence gene combinations were categorized into four types, with Type I combinations being prevalent across seven *Aeromonas* species: *A. veronii*, *A. hydrophila*, *A. salmonicida*, *A. sobria*, *A. caviae*, *A. enteropelogenes*, and *A. jandaei*. *A. veronii* strains consistently carried Types I and II virulence gene combinations across all three intervals, indicating a stable virulence profile over time. Similar trends were observed in *A. hydrophila* and *A. caviae* strains in 2012–2014 and 2020–2022. Additionally, *A. sobria* and *A. salmonicida* strains exhibited Types I and II combinations in 2015–2018 and 2020–2022. In 2020–2022, *A. enteropelogenes* carried Types I, II, and III combinations, indicating a broader virulence profile than the other species. *A. jandaei* predominantly carried Type I combinations, whereas *A. bestiarum* primarily carried Type II combinations. Overall, *Aeromonas* strains predominantly carried Types I and II virulence gene combinations throughout the decade, suggesting a stable polymorphism in the carriage of VFGs. Despite fluctuations in the prevalence of specific genes, the overall composition of virulence gene combinations remained consistent over time.

In *A. veronii*, the types and numbers of virulence and resistance genes showed a consistent trend over the decade, with a decrease observed in 2015–2018, followed by an increase in 2020–2022. In contrast, *A. hydrophila* strains maintained the same types of virulence genes across intervals but exhibited a significant increase in the carriage of resistance genes in 2020–2022 compared to 2012–2014 (Figures 1 and 2).

3.3 Significant enrichment of ARG types across different periods

Initially, six types of ARGs belonging to four classes of antibiotics were detected from 2012 to 2014, namely *tetA*, *tetB*, *tetE*, CHL (*catF*), *qnrS*, and *Sul2*. This expanded to six types corresponding to five antibiotic classes in 2015–2018 and significantly increased to 14 types covering five antibiotic classes in 2020–2022 (Figure 2).

Across all time intervals, the *catF* gene consistently showed the highest detection rate among *Aeromonas* strains, except for *A. enteropelogenes*, where it was 66.67%. Tetracycline resistance genes (*tet*) also showed high detection rates across

all intervals.

In *A. veronii* isolates, there was a significant overall increase in the diversity of ARGs over time, with an expansion from four classes and six types of ARGs in 2012–2014 to five classes and 13 genes in 2020–2022. A similar trend was observed in *A. hydrophila* and *A. caviae* isolates.

A. sobria and *A. salmonicida* isolates displayed varying patterns of ARG distribution over time, with an increase in the number of detected genes and classes in 2015–2018 and 2020–2022.

A. bestiarum, *A. enteropelogenes*, and *A. jandaei* consistently showed the presence of *catF*, *tet*, and *qnrS* genes in 2020–2022, with a detection rate of 50%–100%. Other ARGs, such as *tetA*, *tetB*, *strA-strB*, and *aac(6')-Ib*, were also detected in these species to varying extents.

3.4 Temporal variations in antibiotic-resistance profile

Antimicrobial susceptibility tests were conducted on all 68 *Aeromonas* strains (Table 3). Between 2012 and 2014, only *A. veronii* showed resistance to four types of antibiotics: DOX, THI, ENR, and SXT, with a resistance rate of 12.5%. Other isolates, such as *A. hydrophila* and *A. caviae*, exhibited sensitivity to all tested antibiotics. In 2015–2018, *A. veronii* showed resistance solely to DOX (33.3%), whereas *A. sobria* showed resistance to SMM (50%) and SXT (50%). A more diverse pattern of resistance emerged during 2020–2022. Isolates of *A. veronii*, *A. hydrophila*, and *A. sobria* showed varying degrees of resistance to SMM and SXT. Furthermore, *A. veronii* showed resistance to THI, ENR, and NEO. An escalation in resistance to CHL and sulfonamide drugs was observed among strains during this timeframe. The resistance frequency of all isolates to THI, SMM, and SXT was significantly higher than that observed in 2012–2018. Intermediate resistance, indicative of potentially reduced susceptibility, was also observed across the strains (Table 4). In 2012–2014, *A. veronii* and *A. hydrophila* displayed intermediate resistance to DOX and FFC. Similarly, in the subsequent intervals, *A. veronii* exhibited intermediate resistance to FFC and ENR. *A. veronii* and *A. hydrophila* maintained a consistent pattern of intermediate resistance to DOX and FFC throughout 2012–2022. Overall, intermediate resistance across all strains predominantly centered on FFC and ENR.

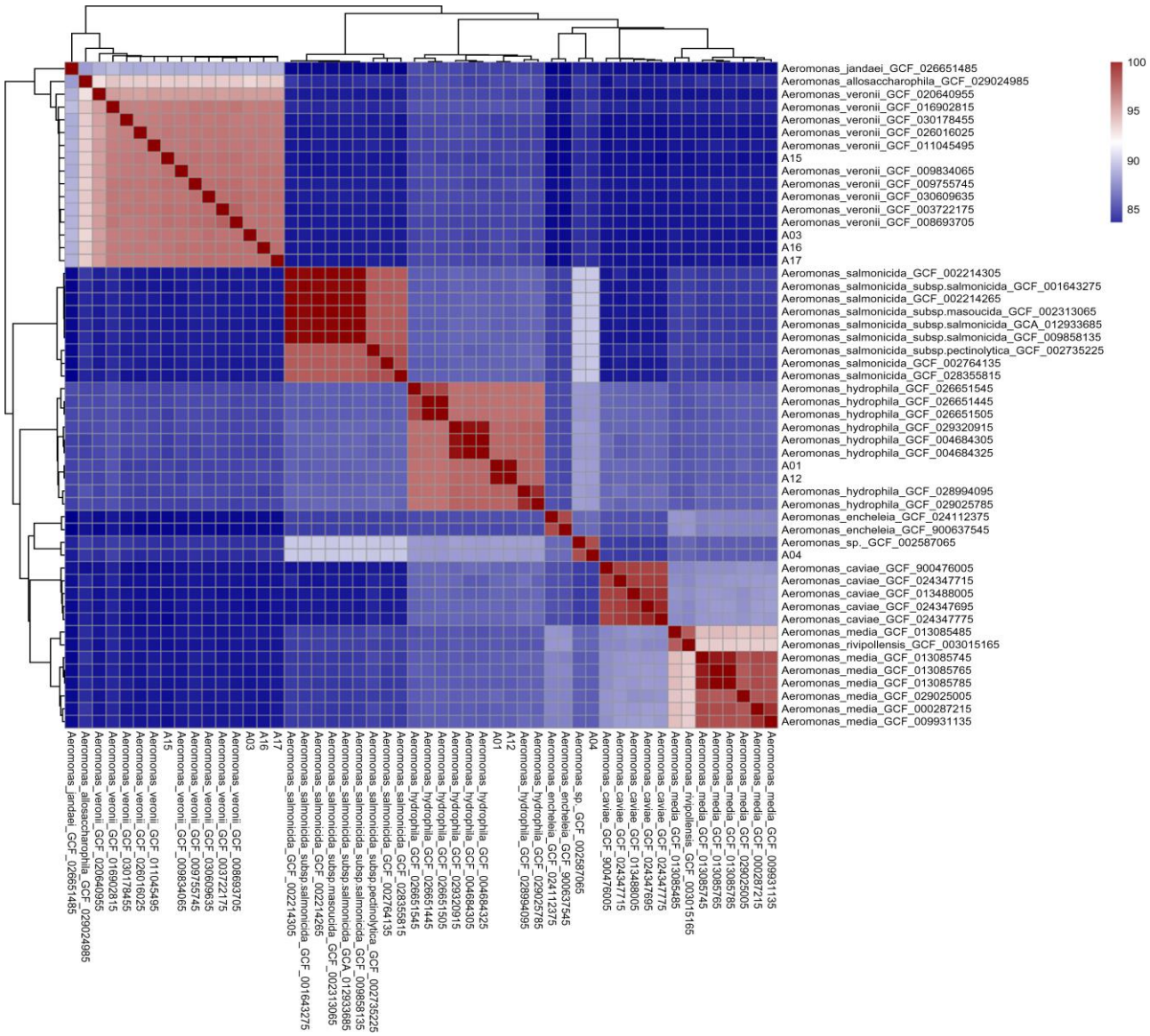

Figure 3. Heatmap of average nucleotide identity values among the seven isolates and 45 reference genome assemblies

3.5 Genomic characterization and phylogenetic relationships

The genetic characterization of seven sequenced *Aeromonas* isolates and their phylogenetic relationship with other published isolates (human, AS1) was elucidated through core genome similarity analysis (Figure 3). This revealed that these isolates belonged to three distinct species: *A. veronii* (A15, A03, A16, A17), *A. hydrophila* (A01, A12), and a novel species designated as *Aeromonas* sp. (A04). Genome-wide ANI values were above 95%, indicating close relatedness among the isolates (Table S2).

This species pattern was supported by the maximum likelihood phylogenetic tree, which was constructed based on a gene-by-gene core alignment of genes present in more than 95% of all isolates. The phylogenetic tree revealed the clustering of genomes broadly according to sequence type (ST) (Figure 4). The seven *Aeromonas* isolates were assigned to six known STs. Specifically, *A. hydrophila* isolates A01 and A12, obtained from mandarin fish and crucian carp, respectively, clustered together within ST-1826. The four *A. veronii* isolates formed a distinct cluster, and each was assigned to a different ST: ST-2386 (A15), ST-1113 (A03), ST-903 (A16), and ST-1114 (A17). Three of these isolates originated from *L. vannamei*, and the remaining isolate was obtained from crucian carp.

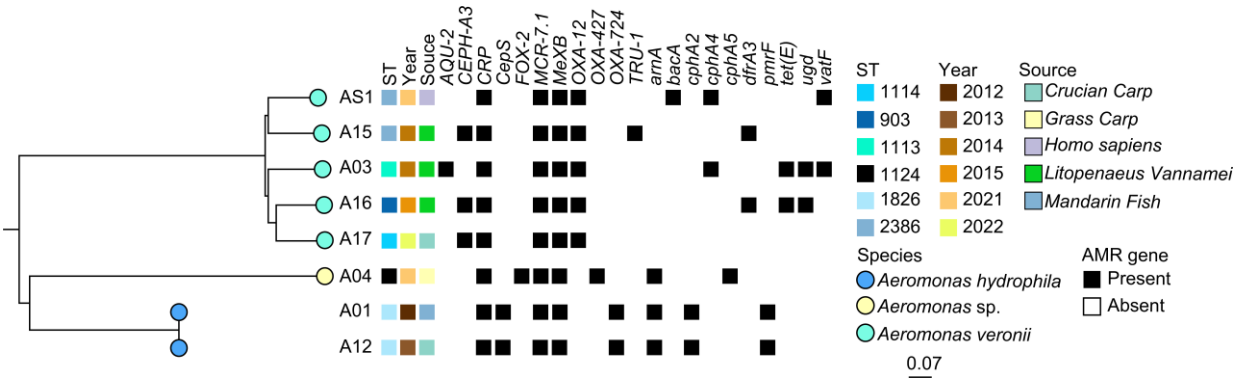

Figure 4. Distribution of antibiotic-resistance genes (ARGs) in the seven isolates

Figure 5. Distribution of virulence genes in the seven isolates

Isolate A15 also exhibited a close phylogenetic relationship with an *A. veronii* reference strain AS1 (NCBI Reference Sequence: NZ_CP114182.1), a clinical isolate from a patient with Crohn's disease.

3.6 Distribution of VFGs

Analysis of VFGs among the *Aeromonas* population (n = 7) revealed variations in gene content among different species (Figure 5). The number of VFGs ranged from 118 to 213 over the study period, with *A. hydrophila* isolates exhibiting more genes (199–213) than *Aeromonas* sp. isolates (168 genes) and *A. veronii* isolates (118–132 genes; Tables S3-S9). *Aeromonas* isolates were categorized into three groups—species classification, ANI, and phylogeny—based on gene presence and absence.

Shared virulence genes among all isolates included those responsible for traits associated with flagella (*flgC*), lipooligosaccharide synthesis (*waaF*), and chemotaxis (*cheA*, *cheB*, *cheR*, *cheV*, *cheW*, *cheY*, *cheZ*). Additionally, unique genes specific to certain strains were identified. For example, the motility accessory factor gene *pseB* was exclusively detected in *A. veronii* strains, whereas genes such as *ToxA* and *tviB* were found only in the *Aeromonas* sp. strain (A04).

The proportion of *A. veronii* and *A. hydrophila* strains carrying the same virulence genes remained consistently high (> 90%) across different years, indicating stability in the distribution of these genes within these species.

3.7 Distribution of antimicrobial resistance

The presence of horizontally acquired genes encoding resistance to various classes of antibiotics among the seven sequenced *Aeromonas* isolates was investigated (Tables S10–S16). WGS analysis confirmed the presence of multiple ARGs within the genomes of all seven *Aeromonas* strains and revealed the presence of different classes of β-lactamases. Among the isolates, *A. hydrophila* strains A01 and A12 exhibited a shared set of eight unique ARGs, spanning five major classes of antibiotics. These included β-lactamases (*blaOXA-724*, *CepS*, and *AQU-2*), tetracycline (*tetE*), polymyxin (*mcr-7.1*), carbapenem (*cphA4*), cephalosporin (*Ceps*), and peptide antibiotic (*pmrF*). In contrast, *Aeromonas* sp. strain A04 exhibited seven unique ARGs, representing three distinct major classes of antibiotics, namely β-lactamases (*blaOXA-427* and *FOX-2*), polymyxins (*arnA* and *bacA*), and carbapenems (*cphA4*).

In the *A. veronii* population, 13 unique ARGs were identified, covering five different major classes of antibiotics, namely β-lactamases, tetracycline, diaminopyrimidine, streptogramin, and polypeptide (Figure 4). All four *A. veronii* isolates carried four horizontally acquired resistance genes, namely *CRP*, *mcr-7.1*, *MexB*, and *blaOXA-12*, with a gene coverage ranging from 96% to 99%. The presence of *tetE* was observed in 50% (2/4) of these *A. veronii* isolates; however, it was absent in the Crohn's disease isolate AS1. Each of the analyzed bacterial strains contained *MexB*, a crucial multidrug efflux transporter protein.

4. DISCUSSION

Aeromonas, an emerging foodborne pathogen, poses a significant threat to food safety, particularly in aquatic products, by contributing to contamination and increasing the risk of foodborne illness [23]. The emergence of antibiotic-resistant strains of *Aeromonas* further heightens these concerns, increases food insecurity, and presents formidable challenges in clinical treatment [6]. Despite these risks, research on pathogenic and antibiotic-resistant strains of *Aeromonas* in the aquaculture products of Eastern China has been limited. This study fills a critical gap by offering the most comprehensive report on antibiotic-resistant *Aeromonas* in aquaculture products from Shanghai, a key city in Eastern China. The report provides a decade-long dataset on virulence genes, antimicrobial resistance, and characteristic resistance genes.

In our study, we examined 68 dominant strains of *Aeromonas* isolated from nine different types of aquaculture products in Shanghai from 2012 to 2022. We systematically selected these strains based on the respective years for species identification, followed by sensitivity testing to eight antibiotics. Our findings align with a previous study by Sadique et al. [24], which showed *Aeromonas* as the predominant strain, exhibiting diversity within cultured ponds. Our results revealed *A. veronii* as the most prevalent strain, followed by *A. hydrophila*. Additionally, we identified other species, such as *A. sobria*, *A. salmonicida*, *A. jandaei*, *A. enteropelogenes*, *A. caviae*, and *A.* sp. These observations are consistent with those of previous studies. For example, a study conducted on farmed Nile tilapia (*Oreochromis niloticus*) in the Philippines also highlighted *A. veronii* as the dominant strain, followed by *A. jandaei* and *A. caviae* [25]. Similarly, research on *Aeromonas* diversity in Thailand, using sequencing and phylogenetic analysis of the *gyrB* gene, confirmed the presence of six *Aeromonas* species, with *A. veronii* comprising the majority (72.1%), followed by *A. jandaei* (11.6%) [14]. These findings demonstrate the global importance of *Aeromonas* as a pathogen in aquatic products.

Aeromonas spp., including *A. veronii*, *A. caviae*, and *A. hydrophila*, are widely recognized pathogens linked to infectious diarrhea [26-29], with a study by the Chinese CDC highlighting the presence of a consistent strain species among adult patients with diarrhea in Beijing [11]. The pathogenesis of *Aeromonas*-induced diarrhea is complex, with no single virulence factor definitively identified as the sole cause [30]. It is generally believed that the carriage of multiple virulence genes contributes to the pathogenicity of these bacteria [8, 9]. For instance, Aslani et al. [31] reported that various genotypes carrying virulence genes, such as *aerA*, *hlyA*, *alt*, *ast*, and *act*, are associated with diarrheal illnesses. In Malaysia, 50% of isolates from cultured freshwater fish carry virulence genes, including *aer*, *alt*, *hly*, *lip*, *fla*, and *act* [32]. Similarly, in Southeast China, *Aeromonas* strains causing bacteremia exhibit a high prevalence of virulence genes such as *aer*, *lip*, *hlyA*, *alt*, *ast*, and *act* [33]. Albert et al. [34] found that 56% of diarrheal isolates from children in Bangladesh contain the *alt* and *ast* genes.

From 2012 to 2022, the genes *aer*, *lip,* and exu were consistently detected at high rates. A prevalent combination of Type I virulence genes was observed in seven *Aeromonas* species; these genes encode exotoxins (such as *alt*, *act*, *ast*, and *aer*), enzymes (including *exu*, *lip*, *gcaT*, *eprCAI*, and *ahyB*), and adhesion factors (such as *fla*). In comparison to the findings of other research, the virulence genes we found in *Aeromonas* strains show regional differences and polymorphisms, indicating genetic diversity [14, 24]. However, these genes remained stable over the studied decade, suggesting a consistent genetic profile of *Aeromonas* in Shanghai's aquaculture environment. The similarity between the virulence patterns of the strains in our study and those from strains isolated from clinical settings underscores the pathogenic potential of these bacteria. Therefore, heightened

awareness is crucial for identifying *Aeromonas* as a possible foodborne pathogen that poses a risk to human health. Additional research is necessary to elucidate the role of these genes in the pathogenicity of these bacteria.

In addition to the identified virulence genes, WGS analysis revealed previously unreported potential pathogenic genes, such as chemotaxis genes (*cheA*, *cheB*, *cheR*, *cheV*, *cheW*, *cheY*, and *cheZ*). Chemotaxis genes are closely related to flagella and are recognized as important virulence factors in enteric pathogens. They play crucial roles in pathogenesis and host colonization [35, 36], thereby affecting the virulence and infection potential of pathogenic bacteria [37], which ultimately impacts human health. Our study demonstrated that all seven *Aeromonas* strains carried chemotaxis genes, suggesting that chemotaxis represents a novel potential pathogenic factor in *Aeromonas*. These genes have been implicated in colistin resistance in *A. hydrophila* [38].

Many studies have focused on well-known virulence genes, such as *act*, *aer*, *hylA*, and *ast*, which are typically detected using PCR. However, this study has revealed the presence of additional virulence determinants, such as *waa* and *che* genes, which have received less attention. This demonstrates the ability of WGS to predict the pathogenic genes in seven strains of *Aeromonas* and provides a novel perspective on understanding the pathogenicity of this bacterium.

The resistance phenotype analysis revealed a significant shift in trends. From 2012 to 2018, all bacterial strains exhibited decreased resistance rates. Nonetheless, a notable surge in resistance was observed in all strains between 2020 and 2022, indicating evolving resistance patterns. Notably, sulfonamide resistance rates steadily increased over the years, indicating that this class of antibiotics should not be overlooked. Moreover, during the 2020–2022 period, all isolates showed higher resistance to CHL and sulfonamides (SMM/SXT) compared to the 2012–2018 period, suggesting a rise in the usage or environmental exposure to these antibiotics over the last few years. A study by Liu et al. [39] detected high concentrations of sulfonamides in fish from the Hangbu-Fengle River, highlighting significant environmental exposure. This exposure may be contributing to the increasing resistance observed in *Aeromonas* species. It is crucial to raise awareness of *Aeromonas* as a potential foodborne pathogen threatening human health, as our findings imply a possible increase in the use or exposure to these drugs in the last part of the studied period.

By comparing ARGs carried by different *Aeromonas* strains over the three time intervals, we observed that the CHL *catF* gene exhibited the highest and most sustained detection rate. Specifically, this gene was present in 100% of all strains examined, except for *A. enteropelogenes*, where its detection rate was 66.67%. This finding aligns with the consistently high detection rates of CHL resistance phenotypes observed across the strains. Although China has prohibited the use of CHL in aquaculture since 1999, many studies have indicated that historically, residual CHL resistance genes have persisted and transferred among diverse microbes in aquatic and food environments without significant selective pressure [40, 41]. After CHL, the next highest detection rate was observed for *tet* resistance genes, which were distributed among all strains in 2012–2022. Various studies have consistently reported the prevalence of different types of *tet* family genes in water environments, fresh vegetables, and aquatic products [42]. Our study also revealed an increase in the diversity of antibiotic-resistant types and genes over time. Given the widespread use of sulfonamides, quinolones, tetracyclines, and other antibiotics in Chinese aquaculture [43], these changes were possibly affected by changes in the types and habits of drug usage. Although there is diversity in resistance genes, not all resistance phenotypes are expressed, indicating that the presence of various resistance genes does not necessarily correlate with the occurrence of all corresponding resistances.

The study showed no correlation between the antimicrobial resistance phenotypes and genotypes of all strains, suggesting that potential factors contributing to this discrepancy include the limited sample size or insufficient expression of resistance genes. WGS analysis showed that all seven bacterial strains contained multiple resistance genes, including four types of β-lactamase extended-spectrum β-lactamases, labeled A, B, C, and D. This suggests that β-lactamases are prevalent in *A. veronii* and *A. hydrophila*. However, *CphA* is exclusive to *A. hydrophila* and absent in *A. veronii*, which was consistent with a previous study [25]. Additionally, the types of resistance genes carried by two strains of *A. hydrophila* remained consistent over at least two years, indicating stability in their resistance gene profile. Similarly, four strains of *A. veronii* isolated from different hosts in 2014–2022 consistently carried *CRP*, *mcr-7.1*, *MexB*, and *OXA-427*, demonstrating a persistent ability to carry resistance genes. Despite the ban on β-lactamase antibiotics in Chinese aquaculture, our WGS results reveal the continued presence of these resistance factors, suggesting the need for further research to assess their potential harm to human health.

China has not officially approved the use of colistin, which can contaminate the aquatic environment through runoff carrying animal excreta containing residual colistin in aquaculture [44, 45]. Additionally, antibiotic environments facilitate horizontal gene transfer among bacteria, amplifying bacterial resistance [46]. Fish and seafood may serve as reservoirs for antibiotic-resistant bacteria, contributing to the spread of ARGs [47-49], particularly since resistant *Aeromonas* poses a significant public health risk to humans [28].

The primary limitations of this study include the inconsistent number of fish across groups and the varying duration of sample collection. Furthermore, we did not statistically analyze the distribution of *Aeromonas* spp. in this study. In the future, pathogenicity testing and genomic studies should be conducted to validate *A. veronii* and *A. hydrophila* as the principal pathogens.

5. CONCLUSIONS

This decade-long study provides critical insights into the diversity, virulence, and antimicrobial resistance profiles of *Aeromonas* spp. in aquaculture products from Eastern China. The consistent presence of key virulence genes (e.g., *aer*, *lip*, and *exu*) and the increasing prevalence of ARGs (e.g., CHL and sulfonamide resistance genes) highlight the evolving pathogenic potential and environmental adaptability of these bacteria. These findings underscore the importance of continuous surveillance and the need for stringent antibiotic stewardship in aquaculture to mitigate the risk of foodborne infections and preserve the efficacy of existing antimicrobials. The potential for cross-species transmission of high-risk *Aeromonas* strains further supports the necessity of a One Health approach, integrating efforts across human, animal, and environmental health sectors. Our findings emphasize the

urgent need for coordinated, multi-sectoral strategies to address antimicrobial resistance, ensuring the sustainability of aquaculture resources and safeguarding public health.

INSTITUTIONAL REVIEW BOARD STATEMENT

The animal study protocol was approved by the Institutional Review Board of the Shanghai Fisheries Research Institute (protocol code SFRI-202303).

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REFERENCES

[1] Food and Agriculture Organization of the United Nations (2020). The State of World Fisheries and Aquaculture 2020: Sustainability in Action. Food and Agriculture Organization: Rome, Italy. https://openknowledge.fao.org/items/b752285b-b2ac-4983-92a9-fdb24e92312b.

[2] Ofoegbu, C., Chirwa, P.W., Francis, J., Babalola, F.D. (2016). Assessing forest-based rural communities' adaptive capacity and coping strategies for climate variability and change: the case of Vhembe district in South Africa. Environmental Development, 18: 36-51. https://doi.org/10.1016/j.envdev.2016.03.001

[3] Tacon, A.G.J., Metian, M. (2012). Fish matters: Importance of aquatic foods in human nutrition and Global Food Supply. Reviews in Fisheries Science, 21(1): 22-38. https://doi.org/10.1080/10641262.2012.753405

[4] Collignon, P.J., McEwen, S.A. (2019). One health—Its importance in helping to better control antimicrobial resistance. Tropical Medicine and Infectious Disease, 4(1): 22. https://doi.org/10.3390/tropicalmed4010022

[5] Hernando-Amado, S., Coque, T.M., Baquero, F., Martínez, J.L. (2019). Defining and combating antibiotic resistance from one health and global health perspectives. Nature Microbiology, 4(9): 1432-1442. https://doi.org/10.1038/s41564-019-0503-9

[6] Bernabé, G., Brun, P., Pietra, G.D., Zatta, V., Asad, S., Meneghello, S., Cordioli, G., Lavezzo, E., Valente, E., Mietto, S., Besutti, V., Castagliuolo, I. (2023). Prevalence and virulence potential of Aeromonas spp. isolated from human diarrheal samples in North East Italy. Microbiology Spectrum, 11(6): e0080723. https://doi.org/10.1128/spectrum.00807-23.

[7] Janda, J.M., Abbott, S.L. (2010). The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. Clinical Microbiology Reviews, 23(1): 35-73. https://doi.org/10.1128/cmr.00039-09

[8] Tomás, J.M. (2012). The main *Aeromonas* pathogenic factors. ISRN Microbiology, 2012: 256261. https://doi.org/10.5402/2012/256261

[9] Fernández-Bravo, A., Figueras, M.J. (2020). An update on the genus Aeromonas: Taxonomy, epidemiology, and pathogenicity. Microorganisms, 8(1): 129. https://doi.org/10.3390/microorganisms8010129

[10] Larsson, D.G.J., Flach, C.-F. (2021). Antibiotic resistance in the environment. Nature Reviews Microbiology, 20(5): 257-269. https://doi.org/10.1038/s41579-021-00649-x

[11] Li, Y., Yu, K., Du, X., Liu, Y., et al. Characterization of Aeromonas Strains isolated from adult patients with diarrhea and aquatic environments — Beijing Municipality, China, 2016–2022. China CDC Weekly, 7(4): 121-129. https://doi.org/10.46234/ccdcw2025.019

[12] Sheng, L., Wang, L. (2020). The microbial safety of fish and fish products: Recent advances in understanding its significance, contamination sources, and control strategies. Comprehensive Reviews in Food Science and Food Safety, 20(1): 738-786. https://doi.org/10.1111/1541-4337.12671

[13] Jones, D.C., Lamartina, E.L., Lewis, J.R., Dahl, A.J., Nadig, N., Szabo, A., Newton, R.J., Skwor, T.A. (2023). One health and global health view of antimicrobial susceptibility through the 'eye' of *Aeromonas*: Systematic review and meta-analysis. International Journal of Antimicrobial Agents, 62(2): 106848. https://doi.org/10.1016/j.ijantimicag.2023.106848

[14] Mursalim, M.F., Budiyansah, H., Raharjo, H.M., Debnath, P.P., 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. Journal of Fish Diseases, 45(8): 1149-1163. https://doi.org/10.1111/jfd.13650

[15] Wickramanayake, M.V.K.S., Dahanayake, P.S., Hossain, S., Heo, G.-J. (2020). Antimicrobial resistance of pathogenic *Aeromonas* spp. isolated from marketed Pacific abalone (*Haliotis discus hannai*) in Korea. Journal of Applied Microbiology, 128(2): 606-617. https://doi.org/10.1111/jam.14485

[16] Colston, S.M., Fullmer, M.S., Beka, L., Lamy, B., Gogarten, J.P., Graf, J. (2014). Bioinformatic genome comparisons for taxonomic and phylogenetic assignments using *Aeromonas* as a test case. mBio, 5(6): e02136. https://doi.org/10.1128/mbio.02136-14

[17] Feil, E.J., Spratt, B.G. (2001). Recombination and the population structures of bacterial pathogens. Annual Review of Microbiology, 55: 561-590. https://doi.org/10.1146/annurev.micro.55.1.561

[18] Maiden, M.C.J., Bygraves, J.A., Feil, E., Morelli, G., Russell, J.E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D.A., Feavers, I.M., Achtman, M., Spratt, B.G. (1998). Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. In Proceedings of the National Academy of Sciences, 95(6): 3140-3145. https://doi.org/10.1073/pnas.95.6.3140

[19] Treangen, T.J., Ondov, B.D., Koren, S., Phillippy, A.M. (2014). The harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. Genome Biology, 15(11): 524. https://doi.org/10.1186/s13059-014-0524-x

[20] Yañez, M.A., Catalán, V., Apráiz, D., Figueras, M.J., Martínez-Murcia, A.J. (2003). Phylogenetic analysis of members of the genus Aeromonas based on gyrB gene sequences. International Journal of Systematic and Evolutionary Microbiology, 53(3): 875-883. https://doi.org/10.1099/ijs.0.02443-0

[21] Nawaz, M., Khan, S.A., Khan, A.A., Sung, K., Tran, Q.,

Kerdahi, K., Steele, R. (2010). Detection and characterization of virulence genes and integrons in Aeromonas veronii isolated from catfish. Food Microbiology, 27(3): 327-331. https://doi.org/10.1016/j.fm.2009.11.007

[22] Glaize, A., Gutierrez-Rodriguez, E., Hanning, I., Díaz-Sánchez, S., Gunter, C., van Vliet, A.H.M., Watson, W., Thakur, S. (2020). Transmission of antimicrobial resistant non-O157 *Escherichia coli* at the interface of animal-fresh produce in sustainable farming environments. International Journal of Food Microbiology, 319: 108472. https://doi.org/10.1016/j.ijfoodmicro.2019.108472

[23] Park, S.M., Kim, H.W., Choi, C., Rhee, M.S. (2021). Pathogenicity and seasonal variation of Aeromonas hydrophila isolated from seafood and ready-to-eat sushi in South Korea. Food Research International, 147: 110484. https://doi.org/10.1016/j.foodres.2021.110484

[24] Sadique, A., Neogi, S.B., Bashar, T., Sultana, M., et al. (2021). Dynamics, diversity, and virulence of Aeromonas spp. in homestead pond water in coastal Bangladesh. Frontiers in Public Health, 9: 692166. https://doi.org/10.3389/fpubh.2021.692166

[25] Legario, F.S., Choresca Jr, C.H., Grace, K., Turnbull, J.F., Crumlish, M. (2023). Identification and characterization of motile *Aeromonas* spp. Isolated from farmed Nile tilapia (*Oreochromis niloticus*) in the Philippines. Journal of Applied Microbiology, 134(12): lxad279. https://doi.org/10.1093/jambio/lxad279

[26] Gracey, M., Burke, V., Robinson, J. (1982). Aeromonas-associated gastroenteritis. The Lancet, 320(8311): 1304-1306. https://doi.org/10.1016/s0140-6736(82)91510-0

[27] Pablos, M., Remacha, M.A., Rodríguez-Calleja, J.M., Santos, J.A., Otero, A., García-López, M.L. (2010). Identity, virulence genes, and clonal relatedness of Aeromonas isolates from patients with diarrhea and drinking water. European Journal of Clinical Microbiology & Infectious Diseases, 29(9): 1163-1172. https://doi.org/10.1007/s10096-010-0982-3

[28] Pessoa, R.B.G., de Oliveira, W.F., Correia, M.T.D.S., Fontes, A., Coelho, L.C.B.B. (2022). *Aeromonas* and human health disorders: Clinical approaches. Frontiers in Microbiology, 13: 868890. https://doi.org/10.3389/fmicb.2022.868890

[29] Senderovich, Y., Ken-Dror, S., Vainblat, I., Blau, D., Izhaki, I., Halpern, M. (2012). A molecular study on the prevalence and virulence potential of Aeromonas spp. recovered from patients suffering from diarrhea in Israel. PLOS One, 7(2): e30070. https://doi.org/10.1371/journal.pone.0030070

[30] Gonçalves Pessoa, R.B., de Oliveira, W.F., Marques, D.S.C., Dos Santos Correia, M.T., de Carvalho, E.V.M.M., Coelho, L.C.B.B. (2019). The genus Aeromonas: A general approach. Microbial Pathogenesis, 130: 81-94. https://doi.org/10.1016/j.micpath.2019.02.036

[31] Aslani, M.M., Seyyed Hamzeh, H. (2004). Characterization and distribution of virulence factors in Aeromonas hydrophila strains isolated from fecal samples of diarrheal and asymptomatic healthy persons, in Ilam, Iran. Iranian Biomedical Journal, 8(4): 199-203.

[32] Azzam-Sayuti, M., Ina-Salwany, M.Y., Zamri-Saad, M., Yusof, M.T., et al. (2021). The prevalence, putative virulence genes and antibiotic resistance profiles of Aeromonas spp. isolated from cultured freshwater fishes in peninsular Malaysia. Aquaculture, 540: 736719. https://doi.org/10.1016/j.aquaculture.2021.736719

[33] Sun, Y., Zhao, Y., Xu, W., Fang, R., et al. (2021). Taxonomy, virulence determinants and antimicrobial susceptibility of *Aeromonas* spp. isolated from bacteremia in Southeastern China. Antimicrobial Resistance & Infection Control, 10: 43. https://doi.org/10.1186/s13756-021-00911-0

[34] Albert, M.J., Ansaruzzaman, M., Talukder, K.A., Chopra, A.K., et al. (2000). Prevalence of enterotoxin genes in Aeromonas spp. Isolated from children with diarrhea, healthy controls, and the environment. Journal of Clinical Microbiology, 38(10): 3785-3790. https://doi.org/10.1128/JCM.38.10.3785-3790.2000

[35] Duan, J., Zhao, Q., Wang, Y., Chi, Z., Li, W., Wang, X., Liu, S., Bi, S. (2023). The dCache domain of the chemoreceptor Tlp1 in Campylobacter jejuni binds and triggers chemotaxis toward formate. mBio, 14(3): e0356422. https://doi.org/10.1128/mbio.03564-22

[36] Porter, S.L., Wadhams, G.H., Armitage, J.P. (2011). Signal processing in complex chemotaxis pathways. Nature Reviews Microbiology, 9(3): 153-165. https://doi.org/10.1038/nrmicro2505

[37] Chen, H.H., Wang, Y.X., Li, D.F., Liu, C., Bi, S.Y., Jiang, C.Y., Liu, S.J. (2023). Chemoreceptors from the commensal gut *Roseburia rectibacter* bind to mucin and trigger chemotaxis. Environmental Microbiology, 25(7): 1329-1343. https://doi.org/10.1111/1462-2920.16365

[38] Li, W., Ali, F., Cai, Q., Yao, Z., Sun, L., Lin, W., Lin, X. (2018). Quantitative proteomic analysis reveals that chemotaxis is involved in chlortetracycline resistance of Aeromonas hydrophila. Journal of Proteomics, 172: 143-151. https://doi.org/10.1016/j.jprot.2017.09.011

[39] Shen, M., Yu, B., Hu, Y., Liu, Z., et al. (2023). Occurrence and health risk assessment of sulfonamide antibiotics in different freshwater fish in Northeast China. Toxics, 11(10): 835. https://doi.org/10.3390/toxics11100835

[40] Liu, J., Wu, Q., Xu, H., Pan, Y., Malakar, P.K., Zhao, Y., Zhang, Z. (2024). Change of antibiotic resistance in Vibrio spp. During shrimp culture in Shanghai. Aquaculture, 580: 740303. https://doi.org/10.1016/j.aquaculture.2023.740303

[41] Wang, X.M., Liao, X.P., Liu, S.G., Zhang, W.J., et al. (2025). Serotypes, virulence genes, and antimicrobial susceptibility of Escherichia coli isolates from pigs. Foodborne Pathogens and Disease, 8(6): 687-692. https://doi.org/10.1089/fpd.2010.0739

[42] Musovic, S., Klümper, U., Dechesne, A., Magid, J., Smets, B.F. (2013). Long-term manure exposure increases soil bacterial community potential for plasmid uptake. Environmental Microbiology Reports, 6(2): 125-130. https://doi.org/10.1111/1758-2229.12138

[43] Walsh, T.R., Stunt, R.A., Nabi, J.A., MacGowan, A.P., Bennett, P.M. (1997). Distribution and expression of beta-lactamase genes among Aeromonas spp. Journal of Antimicrobial Chemotherapy, 40(2): 171-178. https://doi.org/10.1093/jac/40.2.171

[44] Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A. (2018). Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. Molecules, 23(4): 795. https://doi.org/10.3390/molecules23040795

[45] Shen, Y., Lv, Z., Yang, L., Liu, D., et al. (2019). Integrated aquaculture contributes to the transfer of *mcr-1* between animals and humans via the aquaculture supply chain. Environment International, 130: 104708. https://doi.org/10.1016/j.envint.2019.03.056.
[46] Tao, S., Chen, H., Li, N., Wang, T., Liang, W. (2022). The spread of antibiotic resistance genes in vivo model. Canadian Journal of Infectious Diseases and Medical Microbiology, 2022(1): 3348695. https://doi.org/10.1155/2022/3348695
[47] Chenia, H.Y. (2016). Prevalence and characterization of plasmid-mediated quinolone resistance genes in Aeromonas spp. isolated from South African freshwater fish. International Journal of Food Microbiology, 231: 26-32. https://doi.org/10.1016/j.ijfoodmicro.2016.04.030
[48] Woo, S.J., Kim, M.S., Jeong, M.G., Do, M.Y., Hwang, S.D., Kim, W.J. (2022). Establishment of epidemiological cut-off values and the distribution of resistance genes in *Aeromonas hydrophila* and *Aeromonas veronii* isolated from aquatic animals. Antibiotics, 11(3): 343. https://doi.org/10.3390/antibiotics11030343
[49] Yoo, M.H., Huh, M.D., Kim, E.H., Lee, H.H., Jeong, H.D. (2003). Characterization of chloramphenicol acetyltransferase gene by multiplex polymerase chain reaction in multidrug-resistant strains isolated from aquatic environments. Aquaculture, 217(1-4): 11-21. https://doi.org/10.1016/S0044-8486(02)00169-2

NOMENCLATURE

MLST	Multilocus sequence typing
ARG	Antibiotic-resistance gene
WGS	Whole-genome sequencing
ANI	Average nucleotide identity

APPENDIX

Supplementary Materials: Table S1: All strain species, years, types of virulence genes, and resistance genes; Table S2: ANI details; Table S3: Genome annotation by VFDB(A01); Table S4: Genome annotation by VFDB(A03); Table S5: Genome annotation by VFDB(A04); Table S6: Genome annotation by VFDB(A12); Table S7: Genome annotation by VFDB(A15); Table S8: Genome annotation by VFDB(A16); Table S9: Genome annotation by VFDB(A17); Table S10: Genome annotation by CARD(A01); Table S11: Genome annotation by CARD(A03); Table S12: Genome annotation by CARD(A04); Table S13: Genome annotation by CARD(A12); Table S14: Genome annotation by CARD(A15); Table S15: Genome annotation by CARD(A16); Table S16: Genome annotation by CARD(A17).