



## RESEARCH ARTICLE

# Molecular characterization of *Aeromonas hydrophila* detected in *Channa marulius* and *Sperata sarwari* sampled from rivers of Punjab in Pakistan

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## Abstract

*Aeromonas hydrophila* is one of the major pathogenic bacteria responsible for causing severe outbreaks at fish farms and is also a major global public health concern. This bacterium harbors many virulence genes. The current study was designed to evaluate the anti-drug and virulence potential of *A. hydrophila* by amplifying its antimicrobial resistance and virulence genes using PCR and examining their effects on fish tissues and organs. A total of 960 fish samples of *Channa marulius* and *Sperata sarwari* were collected from four sites of the rivers of the Punjab, Pakistan. *A. hydrophila* isolates were subjected to biochemical identification and detection of virulence and antimicrobial resistance (AMR) genes by PCR. We retrieved 181 (6.46%) *A. hydrophila* isolates from *C. marulius* and 177 (6.25%) isolates from *S. sarwari*. Amplification through PCR revealed the incidence of virulence genes in 95.7% of isolates in *C. marulius* and 94.4% in *S. sarwari*. Similarly, amplification through PCR also revealed occurrence of AMR genes in 87.1% of isolates in *C. marulius* and 83.9% in *S. sarwari*. Histopathological examination revealed congestion (5.2%) and hepatocyte necrosis (4.6%) in liver, lamellar fusion (3.3%) and the presence of bacterial colonies (3.7%) in gills, fin erosion (6%), and the presence of biofilms (3.5%) in tail fins of infected fish. Phylogenetic tree analysis of *16S rRNA* and *gyrB* gene of *A. hydrophila* revealed 100% and 97% similarity, respectively, with *16S rRNA* gene and *gyrB* of *A. hydrophila* isolated in previous studies. The results of antimicrobial susceptibility testing showed that all isolates demonstrated resistance to sulfamethoxazole, ampicillin, neomycin, and norfloxacin, while susceptibility to gentamicin, chloramphenicol, and tetracycline, and intermediate resistance was observed against cefotaxime. The results concluded that examined fish samples were markedly contaminated with virulent and multidrug strains of *A. hydrophila* which may be of a potential health risk. The study emphasizes the responsible antimicrobial use in aquaculture and the urgent need for effective strategies to control the spread of virulence and antimicrobial resistance genes in *A. hydrophila*.

## Introduction

The fast-growing aquaculture industry plays a vital role in global food security, offering high-quality protein, economic benefits, and jobs opportunities [1,2]. It also provides essential nutrients and a variety of food products [3,4]. In 2020, aquaculture contributed 122.6 million metric tons of aquatic products valued at USD 281.5 billion, with an annual growth rate of 6.7%. The current worldwide per capita fish consumption is 20.5 kg. Fish, in particular, is a cost-effective protein source, ranking second globally and accounting for 60% of protein intake [5]. It plays a crucial role in ensuring food security for the growing global population [6]. To meet demand, there has been a substantial increase in freshwater and marine fish production [7]. However, this expansion has intensified aquaculture systems, leading to water resource challenges and increased bacterial infections among cultivated aquatic organisms [8]. Despite these challenges, aquaculture remains a sustainable solution for global food security, helping mitigate food shortages driven by population growth [9].

Freshwater fish farming has been vital to the aquaculture industry's growth, especially in Asia, providing food security, jobs, and economic benefits [10]. Various freshwater fish species like *Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Channa marulius*, *Sperata sarwari*, *Catla catla*, and *Pangasianodon hypophthalmus* have contributed to significant global commercial production [11]. These fish are prime candidates for aquaculture and have been widely cultivated [12,13]. However, it's important to note that freshwater bodies, their primary habitat, also host the zoonotic pathogen *Aeromonas hydrophila*, which can infect fish, bivalves, amphibians, reptiles, and humans [14–16]. Two successful candidates in freshwater aquaculture are *Sperata sarwari* and *Channa marulius*, cultivated commercially in various regions, including Pakistan, India, Bangladesh, China, and Indonesia, where they are top producers [17].

*Channa marulius*, commonly known as the "Sole," thrives in diverse aquatic habitats like marshes, ponds, rivers, and rice fields, found in countries like China, India, Pakistan, Cambodia, and Thailand [18]. Belonging to the Channidae family, *C. marulius* is well-suited for intensive rearing systems due to its survival rate and rapid growth [19]. In Pakistan, it's been introduced for commercial farming, standing out for its potential size of up to 30 kg [20,21]. In the Indus River, *Sperata sarwari* dominates the Bagridae catfishes, prized for its large size, valuable flesh, and low intramuscular bones [22]. Advances in aquaculture have enabled captive breeding for *S. sarwari* [23]. While most production relies on capturing, young *S. sarwari* occasionally enters the ornamental fish trade, easily distinguishable from other Bagridae catfish [24].

*Aeromonas hydrophila* is an emerging Gram-negative pathogen found in nature, belonging to the Aeromonadaceae family [25,26]. It is prevalent in aquatic environments, food sources, and mineral water bottles. This bacterium poses threats to both aquatic organisms, mainly fish, causing conditions like motile *Aeromonas* septicemia (MAS), ulcerative disease, and hemorrhagic septicemia [27], as well as humans, leading to wound infections, septicemia, and gastroenteritis. Factors contributing to its virulence include host susceptibility, environmental stressors, and virulence genes [28,29]. *A. hydrophila* is also a significant public health concern due to its potential for transferring virulence genes to humans. It can be found in various sources such as food, groundwater, wastewater, aquatic, and terrestrial animals [30,31]. Identification of *A. hydrophila* involves phenotypic methods and characterizing its *16S rRNA* gene and virulence genes [32,33]. Typically, its identification relies on the presence of virulence genes like the *aerolysin* gene (*aer*), *enterotoxin* gene (*ast*), *hemolysin A* gene (*hylA*), and

*cytotoxic enterotoxin* gene (*act*) [33]. These virulence factors cause histopathological effects in fish [34]. The potential pathogens are associated with serious zoonotic infections [35].

The close interaction between naturally resistant bacteria in terrestrial and aquatic environments facilitates the rapid transfer of antimicrobial resistance (AMR) genes to pathogenic fish bacteria [36,37], making fish a vehicle for AMR bacteria and genes dissemination [38]. This results from fish farmers frequently using multiple antimicrobials to combat AMR bacteria [39], which, unfortunately, leads to an increase in antimicrobial-resistant (AMR) bacteria and their genes in aquaculture [40,41]. Addressing antimicrobial resistance within the One Health framework is crucial due to its interconnected impact on human, animal, and environmental health, requiring collaborative efforts for comprehensive solutions [42]. The emergence of AMR bacteria poses a significant challenge to public health [43,44], as they employ genetic strategies to resist antimicrobials [45]. Meanwhile, pathogenic bacterial diseases are a major cause of mass fish mortality in both cultured and farmed species [46], driven by virulence genes controlling factors like enzyme production [47], biofilm formation [48], immune system suppression, bloodstream infections [49], host-pathogen interactions [50], adaptation to various conditions [51], specificity to hosts [52], and epithelial cell lesions [53]. These factors directly impact nutrition, oxygen levels [54], growth phases [55], temperature [56], and pH in fish environments [57]. Regular monitoring and investigation of physicochemical parameters play a crucial role in controlling the prevalence of pathogenic bacteria [34,58].

The current study was designed to evaluate the antidrug and virulence potential of *A. hydrophila* by amplifying its antimicrobial resistance and virulence genes using PCR and examining their effects on fish tissues and organs.

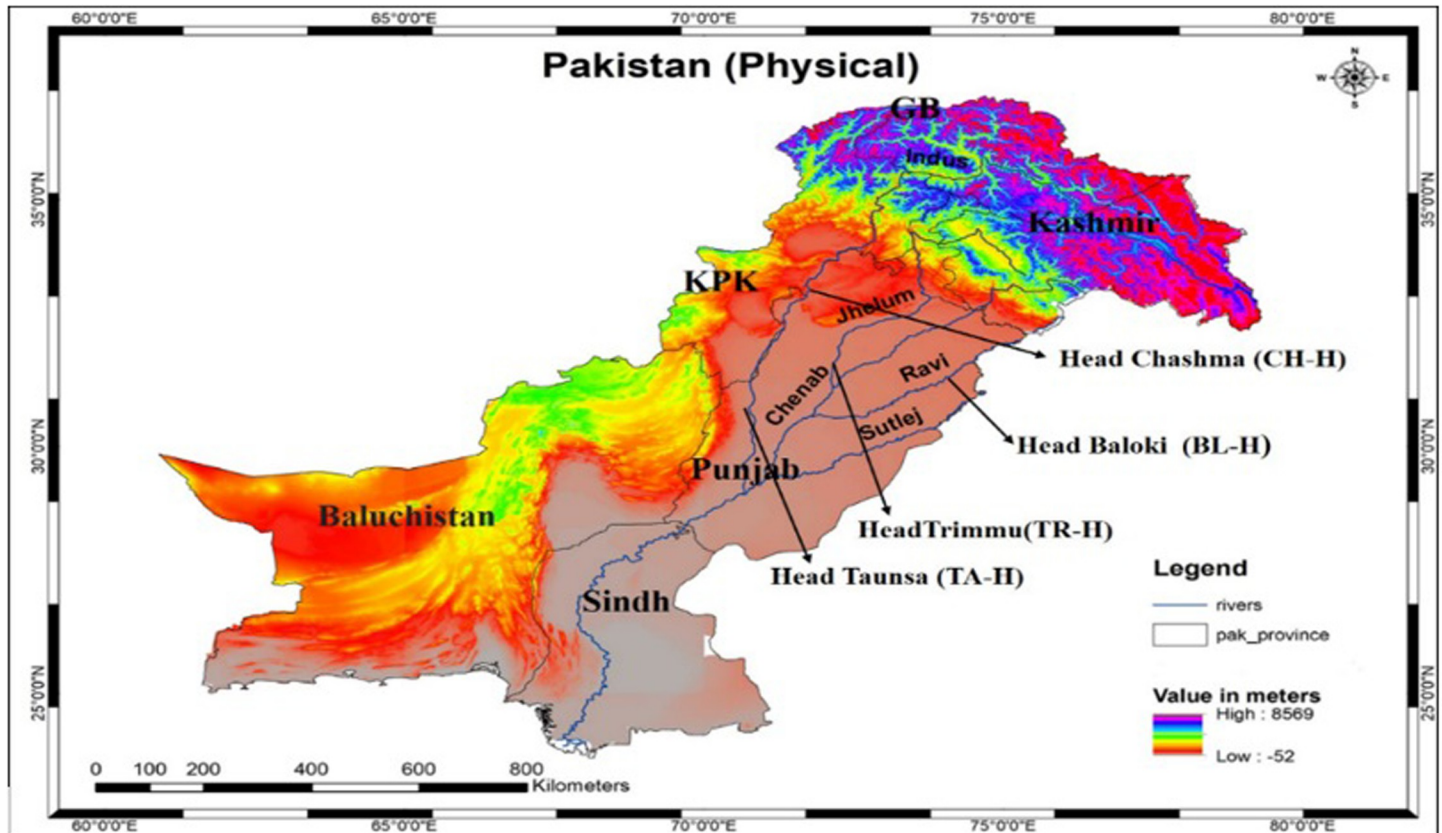
## Materials and methods

### Ethical approval and, fish sampling

All protocols and procedures were approved by the Guidelines for the Care and Use of Laboratory Animals Committee of the University of Veterinary and Animal Sciences, Lahore, Pakistan (DAS/358, 02-03-2023). A total of 960 fish samples (480 from each of *C. marulius* and *S. sarwari*) were collected using a nylon drag net from four selected sites: Head Baloki (BL-H), Head Taunsa (TA-H), Head Chashma (CH-H), and Head Trimmu (TR-H) of the riverine system of Punjab, Pakistan. Sampling was conducted from April 2022 to December 2022, categorized seasonally as 280 in summer, 120 in autumn, and 80 in winter. 120 fish samples of each species were collected from each sampling site. Soon after netting, the fish samples were measured for weight and length parameters outdoors. Water temperature of BL-H was measured as 25.42°C, 26.62°C at TR-H, 24.79°C at TA-H, and 22.98°C at CH-H. The sampling sites for the current study are depicted in Fig 1. The fish samples were placed in plastic containers with ice packs and transported directly (within 24 hours) to the laboratory of the Department of Zoology, University of Education, Faisalabad Campus, Pakistan.

### Isolation, phenotypic, morphological, and biochemical characterization of *Aeromonas hydrophila*

The collected fish was disinfected by rinsing with clean water and sodium hypochlorite following regulations and guidelines recommended by Noga, [59]. The internal organs (skin, stomach, kidney, liver, intestine, spleen, and gills) of the collected fish were subjected to bacteriological examination. Swabs were randomly collected from suspected organs and were inoculated onto Trypticase soy agar (TSA LAB, UK) media by plate streaking method and were incubated at 37°C overnight according to the method described by Lima, and Muratori,



**Fig 1.** The sampling sites for the current study are depicted on the map.

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[60,61]. A single colony from freshly obtained bacterial culture was inoculated onto Trypticase soy agar (TSA LAB, UK) media plates to obtain a pure culture of *A. hydrophila*, which was then incubated at 37°C for 24 h following the method recommended by Muratori, [61]. Pure culture of *A. hydrophila* was subjected to Gram-staining and viewed microscopically (Euro-mex, 100X). Colony morphology, culture, and microscopic characteristics of *A. hydrophila* were observed according to the protocol recommended by Muratori, and Xiao, [61,62]. The isolates of *A. hydrophila* were characterized by biochemical tests like indole, motility, oxidase, H<sub>2</sub>S production, catalase, and urease tests as for identification as previously performed by Fang, [63].

### DNA extraction

DNA was isolated using a Genomic DNA Purification Kit (Thermo Scientific, GeneJET, USA) and DNA samples were evaluated by gel electrophoresis on 1% agarose gel stained with ethidium bromide (Sigma-Aldrich E7637, USA) and utilizing a standard-sized molecular marker [1Kb DNA Ladder RTU (Ready-to-Use) GeneDireX, Taiwan]. Isolated DNA was stored at -20°C for further use.

## Amplification, sequencing, and phylogenetic tree analysis of *16S rRNA* and *gyrB* gene of *A. hydrophila*

One microliter of template DNA was added into a total of 25  $\mu$ l reaction solution for PCR containing two primers of *16S rRNA*; 1  $\mu$ l forward primer (27F): AGAGTTTGTATCCTGGCTCAG, 1  $\mu$ l reverse primer (1492R): GGTTACCTTGTTACGACTT, 10  $\mu$ l PCR-grade water, and 12  $\mu$ l GoTaq Green Master Mix (Promega, USA) (Table 1). Similarly, *gyrB* gene was also amplified by species-specific primers. PCR products were electrophoresed in 1% agarose gel stained with ethidium bromide (Sigma-Aldrich E7637, USA) and utilizing a standard-sized molecular marker (1Kb DNA Ladder RTU, GeneDireX). PCR products revealing the thickest bands were sequenced by Sanger's method at BGI Hong Kong Co. Ltd. China. The obtained sequences were analyzed and compared for taxonomic identification using National Centre for Biotechnology Information-Basic Local Alignment Search Tool (NCBI-BLAST), and subsequently, submitted to the GenBank® database. To determine the phylogenetic relationship of *A. hydrophila*, a phylogenetic tree analysis was conducted on the *16S rRNA* and *gyrB* genes of *A. hydrophila*. This analysis employed the bootstrap method with 1,000 bootstrap replications, and it was carried out using MEGA 11.0 (Molecular Evolutionary Genetic Analysis), as described by Chen, [64].

## Molecular identification of virulence and antimicrobial resistance (AMR) genes of *A. hydrophila*

Virulence genes of *A. hydrophila* (including *hemolysin (hlyA)*, *aerolysin (aerA)*, and *cytotoxic enterotoxin (act)*) and antimicrobial resistance genes (such as *sul1*, *sul3*, *qnrA*, *qnrB*, *blaTEM*,

**Table 1. Conditions for amplification of *16S rRNA*, *gyrB*, virulence, and AMR genes of *A. hydrophila*.**

Gene Class	Gene Name	Primer sequence (5–3)	Target bp size	Annealing Temperature	Accession Number	References
rrs gene	<i>16S rRNA</i>	F-AGAGTTTGTATCCTGGCTCAG	1503	52°C for 1min	OR509789; OR509790 OR509791; OR509792 OR509793; OR509794	[65]
		R-ACGGCTACCTTGTTACGACTT				
Antimicrobial Resistance genes (AMR)	<i>gyrB</i>	F-GAGGACTACAGCAAGAAGGCCA	1124	55°C for 90 s	OQ699127	[66]
		R-GACTTGGCCTTCTTGCTGTAGTC				
	<i>tetA</i>	F-GCTACATCCTGCTTGCCTTC	813	55°C for 1min	OR061081	[67]
		R-CATAGATCGCCGTGAAGAGG				
	<i>blaTEM</i>	F-CATTTCCTGTCGCCCTTATTC	873	55°C for 90 s	OQ726106	
		R-CGTTTCATCCATAGTTGCCTGAC				
	<i>qnrA</i>	F-ATTCTCACGCCAGGATTTG	654	60°C for 1 min	OQ729990	[68]
		R-GATCGGCAAGGTTAGGTCA				
	<i>qnrB</i>	F-GGMATHGAAATTCGCCACTG	642	53°C for 30 s	OR515644	[69]
		R-TTTGCGYGYCGCCAGTCGAAC				
<i>sul1</i>	F-CGGCGTGGCTACCTGAACG	444	55°C for 30 s	OR515645	[70]	
	R-GCCGATCGCGTGAAGTTCCG					
<i>sul3</i>	F-AGATGTGATTGATTGGGAGC	444	54.2°C for 30 s	OR061080	[68]	
	R-TAGTTGTTTCTGGATTAGAGCCT					
Virulence gene	<i>hlyA</i>	F-GGCAAACAGCGAAACAAATACC	585	55.5°C for 30 s	OR515643	[71]
		R-CTCAGCGGGCTAATACGGTTTA				
	<i>aerA</i>	F-GTCACCTTCTCGCTCAGGC	417	55°C for 30 s	OR515642	[72]
		R-TGATTCCCGAAGGCACTCCC				
	<i>act</i>	F-GAGAAGGTGACCACCAAGAACA	675	58°C for 30 s	OR515641	
		R-AACTGACATCGGCCTTGAACCTC				

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and *tetA*) of *A. hydrophila* were identified through PCR analysis using species-specific primers (Macrogen, Korea) and were compared with a standard-sized molecular marker DNA ladder (Table 1). A total of 25  $\mu$ l of PCR reaction solution, comprising 1  $\mu$ l of template DNA, 1  $\mu$ l forward primer, 1  $\mu$ l reverse primer, 10  $\mu$ l PCR-grade water, and 12  $\mu$ l GoTaq Green Master Mix (Promega, USA), was utilized for the detection of the AMR genes in *A. hydrophila* (Table 1). Amplified PCR products were analyzed on 1% agarose gel stained with ethidium bromide (Sigma-Aldrich E7637, USA) and utilizing a standard-sized molecular marker (1Kb DNA Ladder RTU, GeneDireX). PCR products revealing the thickest bands were sequenced by Sanger's method at BGI Hong Kong Co. Ltd., China as previously analyzed by Wang, [73].

### Minimal inhibitory concentration (MIC) and antimicrobial susceptibility testing of *A. hydrophila*

*A. hydrophila* isolates were subjected to microtiter plates and Kirby Bauer disc diffusion method for antimicrobial sensitivity testing on Mueller-Hinton agar plates according to the method carried out by Bauer, [74] using antimicrobials norfloxacin, streptomycin, gentamicin, chloramphenicol, ciprofloxacin, doxycycline, ampicillin, flumequine, neomycin, tetracycline, sulfamethoxazole, and cefotaxime. The plates were incubated for twenty-four hours at 37°C. Diameter of the inhibition zone were measured and interpreted to classify bacteria as resistant, moderately susceptible, and susceptible according to clinical and laboratory standards institute (CLSI), [75].

### Histopathological effect of *A. hydrophila*

Tissue samples were collected from the liver, stomach, spleen, and small intestine of infected *C. marulius* and *S. sarwari*. These collected tissue specimens were disinfected and preserved in a 10% neutral buffered formalin solution with a 1:10 ratio (formalin and distilled water, respectively) in plastic sample containers, labeled against each respective tissue specimen. The preserved tissue samples were submitted to the laboratory of the Department of Pathology, City Campus, University of Veterinary and Animal Sciences (UVAS) Lahore, and examined for histopathological changes due to *A. hydrophila* infection, specifically motile *Aeromonas* septicemia (MAS). The obtained slides were viewed under a light microscope (Euromex 100X, Netherlands) to observe histopathological changes caused by *A. hydrophila* and stored for future use.

### Statistical analysis

Chi-square test of independence was applied in comparing the prevalence/occurrence of *A. hydrophila* with respect to sampling site, fish sex, season, and organs. Descriptive statistics such as proportions and frequency were employed in summarizing the data.

## Results

### Physicochemical parameters, analysis of weight and length of *C. marulius* and *S. sarwari*

Maximum and minimum temperature was recorded as 26.62°C (TR-H) and 22.98°C (CH-H) respectively. Maximum and minimum pH was recorded as 8.23 (CH-H) and 7.18 (TR-H) respectively. Samples of *S. sarwari* collected from CH-H showed maximum weight (307 g) and minimum by fish collected from TR-H (303.8 g) while maximum length (27.4 cm) was shown by samples of *S. sarwari* collected from TR-H and minimum length (25.6 cm) by fish collected from BL-H. Similarly, samples of *C. marulius* collected from CH-H showed maximum weight

(175 g) and minimum by fish collected from BL-H (151.2 g) while maximum length (34.2 cm) was shown by samples of *C. marulius* collected from CH-H and minimum length (27.4 cm) by fish collected from BL-H. Results of physicochemical parameters, weight and length of *C. marulius* and *S. sarwari* are shown in [S1 Table](#).

### Isolation, phenotypic and biochemical characterization

We collected swabs from the organs of 480 fish samples of each of *S. sarwari* and *C. marulius*. We isolated *A. hydrophila* by direct plating on TSA plates. We recovered *A. hydrophila* in 31 fish samples of *C. marulius* and 30 of *S. sarwari* collected from all sampling sites. Phenotypic characterization of *A. hydrophila* showed rod-shaped, round, smooth, and grayish-white colored colonies on TSA media plates. Biochemical characterization of *A. hydrophila* isolates revealed it as motile, Gram-negative, rod-shaped, and facultatively anaerobic bacterium bearing Peritrichous flagella, by biochemical tests. All the isolates of *A. hydrophila* were found positive against catalase, oxidase, glucose, sucrose, lactose, urease, indole, and H<sub>2</sub>S production tests represented in [S2 Table](#).

### Prevalence of *A. hydrophila*

Overall prevalence of *A. hydrophila* was recorded as 6.35% in fish samples of both fish species. The maximum prevalence of *A. hydrophila*, 6.46% was observed in the intestine of infected *C. marulius* while, the minimum prevalence, 4.17% was noted in gills of infected *S. sarwari* ([Table 2](#)). Overall *A. hydrophila* infected 15 fish samples (12.5%) of *S. sarwari* collected from BL-H while, the minimum infection rate, 1.67% was observed in *C. marulius* collected from CH-H. Among the fish, *A. hydrophila* infected 9.78% of males in *C. marulius* and 4.7% of females in *S. sarwari*. Furthermore, *A. hydrophila* infected 6.43% and 5.71% of fish samples of *S. sarwari* and *C. marulius* respectively during the summer while 5% and 6.25% of *S. sarwari* and *C. marulius* respectively during the winter [Table 3](#).

### Occurrence of virulence and antimicrobial resistance genes of *A. hydrophila*

Virulence genes (*aerA*, *hylA*, and *act*) and antimicrobial resistance genes (*sul1*, *sul3*, *qnrA*, *qnrB*, *tetA*, and *blaTEM*) of *A. hydrophila* were amplified by PCR. Among all the AMR genes, maximum occurrence, 6.04% of *tetA* gene was recorded in *A. hydrophila* isolates isolated from *C. marulius* ([Table 4](#)). Similarly, among all the virulence genes, maximum occurrence, 6.46% of *aerA* gene was recorded in *A. hydrophila* isolates isolated from *C. marulius*. The chi-square test of independence showed insignificant difference ( $P > 0.05$ ) in occurrence of antimicrobial resistance (AMR) genes [Table 5](#).

### Multiple-drug resistance (MDR) and antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on a total of 30 *S. sarwari* and 31 *C. marulius* isolates of *A. hydrophila*. All the isolates of *A. hydrophila* demonstrated resistance to

**Table 2. Prevalence of *A. hydrophila* with respect to fish organs.**

Fish Species	Fish Organs						
	Skin	Liver	Intestine	Stomach	Gills	Kidney	Spleen
<i>S. sarwari</i>	21 (4.37%)	26 (5.42%)	28 (5.83%)	30 (6.25%)	20 (4.17%)	29 (6.04%)	23 (4.79%)
<i>C. marulius</i>	24 (5%)	25 (5.21%)	31 (6.46%)	29 (6.04%)	23 (4.79%)	27 (5.62%)	22 (4.58%)

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Table 3. Prevalence of *A. hydrophila* with respect to sampling sites, sex, seasons and overall prevalence.

Fish Species	Sampling sites				SEX		SEASONS			Overall prevalence
	Head Baloki (BL-H)	Head Trimmu (TR-H)	Head Taunsa (TA-H)	Head Chashma (CH-H)	Male	Female	Summer	Autumn	Winter	
<i>S. sarwari</i>	15 (12.5%)	8 (6.67%)	4 (3.33%)	3 (2.5%)	18 (8%)	12 (4.7%)	18 (6.43%)	8 (6.67%)	4 (5%)	30 (6.25%)
<i>C. marulius</i>	13 (10.83%)	11 (9.17%)	5 (4.17%)	2 (1.67%)	22 (9.78%)	9 (3.53%)	16 (5.71%)	10 (8.33%)	5 (6.25%)	31 (6.40%)

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amoxicillin, ampicillin, sulfamethoxazole, erythromycin, flumequine, ciprofloxacin, neomycin, and norfloxacin. In contrast, *A. hydrophila* isolates demonstrated susceptible to gentamicin, doxycycline, chloramphenicol, and tetracycline, with intermediate resistance observed against cefotaxime and streptomycin shown in Tables 6–8.

### Phylogenetic tree analysis

Phylogenetic tree of *16S rRNA* gene *A. hydrophila* revealed 100% similarity among all the *A. hydrophila* strains isolated in the current study, as well as with strains isolated in earlier studies (Fig 2). Furthermore, phylogenetic tree analysis of *gyrB* gene of *A. hydrophila* revealed 97% similarity among all the *A. hydrophila* strains isolated in the current study, as well as with strains previously isolated (Fig 3).

### Histopathological effect of *A. hydrophila*

Histopathological examination revealed various abnormalities in the infected fish. In the liver, findings included congestion (5.2%), hepatocyte necrosis (4.6%), granuloma formation (4.3%), and inflammation (5%). The gills exhibited epithelial hyperplasia (3.5%), lamellar fusion (3.3%), edema (3%), and the presence of *A. hydrophila* colonies (3.7%). Tail fins displayed issues such as fin erosion (6%), hemorrhage (6.2%), loss of fin rays (4.8%), and the presence of biofilms (3.5%). *A. hydrophila* infection also led to fibrosis (4%), abscess formation (3.7%), fatty degeneration (3.5%), and the infiltration of inflammatory cells (4.7%) in spleen (Fig 4).

### Discussion

Fish is one of the most important sources of food that provides easy digestion, high palatability, and high nutritional value. However, it is also considered an important vehicle for many types of pathogens, raising public health concerns. The prevalence of *A. hydrophila* is directly proportional to an increase in temperature, but there is no association between its prevalence and the weight and length of the fish. In the current study, overall 61 *A. hydrophila* (6.3%) were recovered in both fish species, *C. marulius* and *S. sarwari*. The intestine and stomach were reported as the organs with a high prevalence of *A. hydrophila*, as 31 isolates of intestine (6.5%) from *C. marulius* and 30 isolates of stomach (6.2%) from *S. sarwari* showed the maximum prevalence. An increase in temperature during the summer also favors a higher

Table 4. Occurrence of antimicrobial resistance (AMR) genes, *gyrB*, and *16S rRNA* gene of *A. hydrophila*.

Fish Species	<i>tetA</i>	<i>blaTEM</i>	<i>qnrA</i>	<i>qnrB</i>	<i>sul1</i>	<i>sul3</i>	<i>aerA</i>	<i>hlyA</i>	<i>act</i>	<i>gyrB</i>	<i>16S rRNA</i>
<i>S. sarwari</i>	22 (4.58%)	25 (5.21%)	28 (5.83%)	23 (4.79%)	28 (5.83%)	24 (5%)	30 (6.25%)	28 (5.83%)	27 (5.62%)	27 (5.62%)	30 (6.25%)
<i>C. marulius</i>	29 (6.04%)	19 (3.96%)	27 (5.62%)	21 (4.37%)	26 (5.42%)	21 (4.37%)	31 (6.46%)	30 (6.25%)	28 (5.83%)	31 (6.46%)	31 (6.46%)

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Table 5. The results of the chi-square test of independence show the  $X^2$ -value and P-value in relation to the parameters.

Parameter	<i>S. sarwari</i>		<i>C. marulius</i>	
	Chi-squared value	p-value	Chi-squared value	p-value
Organs	35.00	0.243 <sup>ns</sup>	108.5	0.628 <sup>ns</sup>
Bacterial Species	20.00	0.220 <sup>ns</sup>	88.3	0.158 <sup>ns</sup>
Sampling Sites	12.00	0.213 <sup>ns</sup>	37.3	0.408 <sup>ns</sup>
Fish Sex	2.00	0.157 <sup>ns</sup>	8.0	0.433 <sup>ns</sup>
Seasons	6.00	0.199 <sup>ns</sup>	30.0	0.268 <sup>ns</sup>
Occurrence of AMR Genes	8.00	2.38 <sup>ns</sup>	36.0	0.607 <sup>ns</sup>

Note; ns indicate Non-significant.

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prevalence, as 34 isolates were recorded during this season (3.5%). *A. hydrophila* affects males more than females, as the maximum prevalence was recorded in 40 (4.2%) male fish samples from both species. Additionally, the highest prevalence was found in 28 fish samples (2.9%) at Head Baloki (BL-H) in Kasur.

Phenotypic characterization in the current study confirmed *A. hydrophila* isolates as circular, smooth, Gram-negative, rod-shaped, motile, and facultatively anaerobic bacteria bearing peritrichous flagella. Biochemical tests showed that all *A. hydrophila* isolates were positive for catalase, oxidase, glucose, sucrose, lactose, urease, indole, and H<sub>2</sub>S production tests. In a previous study, Wamala, [76] identified *A. hydrophila* isolates as Gram-negative, motile, and positive in catalase, oxidase, and indole production tests in Uganda. However, they observed negative results in urease and H<sub>2</sub>S production tests, which contradicted our findings. Li, [77] in a study conducted in China, observed positive results in glucose and H<sub>2</sub>S production tests but negative results in the urease test, again differing from our findings.

In the current study, we detected three virulence genes, namely *aerolysin* (*aerA*), *hemolysin* (*hlyA*), and *cytotoxic enterotoxin* (*act*) genes, in *A. hydrophila* isolates recovered from a total of

Table 6. Results of antimicrobial susceptibility of *A. hydrophila*.

Antibiotics	Concentration (µg)	Class of Antimicrobial	Susceptible	Intermediate	Resistant	MIC <sub>90</sub> (µg/ml)	MIC <sub>50</sub> (µg/ml)
AMX	25	Penicillin	0	0	100%	>128	>64
AMP	10	Penicillin	0	0	100%	>64	>32
CTX	5	Cephalosporin	0	50%	0	<32	<16
C	30	Miscellaneous Antibiotics	100%	0	0	1	0.5
CIP	5	Fluoroquinolones	25%	0	75%	>32	>16
DO	30	Tetracycline	100%	0	0	1	0.5
E	15	Miscellaneous Antibiotics	0	0	100%	16	8
FLU	30	Quinolones	0%	0	100%	>128	>64
GM	10	Aminoglycosides	100%	0	0	4	2
N	30	Aminoglycosides	0	0	100%	>128	>64
NOR	10	Fluoroquinolones	0	0	100%	>64	>32
S	10	Aminoglycosides	0	50%	0	4	2
SXT	25	Sulfonamides	0	0	100%	>64	>32
T	10	Tetracycline	100%	0	0	<4	<2

Note: AMX indicates Amoxicillin, AMP Ampicillin, CTX Cefotaxime, C Chloramphenicol, CIP Ciprofloxacin, DO Doxycycline, E Erythromycin, FLU Flumequine, GM Gentamicin, N Neomycin, NOR Norfloxacin, S Streptomycin, SXT Sulfamethoxazole and, T Tetracycline.

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Table 7. MDR profile for *A. hydrophila* isolated from indus riverine fish Punjab-Pakistan.

Antibiotic Combination	No of Isolates	No of Antibiotic Resistance	%age
AMY	1	1	1.6
AMP, E	1	2	11.5
FLU, N	1	2	
N, NOR	2	2	
AMY, SXT	3	2	
AMY, AMP, E	1	3	
FLU, N, NOR	1	3	8.2
SXT, E, AMP	1	3	
AMY, NOR, N	2	3	
AMY, AMP, E, FLU	1	4	
N, NOR, SXT, AMY	2	4	
AMP, E, FLU, N	4	4	14.8
NOR, SXT, AMY, E	2	4	
AMY, AMP, E, FLU, N	1	5	
AMP, E, FLU, N, NOR	2	5	
E, FLU, N, NOR, SXT	1	5	
FLU, N, NOR, SXT, AMY	1	5	18
N, NOR, SXT, AMY, AMP	3	5	
NOR, SXT, AMY, AMP, E	2	5	
SXT, E, AMP, AMY, FLU	1	5	
AMY, AMP, E, FLU, N, NOR	2	6	
AMY, AMP, E, FLU, N, SXT	1	6	36
AMY, AMP, E, FLU, NOR, SXT	1	6	
AMY, AMP, E, N, NOR, SXT	6	6	
AMY, AMP, FLU, N, NOR, SXT	1	6	
AMY, E, FLU, N, NOR, SXT	8	6	
AMP, E, FLU, N, NOR, SXT	3	6	9.8
AMY, AMP, E, FLU, N, NOR, SXT	6	7	
TOTAL	61	121	100%

Note. AMX indicates Amoxicillin, AMP Ampicillin, CTX Cefotaxime, C Chloramphenicol, CIP Ciprofloxacin, DO Doxycycline, E Erythromycin, FLU Flumequine, GM Gentamicin, N Neomycin, NOR Norfloxacin, S Streptomycin, SXT Sulfamethoxazole and, T Tetracycline.

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31 samples (6.25%) of *C. marulius* and 30 samples (6.46%) of *S. sarwari*. Specifically, we observed *aerA* gene in 31 isolates (6.45%), *hlyA* gene in 30 isolates (6.25%), and *act* gene in 28 isolates (5.83%) of infected *C. marulius*. Similarly, we recorded *aerA* gene in 30 isolates (6.25%), *hlyA* gene in 28 isolates (5.8%), and *act* gene in 27 isolates (5.6%) of infected *S. sarwari*. In a recent study, Morshdy, [78] recovered *A. hydrophila* in 20% of catfish samples in Egypt. They also detected *aerolysin* and *hemolysin* genes in 25% and 75% of retail fish samples, respectively. The main reason behind the high prevalence of *A. hydrophila* was contamination caused by marketing and transportation. Similarly, El-Hossary, [79] detected *aerolysin* (*aerA*) and *hemolysin* (*hlyA*) genes in *A. hydrophila* isolated from infected Nile tilapia (*Oreochromis niloticus*) collected from local fish markets in Egypt. They found *A. hydrophila* in 28.8% of market fish samples. The variations in the prevalence of *A. hydrophila* could be attributed to various factors, including sampling conditions (such as location and time), post-capture contamination, fish species, handling, water type, geographic location, manipulations during

Table 8. Resistance genes profile for *A. hydrophila* isolated from indus riverine fish Punjab-Pakistan.

Resistance gene	No of Isolates	No of Resistance genes	%age
<i>tetA, blaTEM, qnrA, qnrB</i>	1	4	24.6
<i>blaTEM, qnrA, qnrB, Sul1</i>	1	4	
<i>qnrA, qnrB, Sul1, Sul3</i>	6	4	
<i>qnrB, Sul1, Sul3, tetA</i>	1	4	
<i>Sul1, Sul3, tetA, blaTEM</i>	4	4	
<i>Sul3, tetA, blaTEM, qnrA</i>	2	4	
<i>tetA, blaTEM, qnrA, qnrB, Sul1</i>	2	5	60.7
<i>blaTEM, qnrA, qnrB, Sul1, sul3</i>	8	5	
<i>qnrA, qnrB, Sul1, Sul3, tetA</i>	5	5	
<i>qnrB, Sul1, Sul3, tetA, blaTEM</i>	6	5	
<i>Sul1, Sul3, tetA, blaTEM, qnrA</i>	6	5	
<i>Sul3, tetA, blaTEM, qnrA, qnrB</i>	10	5	
<i>tetA, blaTEM, qnrA, qnrB, Sul1, sul3</i>	9	6	14.7
<b>Total</b>	61	60	100%

Note; tetA indicate tetracycline, β-lactamase *blaTEM*, Quinolones *qnrA, qnrB* and sulfonamide resistance gene *Sul1, sul3*.

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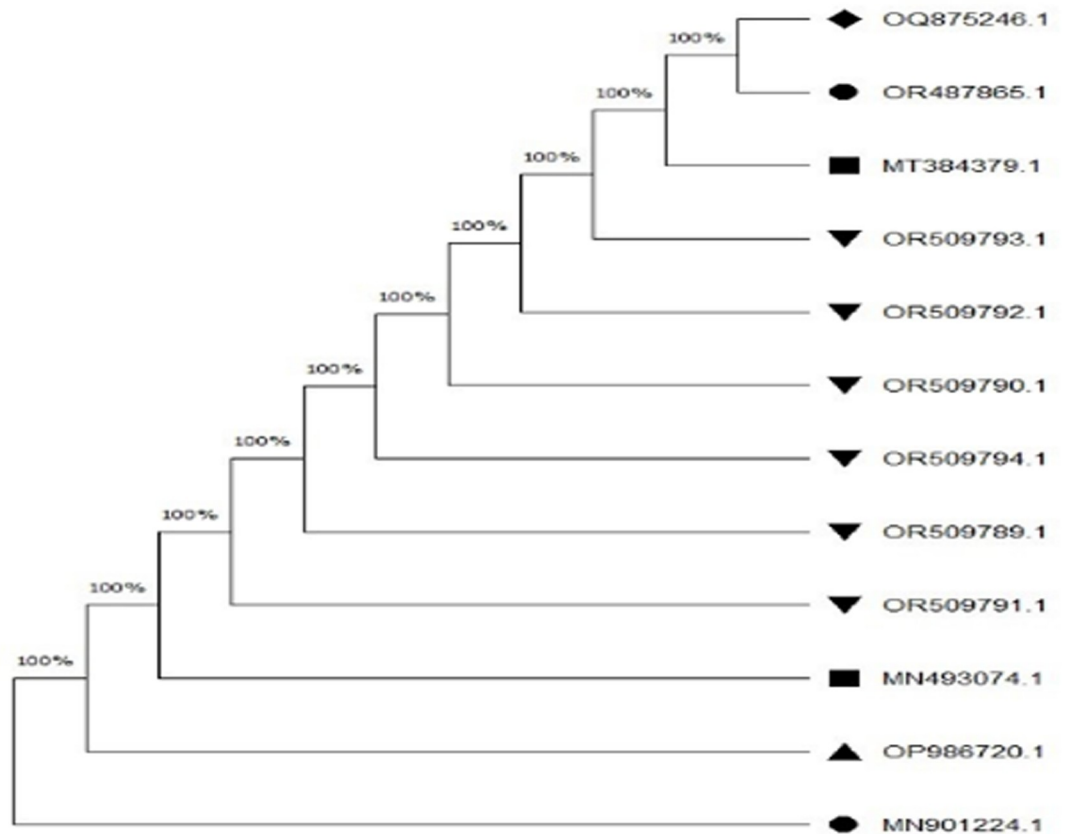


Fig 2. Phylogenetic tree analysis of 16S rRNA gene of *A. hydrophila*.

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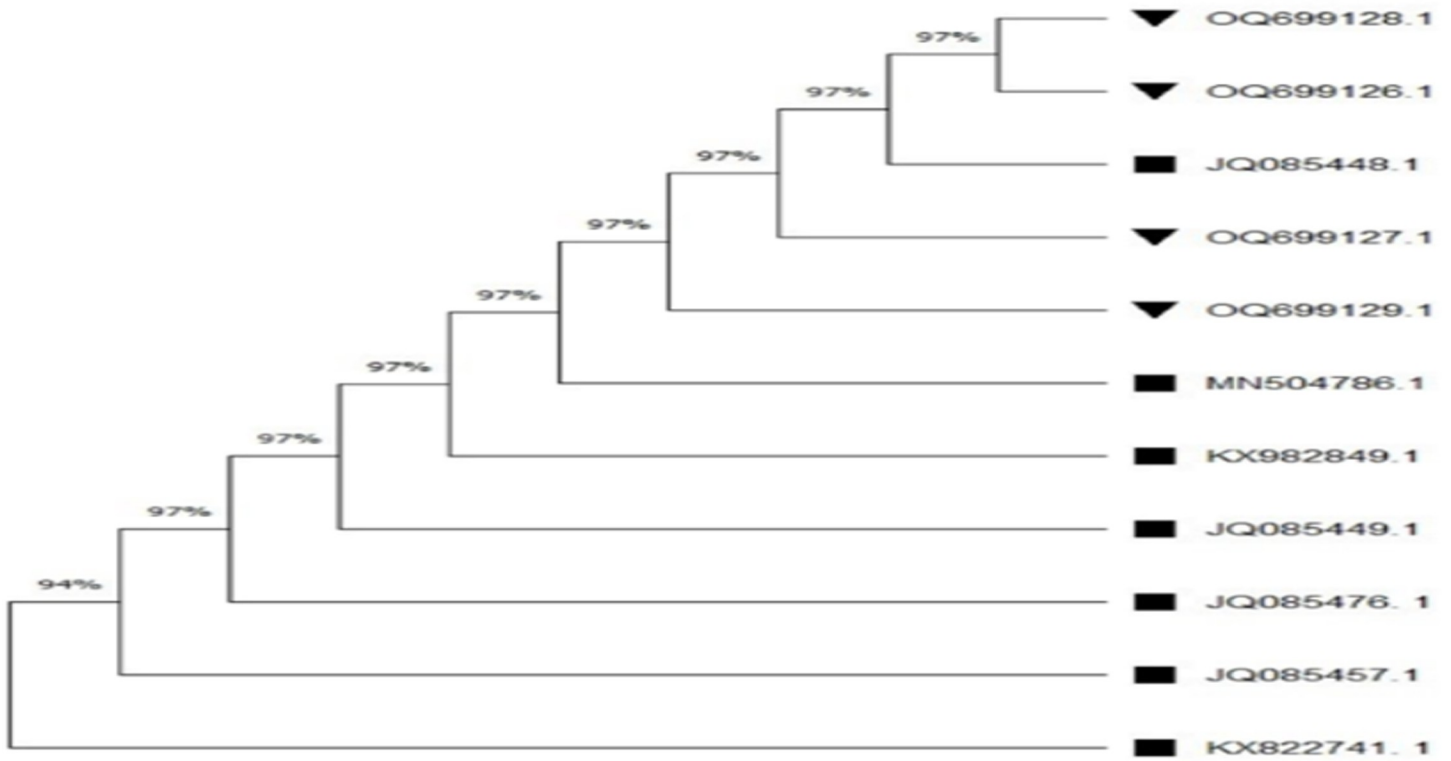


Fig 3. Phylogenetic tree analysis of *gyrB* gene of *A. hydrophila*.

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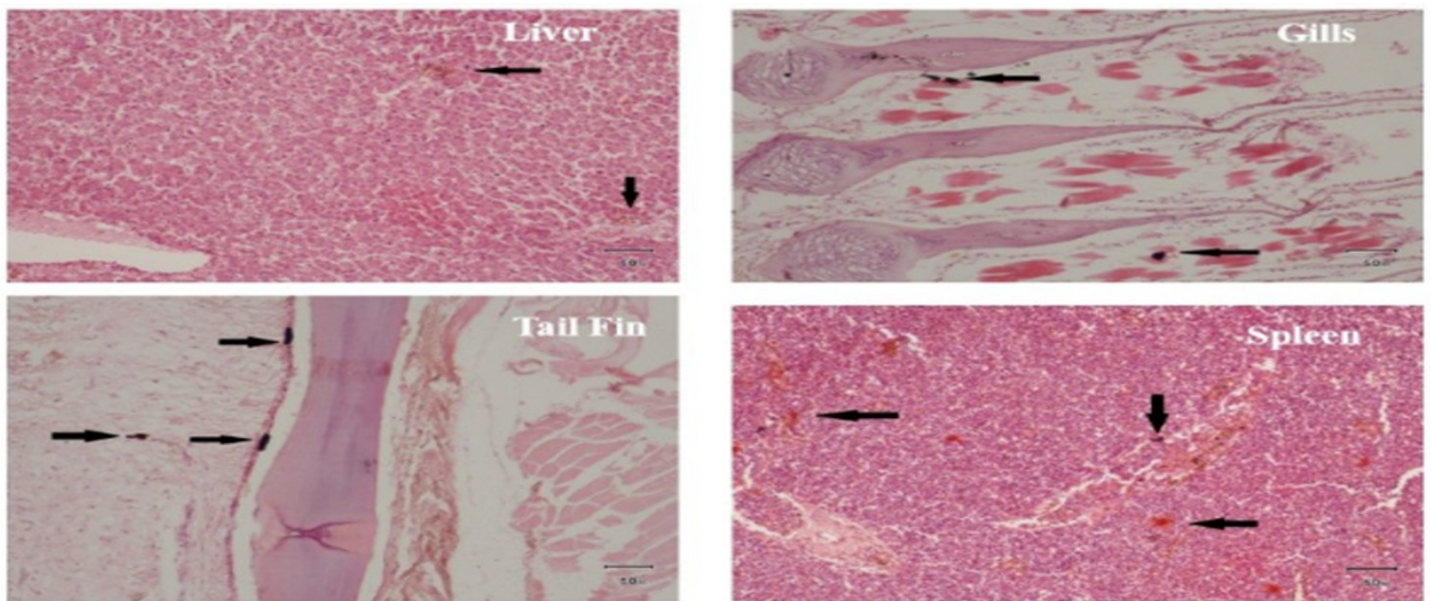


Fig 4. Histopathological impact of *A. hydrophila* on tissues of infected fish samples.

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capture, storage, marketing, and transportation. Moreover, Thaotumpitak, [80] recovered 15 isolates (5.39%) of *A. hydrophila* in hybrid tilapia collected from cage culture in Thailand. They also detected *aerolysin* (*aerA*) and *hemolysin* (*hlyA*) genes in *A. hydrophila* in infected hybrid tilapia. Additionally, Suresh and Pillai, [29] recovered *A. hydrophila* from 27% of samples of Indian major carps (*Cirrhinus mrigala*, *Labeo rohita*, and *Catla catla*) in India. They identified ten virulence genes, including *aerolysin* (*aerA*), *hemolysin* (*hlyA*), and *cytotoxic enterotoxin* (*act*) genes, in the infected fish. The variation in the prevalence of *A. hydrophila* could be attributed to stress, which allows the opportunistic pathogen *A. hydrophila* to cause infections.

As an opportunistic pathogen, *A. hydrophila* infects fish under conditions of stress, high temperature, low water quality, high organic content, and stocking density. In a recent study, Abdella, [28] detected 312 virulence genes in *A. hydrophila* strains, including *aerA*, *hlyA*, and *act* genes in Egypt. In another study, by Ninh, [81] 46.4% of *A. hydrophila* isolates were recovered from 506 diseased (moribund) tilapia, carps (common carp and grass carp), and channel catfish of Vietnam. They also detected the *aerA* gene in 80.1% of cases and the *act* gene in 80.5% of cases. Similarly, Saleh, [82] recovered 53.4% (187/350) of *A. hydrophila* isolates from infected Nile tilapia in Egypt. They detected the *act* and *aerA* genes in virulent *A. hydrophila* strains. In a similar study, Ahmed, [83] found *A. hydrophila* in 34 isolates (7.1%) isolated from Nile tilapia (*O. niloticus*) and *Mugil cephalus* in Egypt. They also identified four virulence genes, including *hly*, *aer*, and *act* genes, in infected fish samples. Additionally, Azzam-Sayuti, [84] recovered 20% of *A. hydrophila* isolated from 270 healthy cultured *Clarias batrachus*, *P. hypophthalmus*, and *O. niloticus* in Malaysia. They detected eight virulence genes, including *aerA*, *hlyA*, and *act* genes. Moreover, Abu-Elala, [85] recovered 20 out of 24 (83.3%) *A. hydrophila* isolates from infected fish in Egypt. They detected 45.45% of virulence genes in *A. hydrophila* isolates, including the *aer* and *act* genes. Similarly, Roges, [86] reported a 92.7% occurrence of virulence genes in 110 *A. hydrophila* isolates isolated from fish, animals, and humans, including the *act*, *aer*, and *hly* genes in Brazil. The major reasons behind these significant variations in results may include contaminated water, severe environmental conditions, bacterial strains, and low water quality parameters.

*A. hydrophila* is a multiple antimicrobial-resistant bacterium and one of the most significant pathogens in fish, causing *Aeromonas* septicemia (MAS) in various freshwater fish species. Its antimicrobial resistance against multiple drugs has made it a global health risk. In the current study, we identified the presence of *bla*TEM, *sul1*, *sul3*, *qnrA*, *qnrB*, and *tetA* genes in *A. hydrophila* isolated from both *C. marulius* and *S. sarwari*. Specifically, we recorded a 6.46% prevalence of the *tetA* gene, 6.25% for *bla*TEM, 5.83% for *sul1*, 5.42% for *sul3*, 5% for *qnrA*, and 4.17% for *qnrB* gene in 31, 30, 28, 26, 24, and 20 samples of infected *C. marulius*, respectively. Similarly, in *S. sarwari*, we recorded a 6.25% prevalence of the *tetA* gene, 6.04% for *bla*TEM, 5.21% for *sul1*, 4.79% for *sul3*, 4.58% for *qnrA*, and 4.37% for *qnrB* gene in 30, 29, 25, 23, 22, and 21 samples of infected fish, respectively.

We observed that all *A. hydrophila* isolates were resistant to amoxicillin, ampicillin, sulfamethoxazole, neomycin, and norfloxacin, while they were susceptible to gentamicin, chloramphenicol, and tetracycline. Additionally, they showed intermediate resistance to cefotaxime. In a recent study, Eid, [87] reported a 53.85% prevalence of *A. hydrophila* collected from Mediterranean seawater in Egypt. They identified *sul1*, *bla*TEM, and *tetA* genes in *A. hydrophila* isolated from *M. cephalus* (striped mullet) in Egypt and also detected the *act* gene in antimicrobial-resistant *A. hydrophila*. These isolates were resistant to  $\beta$ -lactams and sulfonamides (100%), oxytetracycline (90%), and streptomycin (62.22%), but completely susceptible to cefotaxime. In a recent study, Thaotumpitak, [80] identified six antimicrobial resistance genes in *A. hydrophila* isolated from hybrid red tilapia cultured in cages in Thailand, including

*bla*TEM, *sul1*, *sul3*, *qnrA*, *qnrB*, and *tetA*. All *A. hydrophila* isolates were resistant to ampicillin, oxytetracycline, tetracycline, trimethoprim, and oxolinic acid. Similarly, Fauzi, [88] reported the presence of drug resistance genes in *A. hydrophila* isolated from freshwater fish in Malaysia. They identified *sul1*, *bla*TEM, and *tetA* genes in *A. hydrophila*. These isolates were resistant to ampicillin, kanamycin, nalidixic acid, neomycin, oxytetracycline, streptomycin, tetracycline, and sulfamethoxazole. Additionally, they showed intermediate resistance to gentamicin, ciprofloxacin, norfloxacin, and doxycycline, while they were susceptible to chloramphenicol and nitrofurantoin.

Regular exposure to antimicrobials facilitates the spread of slowly curable infections caused by *A. hydrophila*. In a previous study, Elkenany, [89] recovered 14.3% of *A. hydrophila* isolated from aquatic seafood organisms such as shrimp, crab, squid, and octopus in Egypt. They detected the *aer* and *hlyA* genes in *A. hydrophila*. Additionally, they observed that *A. hydrophila* was resistant to amoxicillin, ceftriaxone, chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline. In a recent study, Lee, [90] detected antimicrobial resistance (AMR) genes such as *sul1*, in *A. hydrophila* in Norway. They also found *A. hydrophila* isolates resistant to erythromycin and florfenicol, with reduced susceptibility to oxolinic acid. Another study by Ghariieb, [91] reported an overall 40.67% prevalence of *A. hydrophila* from *Tilapia nilotica* and *M. cephalus* in Egypt. They observed that *A. hydrophila* was resistant to carbenicillin and ampicillin, but susceptible to chloramphenicol, amikacin, ciprofloxacin, ceftaxime, trimethoprim/sulfamethoxazole, and tetracycline. Moreover, Roges, [86] observed that *A. hydrophila* was highly resistant to ceftaxime, nalidixic acid, and tetracycline, with intermediate resistance to ceftaxime, imipenem, and ceftazidime. However, it was least resistant to amikacin, gentamicin, sulfamethoxazole-trimethoprim, ciprofloxacin, and nitrofurantoin. Similarly, Saleh, [82] observed that *A. hydrophila* was resistant to chloramphenicol, amikacin, and gentamicin, while highly susceptible to meropenem, ciprofloxacin, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole.

Virulence genes of pathogenic *A. hydrophila* cause serious histopathological effects in infected fish. In the current study, congestion (5.2%), hepatocyte necrosis (4.6%), granuloma formation (4.3%), and inflammation (5%) were observed in liver of infected fish. Epithelial hyperplasia (3.5%), lamellar fusion (3.3%), edema (3%), and the presence of *A. hydrophila* colonies (3.7%) in the gills. Fin erosion (6%), hemorrhage (6.2%), loss of fin rays (4.8%), and the presence of biofilms (3.5%) were observed in tail fins. Fibrosis (4%), abscess formation (3.7%), fatty degeneration (3.5%), and the infiltration of inflammatory cells (4.7%) were observed in spleen of infected fish. Histopathological effects of *A. hydrophila* infection were not studied in any previous study.

In the current study, we observed 100% and 97% similarity in the phylogenetic relationships of the *16S rRNA* and *gyrB* genes of *A. hydrophila*, respectively, among all the *A. hydrophila* strains isolated in this study, as well as with strains isolated in earlier studies. In a previous study, Wamala, [76] also analyzed the phylogenetic relationships through tree analysis of the *16S rRNA* and *gyrB* genes, revealing 100% and 99% similarity, respectively. Similarly, Esteve, [92] compared the phylogenetic relationships in Spain using the phylogenetic tree of the *16S rRNA* and *gyrB* genes of *A. hydrophila*, showing 100% similarity, consistent with our findings. Likewise, Li, [77] found 100% similarity in the phylogenetic relationships of the *16S rRNA* gene of *A. hydrophila* in China. In a recent study, Sani, [93] also observed 100% similarity in the phylogenetic relationships of the *16S rRNA* gene of *A. hydrophila* in Malaysia, which corroborated our results.

## Conclusion

Our examination of fish samples unveiled a concerning level of contamination with virulent and multidrug-resistant strains of *A. hydrophila*, highlighting the potential health risks associated with this contamination. The presence of pathogenic *A. hydrophila* results in significant histological changes in infected fish. The study underscores the importance of responsible antimicrobial use in aquaculture and the pressing need for effective strategies to curb the spread of virulence and antimicrobial resistance genes in *A. hydrophila*. Further research is imperative to delve into the mechanisms of virulence and resistance of *A. hydrophila* in fish.

## Supporting information

**S1 Table. Mean  $\pm$  S.E of physico-chemical parameters of Indus riverine system in Punjab.**  
(DOCX)

**S2 Table. Phenotypic and biochemical characteristics of *A. hydrophila*.**  
(DOCX)

**S1 Raw data.**  
(XLSX)

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**Writing – original draft:** Shahid Mahmood.

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## References

1. Boyd CE, McNevin AA, Davis RP. The contribution of fisheries and aquaculture to the global protein supply. *Food Secur.* 2022;14. <https://doi.org/10.1007/s12571-021-01246-9> PMID: 35075379
2. Manage PM. Heavy use of antibiotics in aquaculture: Emerging human and animal health problems—A review. *Sri Lanka J Aquat Sci.* 2018;23. <https://doi.org/10.4038/sljas.v23i1.7543>
3. Kibria G. Sustainable Energy for Rural Development in Bangladesh -Economic, Social and Environmental Benefits of Renewable Energy. *Sydneybashi-Bangla; Aust Sci Technol Artic.* 2015;37.
4. Chiu CN, Chen C, Muggleton NG. Provided for non-commercial research and educational use only. Not for reproduction, distribution or commercial use. *Prog Brain Res.* 2017;234.
5. FAO. The State of World Fisheries and Aquaculture 2020. Sustainability in action. FAO. 2020. <https://doi.org/10.4060/ca9229en>.
6. Pech ZG H, Chavez MR C, Reynoso F L. Pathogenic Bacteria in *Oreochromis niloticus* Var. Stirling Tilapia Culture. *Fish Aquac J.* 2017;08. <https://doi.org/10.4172/2150-3508.1000197>
7. Marinho-Neto FA, Claudiano GS, Yunis-Aguinaga J, Cueva-Quiroz VA, Kobashigawa KK, Cruz NRN, et al. Morphological, microbiological and ultrastructural aspects of sepsis by *Aeromonas hydrophila* in *Piaractus mesopotamicus*. *PLoS One.* 2019;14. <https://doi.org/10.1371/journal.pone.0222626> PMID: 31539396
8. Soares MP, Cardoso IL, Ishikawa MM, de Oliveira A da SS, Sartoratto A, Jonsson CM, et al. Effects of *Artemisia annua* alcohol extract on physiological and innate immunity of Nile tilapia (*Oreochromis niloticus*) to improve health status. *Fish Shellfish Immunol.* 2020;105. <https://doi.org/10.1016/j.fsi.2020.07.035> PMID: 32693158
9. Froehlich HE, Runge CA, Gentry RR, Gaines SD, Halpern BS. Comparative terrestrial feed and land use of an aquaculture-dominant world. *Proc Natl Acad Sci U S A.* 2018;115. <https://doi.org/10.1073/pnas.1801692115> PMID: 29712823
10. Buentello JA, Reyes-Becerril M, Romero-Geraldo MDJ, Ascencio-Valle FDJ. Effects of dietary arginine on hematological parameters and innate immune function of channel catfish. *J Aquat Anim Health.* 2007; 19: 195–203. <https://doi.org/10.1577/H07-004.1> PMID: 18201061
11. Cai W, Willmon E, Burgos FA, Ray CL, Hanson T, Arias CR. Biofilm and Sediment are Major Reservoirs of Virulent *Aeromonas hydrophila* (vAh) in Catfish Production Ponds. *J Aquat Anim Health.* 2019; 31: 112–120. <https://doi.org/10.1002/aah.10056> PMID: 30549304
12. SS M. Present Status of Fish Disease Management in Freshwater Aquaculture in India: State-of-the-Art-Review. *Aquac Fish.* 2017; 1: 1–9. <https://doi.org/10.24966/aaf-5523/100003>
13. SS M P C, J D, SN S, A B, BS G, et al. Status of Aqua-medicines, Drugs and Chemicals Use in India: A Survey Report. *Aquac Fish.* 2017; 1: 1–15. <https://doi.org/10.24966/aaf-5523/100004>
14. Abdella B, El-Wazzan E, El-Sersy NA, Sabry SA, El-Helou ER. Pathogenicity and antibiotic susceptibility of two bacterial pathogens associated with the clam *Tapes decussatus* in some Egyptian fisheries. *Ege J Fish Aquat Sci.* 2017;34. <https://doi.org/10.12714/egejfas.2017.34.4.04>
15. Tekedar HC, Abdelhamed H, Kumru S, Blom J, Karsi A, Lawrence ML. Comparative genomics of *Aeromonas hydrophila* secretion systems and mutational analysis of hcp1 and vgrG1 genes from T6SS. *Front Microbiol.* 2019;10. <https://doi.org/10.3389/fmicb.2018.03216> PMID: 30687246
16. El-Wazzan E, Ghareeb DA, Abdella B. Pre-induction of Hsp70 expression to protect the grooved carpet shell clam, *Ruditapes decussatus*, against *Micrococcus luteus*: A trained immunity strategy. *Egypt J Aquat Res.* 2020;46. <https://doi.org/10.1016/j.ejar.2019.10.004>
17. Shirajum Monir M, Yusoff SM, Mohamad A, Ina-Salwany MY. Vaccination of Tilapia against Motile *Aeromonas* Septicemia: A Review. *J Aquat Anim Health.* 2020;32. <https://doi.org/10.1002/aah.10099> PMID: 32331001
18. Rahman MA, Arshad A, Amin SMN, Shamsudin MN. Growth and survival of fingerlings of a threatened snakehead, *Channa striatus* (bloch) in earthen nursery ponds. *Asian J Anim Vet Adv.* 2013; 8: 216–226. <https://doi.org/10.3923/ajava.2013.216.226>
19. Nelson JS, Grande TC, Wilson MVH. *Fishes of the World: Fifth Edition.* Fishes of the World: Fifth Edition. 2016. <https://doi.org/10.1002/9781119174844>
20. Rüber L, Tan HH, Britz R. Snakehead (Teleostei: Channidae) diversity and the Eastern Himalaya biodiversity hotspot. *J Zool Syst Evol Res.* 2020;58. <https://doi.org/10.1111/jzs.12324>
21. Nazir S, Khan N, Azmat H, Saima, Ramzan MM, Davies SJ. Effect of vitamin C (L-ascorbic acid) feed supplementation on the proximate composition, amino acids, and fatty acids profile of bullseye snakehead *Channa marulius*. *Pakistan J Agric Sci.* 2022;59. <https://doi.org/10.21162/PAKJAS/22.116>
22. Mohanty BP, Paria P, Das D, Ganguly S, Mitra P, Verma A, et al. Nutrient profile of giant river-catfish *Sperata seenghala* (Sykes). *Natl Acad Sci Lett.* 2012;35. <https://doi.org/10.1007/s40009-012-0014-1>



23. Rahman MA, Arshad A, Yusoff FM, Amin SMN, Marimuthu K, Ara R. Development of captive breeding and seed production techniques for Giant River Catfish *Sperata seenghala*. *N Am J Aquac*. 2014;76. <https://doi.org/10.1080/15222055.2013.855282>
24. ALI MR. Pond Culture and Reproductive Performance of Seengharee, *Sperata Seenghala* (Sykes). 2015. Available: <https://core.ac.uk/download/pdf/132561411.pdf>.
25. Igbiosa IH, Igumbor EU, Aghdasi F, Tom M, Okoh AI. Emerging *Aeromonas* species infections and their significance in public health. *Sci World J*. 2012;2012. <https://doi.org/10.1100/2012/625023> PMID: 22701365
26. Ormen O, Granum PE, Lassen J, Figueras MJ. Lack of agreement between biochemical and genetic identification of *Aeromonas* spp. *Apmis*. 2005; 113: 203–207. <https://doi.org/10.1111/j.1600-0463.2005.apm1130308.x> PMID: 15799764
27. Bargui H, Marzouk M, Hannachi N, Boukadida J. Antimicrobial resistance and molecular determination of virulence genes of *Aeromonas* isolates from clinical patients and hydric samples. *Euro-Mediterranean J Environ Integr*. 2023. <https://doi.org/10.1007/s41207-023-00369-z>
28. Abdella B, Abozakra NA, Shokrak NM, Mohamed RA, El-Helow ER. Whole spectrum of *Aeromonas hydrophila* virulence determinants and the identification of novel SNPs using comparative pathogenomics. *Sci Rep*. 2023;13. <https://doi.org/10.1038/s41598-023-34887-1> PMID: 37173388
29. Suresh K, Pillai D. Prevalence and characterization of virulence-associated genes and antimicrobial resistance in *Aeromonas hydrophila* from freshwater finfish farms in Andhra Pradesh, India. *Biologia (Bratisl)*. 2023. <https://doi.org/10.1007/s11756-023-01454-y>
30. Preena PG, Dharmaratnam A, Swaminathan TR. Antimicrobial Resistance analysis of Pathogenic Bacteria Isolated from Freshwater Nile Tilapia (*Oreochromis niloticus*) Cultured in Kerala, India. *Curr Microbiol*. 2020;77. <https://doi.org/10.1007/s00284-020-02158-1> PMID: 32776225
31. Neamat-Allah ANF, Mahmoud EA, Mahsoub Y. Effects of dietary white mulberry leaves on hemato-biochemical alterations, immunosuppression and oxidative stress induced by *Aeromonas hydrophila* in *Oreochromis niloticus*. *Fish Shellfish Immunol*. 2021; 108: 147–156. <https://doi.org/10.1016/j.fsi.2020.11.028> PMID: 33301933
32. Fernández-Bravo A, Figueras MJ. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. *Microorganisms*. 2020. <https://doi.org/10.3390/microorganisms8010129> PMID: 31963469
33. El-Bahar HM, Ali NG, Aboyadak IM, Khalil SAES, Ibrahim MS. Virulence genes contributing to *Aeromonas hydrophila* pathogenicity in *Oreochromis niloticus*. *Int Microbiol*. 2019;22. <https://doi.org/10.1007/s10123-019-00075-3> PMID: 30989358
34. Naseem S, Ghaffar A, Hussain R, Khan A. Inquisition of Toxic Effects of Pyriproxyfen on Physical, Hemato-Biochemical and Histopathological Parameters in *Labeo rohita* Fish. *Pak Vet J*. 2022;42. <https://doi.org/10.29261/pakvetj/2022.014>
35. Aguilar-Marcelino L, Bautista-Garfias CR, Zaheer T, Maqsood A, Bamarni SSI, Abdullah BH, et al. Potential of Anisakiasis in Foodborne Zoonosis. *Pak Vet J*. 2022; 42: 433–444. <https://doi.org/10.29261/pakvetj/2022.080>
36. Cantas L, Shah SQA, Cavaco LM, Manaia CM, Walsh F, Popowska M, et al. A brief multi-disciplinary review on antimicrobial resistance in medicine and its linkage to the global environmental microbiota. *Front Microbiol*. 2013;4. <https://doi.org/10.3389/fmicb.2013.00096> PMID: 23675371
37. Malik F, Nawaz M, Anjum AA, Firyal S, Shahid MA, Irfan S, et al. Molecular Characterization of Antibiotic Resistance in Poultry Gut Origin Enterococci and Horizontal Gene Transfer of Antibiotic Resistance to *Staphylococcus aureus*. *Pak Vet J*. 2022;42. <https://doi.org/10.29261/pakvetj/2022.035>
38. Marti E, Variatza E, Balcazar JL. The role of aquatic ecosystems as reservoirs of antibiotic resistance. *Trends in Microbiology*. 2014. <https://doi.org/10.1016/j.tim.2013.11.001> PMID: 24289955
39. Schar D, Klein EY, Laxminarayan R, Gilbert M, Van Boeckel TP. Global trends in antimicrobial use in aquaculture. *Sci Rep*. 2020;10. <https://doi.org/10.1038/s41598-020-78849-3> PMID: 33318576
40. Cabello FC, Godfrey HP, Buschmann AH, Dölz HJ. Aquaculture as yet another environmental gateway to the development and globalisation of antimicrobial resistance. *The Lancet Infectious Diseases*. 2016. [https://doi.org/10.1016/S1473-3099\(16\)00100-6](https://doi.org/10.1016/S1473-3099(16)00100-6) PMID: 27083976
41. Abbas R, Nawaz Z, Siddique AB, Aslam R, Rafique A, Zahoor MA, et al. Molecular Detection of Biofilm Production among Multidrug Resistant Isolates of *Pseudomonas aeruginosa* from Meat Samples. *Pak Vet J*. 2022; 42: 505–510. <https://doi.org/10.29261/pakvetj/2022.074>
42. Velazquez-Meza ME, Galarde-López M, Carrillo-Quiróz B, Alpuche-Aranda CM. Antimicrobial resistance: One Health approach. *Veterinary World*. 2022. <https://doi.org/10.14202/vetworld.2022.743-749> PMID: 35497962

43. Woo SJ, Kim MS, Jeong MG, Do MY, Hwang SD, Kim WJ. Establishment of Epidemiological Cut-Off Values and the Distribution of Resistance Genes in *Aeromonas hydrophila* and *Aeromonas veronii* Isolated from Aquatic Animals. *Antibiotics*. 2022;11. <https://doi.org/10.3390/antibiotics11030343> PMID: 35326806
44. Ullah M, Rasool F, Khan N, Ali S, Sheikh AA. Pakistan Veterinary Journal Antibiotic Resistance and its Gene Profile in *Escherichia coli* Isolated from Diseased Farm-Raised Carps in Punjab, Pakistan. <https://doi.org/10.29261/pakvetj/2023.041>
45. Munita JM, Arias CA. Mechanisms of antibiotic resistance. *Virulence Mech Bact Pathog*. 2016; 481–511. <https://doi.org/10.1128/microbiolspec.VMBF-0016-2015> PMID: 27227291
46. Pérez-Sánchez T, Mora-Sánchez B, Balcázar JL. Biological Approaches for Disease Control in Aquaculture: Advantages, Limitations and Challenges. *Trends Microbiol*. 2018; 26: 896–903. <https://doi.org/10.1016/j.tim.2018.05.002> PMID: 29801773
47. Liu Y, Liu B, Yang P, Wang T, Chang Z, Wang J, et al. LysR-type transcriptional regulator OvrB encoded in O island 9 drives enterohemorrhagic *Escherichia coli* O157:H7 virulence. *Virulence*. 2019;10. <https://doi.org/10.1080/21505594.2019.1661721> PMID: 31502495
48. Mannion A, Shen Z, Fox JG. Comparative genomics analysis to differentiate metabolic and virulence gene potential in gastric versus enterohepatic *Helicobacter* species 06 *Biological Sciences* 0604 *Genetics*. *BMC Genomics*. 2018;19. <https://doi.org/10.1186/s12864-018-5171-2> PMID: 30458713
49. Daga AP, Koga VL, Soncini JGM, De Matos CM, Perugini MRE, Pelisson M, et al. *Escherichia coli* Bloodstream Infections in Patients at a University Hospital: Virulence factors and clinical characteristics. *Front Cell Infect Microbiol*. 2019;9. <https://doi.org/10.3389/fcimb.2019.00191> PMID: 31245301
50. Niu C, Yu D, Wang Y, Ren H, Jin Y, Zhou W, et al. Common and pathogen-specific virulence factors are different in function and structure. *Virulence*. 2013;4. <https://doi.org/10.4161/viru.25730> PMID: 23863604
51. Jubelin G, Desvaux M, Schüller S, Etienne-Mesmin L, Muniesa M, Blanquet-Diot S. Modulation of enterohaemorrhagic *Escherichia coli* survival and virulence in the human gastrointestinal tract. *Microorganisms*. 2018. <https://doi.org/10.3390/microorganisms6040115> PMID: 30463258
52. Pan X, Yang Y, Zhang JR. Molecular basis of host specificity in human pathogenic bacteria. *Emerg Microbes Infect*. 2014; 3: 0. <https://doi.org/10.1038/emi.2014.23> PMID: 26038515
53. Cepeda-Molero M, Berger CN, Walsham ADS, Ellis SJ, Wemyss-Holden S, Schüller S, et al. Attaching and effacing (A/E) lesion formation by enteropathogenic *E. coli* on human intestinal mucosa is dependent on non-LEE effectors. *PLoS Pathog*. 2017;13. <https://doi.org/10.1371/journal.ppat.1006706> PMID: 29084270
54. Paytubi S, Cansado C, Madrid C, Balsalobre C. Nutrient composition promotes switching between pellicle and bottom biofilm in *Salmonella*. *Front Microbiol*. 2017;8. <https://doi.org/10.3389/fmicb.2017.02160> PMID: 29163440
55. Koohsari H, Ghaemi EA, Mozaffari NA, Moradi A, Sadegh-Sheshpoli M, Javid SN. The effect of adding blood on the virulence genes expression of *Staphylococcus aureus* in exponential and stationary growth phase. *Jundishapur J Microbiol*. 2017;10. <https://doi.org/10.5812/ijm.14380>
56. Guijarro JA, Cascales D, García-Torrío AI, García-Domínguez M, Méndez J. Temperature-dependent expression of virulence genes in fish-pathogenic bacteria. *Frontiers in Microbiology*. 2015. <https://doi.org/10.3389/fmicb.2015.00700> PMID: 26217329
57. Do H, Makthal N, VanderWal AR, Saavedra MO, Olsen RJ, Musser JM, et al. Environmental pH and peptide signaling control virulence of *Streptococcus pyogenes* via a quorum-sensing pathway. *Nat Commun*. 2019;10. <https://doi.org/10.1038/s41467-019-10556-8> PMID: 31197146
58. Manzoor K, Sciences A, Rasool F, Khan N, Sciences A. Prevalence and Molecular Detection of *Edwardsiella tarda* in Cultured Tilapia Species of Fish Farms of Punjab in Pakistan and their Postmortem Examination. 2023. <https://doi.org/10.29261/pakvetj/2023.015>
59. Edward J.Noga. *Fish Disease: Diagnosis and Treatment*, Second Edition. Wiley-Blackwell. 2010.
60. Lima LC, Fernandes AA, Costa AAP, Velasco FO, Leite RC, Hackett JL. Isolation and characterization of *Edwardsiella tarda* from *pacu* *Myleus micans*. *Arq Bras Med Vet e Zootec*. 2008;60. <https://doi.org/10.1590/S0102-09352008000100040>
61. Muratori MCS, Martins NE, Peixoto MTD, Oliveira AL, Ribeiro LP, Costa APR, et al. *Edwardsiella* septicemia mortality in tilapia-integrated with pig in fish farming. *Arq Bras Med Vet e Zootec*. 2001;53. <https://doi.org/10.1590/s0102-09352001000600007>
62. Xiao J, Wang Q, Liu Q, Wang X, Liu H, Zhang Y. Isolation and identification of fish pathogen *Edwardsiella tarda* from mariculture in China. *Aquac Res*. 2008;40. <https://doi.org/10.1111/j.1365-2109.2008.02101.x>

63. Fang H, Zhang X, Chen C, Jin X, Wang X. Studies on the edwardsiellosis and characterization of pathogenic bacteria from diseases flounder (*Paralichthys olivaceus* L.) and turbot (*Scophthalmus maximus* L.). *Acta Oceanol Sin*. 2006;25.
64. Chen M, Lan X, Zhu L, Ru P, Xu W, Liu H. PCR Mediated Nucleic Acid Molecular Recognition Technology for Detection of Viable and Dead Foodborne Pathogens. *Foods*. 2022. <https://doi.org/10.3390/foods11172675> PMID: 36076861
65. Armougom F. Exploring Microbial Diversity Using 16S *rRNA* High-Throughput Methods. *J Comput Sci Syst Biol*. 2009;02. <https://doi.org/10.4172/jcsb.1000019>
66. Persson S, Al-Shuweli S, Yapici S, Jensen JN, Olsen KEP. Identification of clinical aeromonas species by *rpoB* and *gyrB* sequencing and development of a multiplex pcr method for detection of *Aeromonas hydrophila*, *A. caviae*, *A. veronii*, and *A. media*. *J Clin Microbiol*. 2015;53. <https://doi.org/10.1128/JCM.01963-14> PMID: 25411168
67. Wimalasena SHMP De Silva BCJ, Hossain S Pathirana HNKS, Heo GJ. Prevalence and characterisation of quinolone resistance genes in *Aeromonas* spp. isolated from pet turtles in South Korea. *J Glob Antimicrob Resist*. 2017;11. <https://doi.org/10.1016/j.jgar.2017.06.001> PMID: 28743647
68. Sedek A, Abd El Tawab A, El-Hofy F, El-Gohary M. Antibiotic resistance genes of *Edwardsiella tarda* isolated from *Oreochromis niloticus* and *Claris gariepinus*. *Benha Vet Med J*. 2020;38. <https://doi.org/10.21608/bvmj.2020.29323.1205>
69. Öktem IMA, Gülay Z, Biçmen M, Gür D, Gültekin M, Öğünç D, et al. qnrA prevalence in extended-spectrum  $\beta$ -lactamase-positive enterobacteriaceae isolates from Turkey. *Jpn J Infect Dis*. 2008;61. <https://doi.org/10.7883/yoken.jjid.2008.13>
70. Kern MB, Klemmensen T, Frimodt-Møller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *J Antimicrob Chemother*. 2002;50. <https://doi.org/10.1093/jac/dkf164> PMID: 12356795
71. Zhu D, Li A, Wang J, Li M, Cai T, Hu J. Correlation between the distribution pattern of virulence genes and virulence of *Aeromonas hydrophila* strains. *Front Biol China*. 2007;2. <https://doi.org/10.1007/s11515-007-0024-4>
72. Ahangarzadeh M, Najafabadi MG, Peyghan R, Houshmand H, Rohani MS, Soltani M. Detection and distribution of virulence genes in *Aeromonas hydrophila* isolates causing infection in cultured carps. *Vet Res Forum*. 2022;13. <https://doi.org/10.30466/vrf.2020.115998.2761> PMID: 35601789
73. Wang K, Liu E, Song S, Wang X, Zhu Y, Ye J, et al. Characterization of *Edwardsiella tarda* RpoN: Roles in  $\sigma 70$  family regulation, growth, stress adaption and virulence toward fish. *Arch Microbiol*. 2012;194. <https://doi.org/10.1007/s00203-011-0786-6> PMID: 22231477
74. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966;45. [https://doi.org/10.1093/ajcp/45.4\\_ts.493](https://doi.org/10.1093/ajcp/45.4_ts.493)
75. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing M100-ED30. Clinical and Laboratory Standards Institute. 2020.
76. Wamala SP, Mugimba KK, Dubey S, Takele A, Munang'andu HM, Evensen Ø, et al. Multilocus sequence analysis revealed a high genotypic diversity of *Aeromonas hydrophila* infecting fish in Uganda. *J Fish Dis*. 2018; 41: 1589–1600. <https://doi.org/10.1111/jfd.12873> PMID: 30074242
77. Li F, Wu D, Gu HR, Yin M, Ge HL, Liu XH, et al. *Aeromonas hydrophila* and *Aeromonas veronii* cause motile *Aeromonas* septicemia in the cultured Chinese sucker, *Myxocyprinus asiaticus*. *Aquac Res*. 2019;50. <https://doi.org/10.1111/are.14028>
78. Morshdy AEMA Ahmed GE, El-Tahlawy AS El Bay-Omi RM, Mahmoud AFA. Virulence and Antimicrobial Resistance Profiling of *Aeromonas hydrophila* Recovered from Retail fish in Sharkia Province, Egypt. *J Adv Vet Res*. 2023; 13: 1320–1324.
79. El-Hossary D, Mahdy A, Elariny EYT, Askora A, Merwad AMA, Saber T, et al. Antibiotic Resistance, Virulence Gene Detection, and Biofilm Formation in *Aeromonas* spp. Isolated from Fish and Humans in Egypt. *Biology (Basel)*. 2023;12. <https://doi.org/10.3390/biology12030421> PMID: 36979113
80. Thaotumpitak V, Sripradite J, Atwill ER, Jeamsripong S. Emergence of colistin resistance and characterization of antimicrobial resistance and virulence factors of *Aeromonas hydrophila*, *Salmonella* spp., and *Vibrio cholerae* isolated from hybrid red tilapia cage culture. *PeerJ*. 2023;11. <https://doi.org/10.7717/peerj.14896> PMID: 36855429
81. Ninh DT, Le DV, Van K Van, Giang NTH, Dang LT, Hoai TD. Prevalence, virulence gene distribution and alarming the multidrug resistance of *Aeromonas hydrophila* associated with disease outbreaks in freshwater aquaculture. *Antibiotics*. 2021;10. <https://doi.org/10.3390/antibiotics10050532> PMID: 34064504

82. Saleh A, Elkenany R, Younis G. Virulent and multiple antimicrobial resistance *Aeromonas hydrophila* isolated from diseased Nile tilapia fish (*Oreochromis niloticus*) in Egypt with sequencing of some virulence-associated genes. *Biocontrol Sci.* 2021;26. <https://doi.org/10.4265/BIO.26.167> PMID: 34556619
83. Ahmed HA, Mohamed MEM, Rezk MM, Gharieb RMA, Abdel-Maksoud SA. *Aeromonas hydrophila* in fish and humans; prevalence, virulotyping and antimicrobial resistance. *Slov Vet Res.* 2018;55. <https://doi.org/10.26873/SVR-636-2018>
84. Azzam-Sayuti M, Ina-Salwany MY, Zamri-Saad M, Yusof MT, Annas S, Najihah MY, et al. The prevalence, putative virulence genes and antibiotic resistance profiles of *Aeromonas* spp. isolated from cultured freshwater fishes in peninsular Malaysia. *Aquaculture.* 2021;540. <https://doi.org/10.1016/j.aquaculture.2021.736719>
85. Abu-Elala N, Abdelsalam M, Marouf S, Setta A. Comparative analysis of virulence genes, antibiotic resistance and *gyrB*-based phylogeny of motile *Aeromonas* species isolates from Nile tilapia and domestic fowl. *Lett Appl Microbiol.* 2015;61. <https://doi.org/10.1111/lam.12484> PMID: 26280543
86. Roges EM, Gonçalves VD, Cardoso MD, Festivo ML, Siciliano S, Berto LH, et al. Virulence-Associated Genes and Antimicrobial Resistance of *Aeromonas hydrophila* Isolates from Animal, Food, and Human Sources in Brazil. *Biomed Res Int.* 2020;2020. <https://doi.org/10.1155/2020/1052607> PMID: 32461959
87. Eid HM, El-Mahallawy HS, Shalaby AM, Elsheshtawy HM, Shetewy MM, Eidaroos NH. Emergence of extensively drug-resistant *Aeromonas hydrophila* complex isolated from wild Mugil cephalus (striped mullet) and Mediterranean seawater. *Vet World.* 2022; 15: 55–64. <https://doi.org/10.14202/vetworld.2022.55-64> PMID: 35369605
88. Fauzi NNFNM, Hamdan RH, Mohamed M, Ismail A, Zin AAM, Mohamad NFA. Prevalence, antibiotic susceptibility, and presence of drug resistance genes in *Aeromonas* spp. isolated from freshwater fish in Kelantan and Terengganu states, Malaysia. *Vet World.* 2021;14. <https://doi.org/10.14202/vetworld.2021.2064-2072> PMID: 34566322
89. Elkenany rasha. Prevalence and antibiotic resistance of *Aeromonas hydrophila* and *Staphylococcus aureus* isolated from seafood in Egypt. *Mansoura Vet Med J.* 2020;21. <https://doi.org/10.21608/mvmj.2020.47607.1009>
90. Lee H-J, Storesund JE, Lunestad B-T, Hoel S, Lerfall J, Jakobsen AN. Whole genome sequence analysis of *Aeromonas* spp. isolated from ready-to-eat seafood: antimicrobial resistance and virulence factors. *Front Microbiol.* 2023;14. <https://doi.org/10.3389/fmicb.2023.1175304> PMID: 37455746
91. Ghareeb H, Zahran E, Elghany S. Occurrence, Molecular Characterization and Antimicrobial Resistance of Pathogenic *Aeromonas hydrophila* from Retail Fish. *Alexandria J Vet Sci.* 2019; 62: 172. <https://doi.org/10.5455/ajvs.49297>
92. Esteve C, Alcaide E, Blasco MD. *Aeromonas hydrophila* subsp. dhakensis isolated from feces, water and fish in Mediterranean Spain. *Microbes Environ.* 2012;27. <https://doi.org/10.1264/jsme2.ME12009> PMID: 22472298
93. Sani B, Yusoff MS, Yasin ISM, Karim MMA, Matori MF, Amal MNA, et al. Pathogenicity of *Aeromonas hydrophila* in High-value Native Pangasius Catfish, *Pangasius nasutus* (Bleeker). *Pertanika J Trop Agric Sci.* 2023;46. <https://doi.org/10.47836/pjtas.46.2.01>