Original Article

Genetic Diversity, Antimicrobial Resistance, and Virulence Genes of *Aeromonas* Isolates from Clinical Patients, Tap Water Systems, and Food*

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Abstract

Objective This study aimed to evaluate the genetic diversity, virulence, and antimicrobial resistance of *Aeromonas* isolates from clinical patients, tap water systems, and food.

Methods Ninety *Aeromonas* isolates were obtained from Ma'anshan, Anhui province, China, and subjected to multi-locus sequence typing (MLST) with six housekeeping genes. Their taxonomy was investigated using concatenated *gyrB-cpn60* sequences, while their resistance to 12 antibiotics was evaluated. Ten putative virulence factors and several resistance genes were identified by PCR and sequencing.

Results The 90 *Aeromonas* isolates were divided into 84 sequence types, 80 of which were novel, indicating high genetic diversity. The *Aeromonas* isolates were classified into eight different species. PCR assays identified virulence genes in the isolates, with the enterotoxin and hemolysin genes *act*, *aerA*, *alt*, and *ast* found in 47 (52.2%), 13 (14.4%), 22 (24.4%), and 12 (13.3%) of the isolates, respectively. The majority of the isolates (≥ 90%) were susceptible to aztreonam, imipenem, cefepime, chloramphenicol, gentamicin, tetracycline, and ciprofloxacin. However, several resistance genes were detected in the isolates, as well as a new *mcr-3* variant.

Conclusions Sequence type, virulence properties, and antibiotic resistance vary in *Aeromonas* isolates from clinical patients, tap water systems, and food.

Key words: *Aeromonas*; Multi-locus sequence typing; Multidrug resistance; Virulence gene; Antimicrobial resistance gene

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INTRODUCTION

Aeromonas spp. are important human opportunistic pathogens that can cause intestinal and extra-intestinal diseases, particularly in immunocompromized individuals, including gastroenteritis, wound infections, and even life-threatening necrotizing fasciitis[1]. *Aeromonas* species are often isolated from freshwater, seafood, and meat products[2-3] and are therefore a primary cause of food contamination and may act as intermediaries in transmitting disease to humans[4].

The *Aeromonas* genus contains over 26 species and has a very complex taxonomy. Although great efforts have been made to correctly identify different *Aeromonas* species, particularly those related to human diseases, this has been difficult to achieve using traditional biochemical methods due to taxonomic complexity[1,5]. In addition, conventional biochemical methods such as matrix assisted laser desorption/ionization flight mass spectrometry (MALDI-TOF MS) are time consuming and tedious for routine use. Moreover, the 16S rDNA sequence used for bacterial identification has high between-species similarity and thus cannot adequately distinguish between *Aeromonas* species[6-7]. Recently studies have shown that housekeeping gene sequencing (*gyrB* and *rpoD*) can be used for the phylogenetic analysis and identification of *Aeromonas* species[8-9]; for instance, Yano et al.[10] used housekeeping gene sequencing to identify 87 *Aeromonas* strains at the species level.

The pathogenesis of *Aeromonas* spp. involves a series of virulence factors[11]. These include hemolytic toxins such as aerolysin-related cytotoxic enterotoxin (*Act*)[12], heat-labile cytotonic enterotoxin (*Alt*), hemolysin (*hlyA*), heat-stable cytotonic toxins (*Ast*)[13], and aerolysin (*aerA*)[14]. In addition, the type III secretion system (*TTSS*), lateral flagella (*laf*), polar flagellum (*fla*)[15-16], elastase (*ela*)[17], and lipase (*lip*)[18] also contribute toward the pathogenicity of *Aeromonas*.

Aeromonas antibiotic resistance has increased globally in recent years; for example, some strains are resistance to aminoglycosides [*aac*(*6'*)-*Ib*], while others harbor plasmid-mediated quinolone resistance (PMQR) determinants[19]. In *Aeromonas* isolates from South Africa and Korea, the prevalence of *aac*(*6'*)-*Ib* was found to be 29.23% and 29.00%, respectively[19-20]. The important PMQR determinant *qnrS* has been reported in *Aeromonas*[21-22], with 73.85% of *Aeromonas* strains in Korea found to harbor *qnrS* genes[19]. Conversely, *qnrS* was found to be present in 21.00% of *Aeromonas* isolates from freshwater fish in South Africa[20]. The resistance of *Aeromonas* to several different classes of antibiotics poses a major problem for human health since the resistant bacteria can be transmitted from the aquatic environment to humans *via* the food chain or direct contact[23]. Therefore, it is necessary to monitor *Aeromonas* antimicrobial resistance to guide clinical treatment.

In this study, we evaluated the characteristics of *Aeromonas* strains isolated from environmental sources, food, and clinical patients in Ma'anshan, Anhui province, China. In addition, we investigated the virulence-associated genes and antimicrobial resistance of these *Aeromonas* spp.

MATERIALS AND METHODS

Ethical Statement

Fecal samples and bodily fluids were acquired from patients who had provided informed consent. This study was reviewed and approved by the ethics committee of the National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention.

Aeromonas Isolates

In 2018, 90 *Aeromonas* isolates were obtained from 33 stool samples from patients with diarrhea, 36 tap water systems, and 21 foods in Ma'anshan Anhui Province, China (Figure 1). The isolated strains were identified using an automatic bacteriologic analyzer (Vitek 2 Compact, BioMerieuX). Bacteria were cultured on Luria-Bertani (LB) broth or brain heart infusion (BHI) agar plates overnight at 37 °C.

Multi-locus Sequence Typing (MLST) and Subtyping of Aeromonas Isolates

To analyze the subtype of the *Aeromonas* isolates, we used the *Aeromonas* MLST scheme (http://pubmlst.org/Aeromonas/) with six housekeeping genes: *gyrB*, *groL*, *gltA*, *metG*, *ppsA*, and *recA*. PCR was carried out using previously described primers and protocols[5]. The sequences of the six loci were compared to those published in the *Aeromonas* MLST database, as well as the STs. New alleles and STs were submitted to the *Aeromonas* MLST database for name assignment.

In this study, 90 *Aeromonas* strains were identified at the species level by analyzing the housekeeping genes *gyrB* and *cpn60*[8,24]. The

Figure 1. Phylogenetic relationships were determined using the concatenated sequences of six genes included in this study. The source, species, virulence genes, antibiotic resistance phenotype, MDR (number of drugs resistant to), and antimicrobial resistance genes of the *Aeromonas* isolates are shown on the right. The phylogenetic tree was constructed using a neighbor-joining algorithm. ST: sequence type.

reference nucleotide sequences of these genes were taken from the GenBank database and included the 28 representative species listed in Supplementary Table S1 (available in www.besjournal.com). A phylogenetic tree was constructed using the neighbor-joining method in Clustal-W[25]. All primers were synthesized by Beijing Tsingke Biological Technology Company (Beijing, China).

Detection of Virulence-associated Genes

To detect virulence-associated genes in the *Aeromonas* isolates, we performed PCR using previously described *alt*, *ast*, *hlyA*, *aerA*, *act*, *ascV*, *aexT*, *laf*, *lip*, *fla,* and *ela* primers. PCR amplification was performed in a 50 μL reaction volume containing 25 μL of Taq PCR MasterMix (Takara Bio, Inc., Japan), 1 μL of 10 μmol/L primer, 21 μL of ddH_2O, and 2 μL of DNA template under the following cycling conditions: pre-denaturation at 95 °C for 5 min, 30 cycles of denaturation at 95 °C for 30 s, annealing at 55–60 °C for 30 s, and extension at 72 °C for 1 min, followed by a final cycle at 72 °C for 5 min. Positive PCR products were confirmed by sequencing, detecting a total of 11 virulence-associated genes.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility tests were carried out using the broth microdilution method according to CLSI guidelines (Clinical and Laboratory Standards Institute, 2018). The minimum inhibitory concentrations (MICs) of the following 13 antibiotics were measured: amoxicillin/clavulanate (AMC), ampicillin (AMP), cefepime (FEP), ceftriaxone (CRO), ceftazidime (CAZ), imipenem (IPM), aztreonam (ATM), gentamycin (GEN), tetracycline (TCY), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (SXT), chloramphenicol (CHL), and colistin (CT). *E. coli* ATCC 25922 was used as the quality-control strain for susceptibility testing.

Detection of Resistance Genes

To detect antimicrobial resistance genes, we performed PCR amplification on tetracycline resistance (*tetA*, *tetB*, and *tetE*), extended-spectrum β-lactamase (ESBL) (*blaTEM*, *blaSHV*, and *blaCTX*)[19], aminoglycoside resistance [*armA*, *aphAI-IAB*, *aac*(*6'*)-*Ib*, and *aac(3)-IIa*][26], sulphonamide resistance (*sul1*and *sul2*)[27], and mobile colistin resistance (*mcr-1*, *mcr-2*, *mcr-3,* and *mcr-4*) genes, as well as PMQR (*qnrA*, *qnrB*, and *qnrS*) genes[19] using previously described primers and protocols (Table 1)[19,28-32]. Positive PCR products were confirmed by sequencing.

RESULTS

MLST of Aeromonas Isolates

The 90 *Aeromonas* isolates were divided into 84 STs of which 80 were novel (ST569-ST644 and ST649-ST652), indicating high genetic diversity. No STs were predominant.

Diversity and Distribution of Aeromonas Species

We evaluated the phylogeny of the 90 *Aeromonas* isolates based on their *gyrB* and *cpn60* sequences (Figure 2). Sequencing analysis classified 82 (91.1%) of the strains into eight different species, of which the three most common were *A. jandaei* (32.2%), *A. veronii* (25.5%), and *A. caviae* (13.3%). Notably, eight strains did not belong to any of the 28 known species and may be regarded as new species. In addition, the distribution of *Aeromonas* species isolated from clinical patients, food, and tap water samples varied (Table 2). *A. caviae* (36.4%) was the most prevalent species in clinical isolates, *A. veronii* (18.1%) was the most common in food isolates, and *A. jandaei* (58.3%) was the most prevalent in environmental isolates, with the of these three species differing significantly between patient-, food-, and environment-derived isolates ($P < 0.05$, χ^2 test).

Distribution of Virulence-associated Genes in Aeromonas Strains

We detected 11 virulence-associated genes in the *Aeromonas* isolates (Table 3), of which 77.8% carried *fla*, 52.2% carried *act*, 44.4% carried *ela*, and 43.3% carried *ascV*. Two additional genes, *laf* and *ast*, were present in 8.9 and 13.3% of the isolates, respectively. The prevalence of *ast*, *lip*, and *ela* differed significantly in the patient-, food-, and environment- derived isolates ($P < 0.05$, Fisher's exact test), while only *lip* and *aexT* were found to be more prevalent in patient-derived isolates than food-derived or environmental isolates. As shown in Table 4, the 11 virulence-associated genes differed significantly among the most common species. The hemolytic gene *act* was prevalent in *A. hydrophila* and *A. veronii*, whereas the enterotoxin gene *alt* was prevalent in *A. aquariorum* and *A. hydrophila*. The enterotoxin gene *ast*, hemolytic gene *aerA,* and hemolytic gene *hlyA* were more prevalent in *A. hydrophila*; however, both extracellular protease genes *ela* and

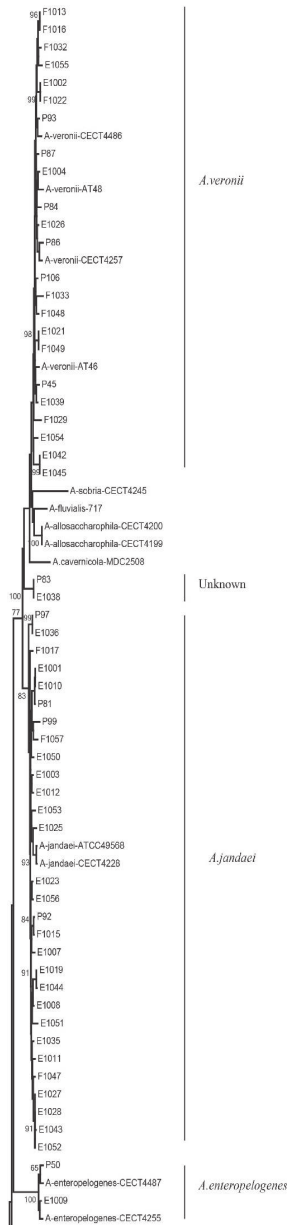
Table 1. Primer sequences used to amplify antimicrobial resistance genes

Targeted gene	Primers	Sequence (5'→3')	Product size (bp)
ESBL			
blaTEM	blaTEM-F	ATAAAATTCTTGAAGACGAAA	1,080
	blaTEM-R	GACAGTTACCAATGCTTAATC	
blaSHV	blaSHV-F	TTATCTCCCTGTTAGCCACC	795
	blaSHV-R	GATTTGCTGATTTCGCTCGG	
blaCTX-M	blaCTX-M-F	CGCTTTGCGATGTGCAG	550
	blaCTX-M-R	ACCGCGATATCGTTGGT	
Tetracycline resistance			
tetA	tetA-F	GTAATTCTGAGCACTGTCGC	1,000
	tetA-R	CTGCCTGGACAACATTGCTT	
tetB	tetB-F	CTCAGTATTCCAAGCCTTTG	400
	tetB-R	CTAAGCACTTGTCTCCTGTT	
tetE	tetE-F	GTGATGATGGCACTGGTCAT	1,100
	tetE-R	CTCTGCTGTACATCGCTCTT	
PMQR			
qnrA	qnrA-F	AGAGGATTTCTCACGCCAGG	580
	qnrA-R	TGCCAGGCACAGATCTTGAC	
qnrB	qnrB-F	GATCGTGAAAGCCAGAAAGG	496
	qnrB-R	ACGATGCCTGGTAGTTGTCC	
qnrS	qnrS-F	GCAAGTTCATTGAACAGGGT	428
	qnrS-R	TCTAAACCGTCGAGTTCGGCG	
Aminoglycoside resistance			
armA	armA-F	AGGTTGTTTCCATTTCTGAG	591
	armA-R	TCTCTTCCATTCCCTTCTCC	
aphAI-IAB	aphAI-IAB-F	AAACGTCTTGCTCGA GGC	500
	aphAI-IAB-R	CAAACCGTTATTCATTCGTGA	
aac(3)-IIa	aac(3)-IIa-F	ATGGGCATC ATTCGCACA	749
	aac(3)-IIa-R	TCTCGGCTTGAACGAATTGT	
aac(6')-Ib	aac(6')-Ib-F	TTGCGATGCTCTATGAGTGGCTA	482
	aac(6')-Ib-R	CTCGAATGCCTGGCGTGTTT	
MCR			
mcr-1	mcr-1-F	CGGTCAGTCCGTTTGTTC	309
	mcr-2-R	CTTGGTCGGTCTGTAGGG	
mcr-2	mcr-2-F	TGTTGCTTGTGCCGATTGGA	567
	mcr-2-R	CAGCAACCAACAATACCATCT	
mcr-3	mcr-3-F	AGTTTGGTTTCGCCATTTCATTAC	1,084
	mcr-3-R	ATATCACTGCGTGGACAGTCAGG	
mcr-4	mcr-4-F	TTACAGCCAGAATCATTATCA	488
	mcr-4-R	ATTGGGATAGTCGCCTTTTT	
Sulfonamide resistance			
sul1	sul1-F	CGGCGTGGGCTACCTGAACG	433
	sul1-R	GCCGATCGCGTGAAGTTCCG	
sul2	sul2-F	GCGCTCAAGGCAGATGGCATT	293
	sul2-R	GCGTTTGATACCGGCACCCGT	

lip were rare in *A. jandaei* and *A. veronii* but very common in other species.

Prevalence of Antimicrobial Resistance

Next, we evaluated the susceptibility of the 90 *Aeromonas* isolates to 13 antibiotics belonging to ten classes of antibiotic using the broth microdilution method according to CLSI recommendations (Table 5). High ampicillin (100%) and amoxicillin/ clavulanic acid (86.7%) resistance was observed in the *Aeromonas* strains; however, the majority of the isolates (≥ 90%) were susceptible to aztreonam, imipenem, cefepime, CHL, gentamicin, tetracycline, and ciprofloxacin. Notably, cefepime and ciprofloxacin resistance were significantly higher in patient isolates than in food or environmental isolates ($P < 0.05$, Fisher's exact test), whereas only one antibiotic (colistin) displayed significantly higher resistance rates in environmental isolates (Table 5). Nineteen isolates (21.1%) were found to be multidrug-resistant (MDR), displaying resistance to at least three of the antibiotics tested in this study. Of these 19 MDR isolates, 10 (52.6%) were isolated from patients, 7 (36.8%) were isolated from the environment, and 2 (10.5%) were isolated from food (Figure 1).



Detection of Aimicrobial Resistance Genes

The PMQR gene *qnrS* was detected in 4 (4.4%) isolates. The ESBL gene *blaCTX-M* was detected in 2 (2.22%) isolates. The aminoglycoside resistance genes *aac*(*6'*)*-Ib* and *armA* were detected in 2 (2.22%) and 1 (1.11%) isolates, respectively. The sulfonamide genes *sul1* and *sul2* were found in 3 (3.33%) and 9 (10%) isolates, respectively (Figure 1). The mobile colistin resistance gene *mcr-3* was detected in 3 (3.33%) isolates. Sequence analysis revealed that one isolate (E1006) harbored *mcr-3.25* (GenBank accession no. KM985469.1) while two (P92 and F1015) harbored a new *mcr-3* variant which differed from the *mcr-3.8* gene by three amino acid changes according to sequence alignment (unpublished data). The ESBL genes *blaTEM* and *blaSHV*, aminoglycoside resistance genes *aphAI-IAB* and *aac(3)-IIa*, tetracycline resistance genes *tetA*, *tetB,* and *tetE*, colistin resistance genes *mcr-1*, *mcr-2,* and *mcr-4* genes, and PMQR genes *qnrA* and *qnrB* were not detected in any isolates.

DISCUSSION

Aeromonas is a genus of bacteria that are ubiquitously present in aquatic environments and have been linked to infections in both humans and animals[1]. In this study, we evaluated 90 *Aeromonas* isolates from patients, tap water, and food, and assessed the genetic diversity, putative virulence genes, and antimicrobial resistance of these isolates.

The 90 isolates were separated into 84 STs of which just four were found to match those published in the *Aeromonas* MLST database, suggesting that 80 were novel (ST569-ST644 and ST649-ST652) and indicating high genetic diversity. We also evaluated the phylogeny of the 90 *Aeromonas* isolates based on the concatenated *gyrB-cpn60* gene sequences (Figure 2), revealing that the isolates were closely related and included *A. jandaei* (29 isolates), *A.*

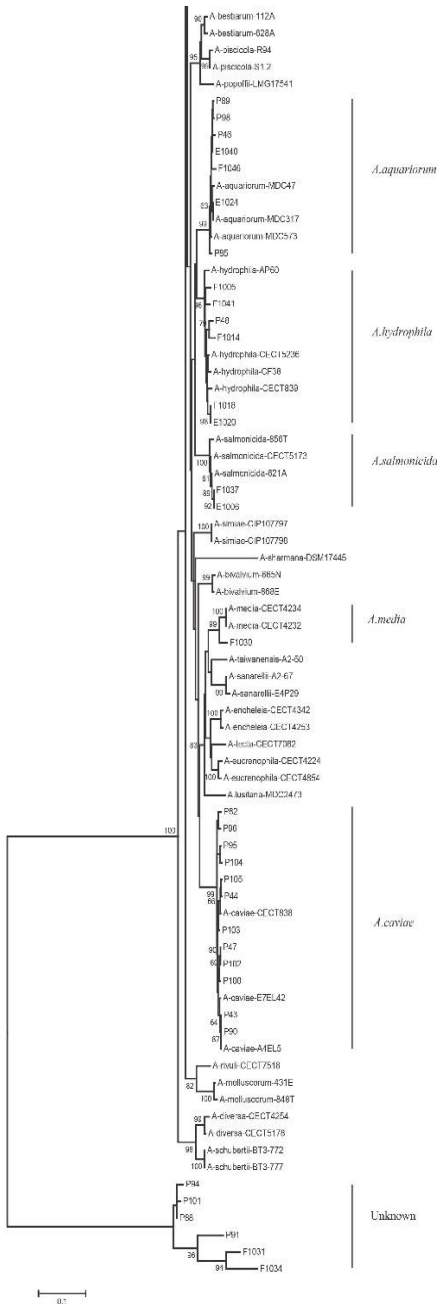


Figure 2. The neighbor-joining phylogenetic tree was constructed using the concatenated sequences of the *gyrB* and *cpn60* genes, revealing the relationships between the 90 *Aeromonas* isolates from clinical patients, tap water systems, and food from Ma'anshan Anhui Province, China. Numbers on or near the nodes represent bootstrap values from 1,000 replicates. Isolates were designated as either P, E, or F to indicate strains isolated from clinical patients, tap water systems (environment), or food, respectively.

veronii (23 isolates), *A. caviae* (12 isolates), *A. aquariorum* (7 isolates), *A. hydrophila* (6 isolates), *A. salmonicida* (2 isolates), *A. enteropelogenes* (2 isolates), *A. media* (1 isolate), and new species (8 isolates). The most common species was *A. jandaei,* which comprised 32% of all isolates and was mostly isolated from tap water systems. The second most prevalent species was *A. veronii*, which is distributed in various environments such as water and fish[1]; indeed, some *A. veronii* strains have been isolated from snail lion, suggesting that this species may be related to invertebrates in aquatic environments. The third most prevalent species was *A. caviae*, which has previously been shown to have clinical relevance. Zhou et al.[33] reported that the four most prevalent species of *Aeromonas* in clinical isolates were *A. caviae* (41.7%), *A. veronii* (31.3%), *A. dhakensis* (13.9%), and *A. hydrophila* (5.2%). Another report[34] about *Aeromonas* recovered from Patients Suffering from Diarrhea in Israel were evaluated for *Aeromonas* species, and the most prevalent species were *A. caviae* (65%) and *A. veronii* (29%). In this study, the most prevalent species in clinical isolates was *A. caviae*, accounting for 36.4% of the isolates, followed by *A. veronii* (18.1%). In brief, the prevalent species of *Aeromonas* in clinical settings reported in this study is correspond with that reported by other scholars.

The pathogenic mechanism of *Aeromonas* is complex and multifactorial, which may be related to some of its virulence-associated genes; therefore, we evaluated the virulence-associated genes present in these isolates (Table 3). The enterotoxin and hemolysin genes *act*, *aerA*, *alt*, and *ast* were present in 47 (52.2%), 13 (14.4%), 22 (24.4%), and 12 (13.3%) of the 90 isolates, respectively. The *act* gene was detected in 91.3% of *A. veronii* isolates and 83.3% of *A. hydrophila* isolates, while the *aerA* gene was detected in 83.3% of *A. hydrophila* isolates and 42.9% of *A. aquariorum* isolates. The *alt* gene was detected in 100% of *A. hydrophila* and *A. aquariorum* strains and 16.7% of *A. caviae*, whereas the *ast* gene was present in 28.6% of *A. aquariorum* strains and all *A. hydrophila*. The *fla*, *ela*, and *lip* genes were present in 70 (77.8%), 40 (44.4%), and 34 (37.7%) of the 90 isolates, with *fla* harbored in the majority of species and *ela* and *lip* both prevalent in *A. aquariorum*, *A. caviae*, and *A. hydrophila* isolates. The TTSS genes *ascV* and *aexT* were detected in 39 (43.3%) and 20 (22.2%) of the 90 isolates, respectively: *ascV* was present in 62.1% of *A. jandaei*, 52.2% of *A. veronii*, and 28.6% of *A. aquariorum*; while *aexT* was present in 56.5% of *A.*

Table 2. Distribution of *Aeromonas* spp. in isolates collected from clinical patients, food, and tap water samples

Species	Total strains (*n*, %)	Clinical isolates (*n*, %)	Environmental isolates (*n*, %)	Food isolates (*n*, %)
A. veronii	23 (25.5)	6 (18.1)	9 (25.0)	8 (38.1)
A. caviae	12 (13.3)	12 (36.4)	0 (0.0)	0 (0.0)
A. aquariorum	7 (7.8)	4 (12.1)	2 (5.6)	1 (4.8)
A. hydrophila	6 (6.7)	1 (3.0)	1 (2.8)	4 (19.0)
A. jandaei	29 (32.2)	4 (12.1)	21 (58.3)	4 (19.0)
A. enteropelogenes	2 (2.2)	1 (3.0)	1 (2.8)	0 (0.0)
A. media	1 (1.1)	0 (0.0)	0 (0.0)	1 (4.8)
A. salmonicida	2 (2.2)	0 (0.0)	1 (2.8)	1 (4.8)
New species	8 (8.9)	5 (15.1)	1 (2.8)	2 (9.5)
Total	90	33	36	21

Table 3. Distribution of virulence-associated genes in *Aeromonas* strains isolated clinical patients, food, and tap water samples

Gene	Total strains (*n*, %)	Clinical strains (*n*, %)	Environmental strains (*n*, %)	Food strains (*n*, %)
act	47 (52.2)	15 (45.5)	18 (50.0)	14 (66.7)
alt	22 (24.4)	11 (33.3)	4 (11.1)	7 (33.3)
ast	12 (13.3)	1 (3.0)	3 (8.3)	8 (38.1)
aerA	13 (14.4)	4 (12.1)	3 (8.3)	6 (28.6)
hlyA	19 (21.1)	6 (18.2)	5 (13.9)	8 (33.3)
ascV	39 (43.3)	12 (39.4)	19 (52.8)	8 (38.1)
aexT	20 (22.2)	10 (30.3)	7 (19.4)	3 (14.3)
fla	70 (77.8)	29 (87.9)	26 (72.2)	15 (71.4)
lip	34 (37.7)	22 (69.7)	4 (11.1)	8 (38.1)
ela	40 (44.4)	22 (66.7)	8 (22.2)	10 (47.6)
laf	8 (8.9)	2 (6.1)	4 (11.1)	2 (9.5)

Table 4. Distribution of virulence genes in the five most common *Aeromonas* spp.

Gene	*A. jandaei* (*n*, %)	*A. veronii* (*n*, %)	*A. caviae* (*n*, %)	*A. aquariorum* (*n*, %)	*A. hydrophila* (*n*, %)
act	8 (27.6)	21 (91.3)	2 (16.7)	3 (42.9)	5 (83.3)
alt	0 (0.0)	1 (4.3)	2 (16.7)	7 (100.0)	6 (100.0)
ast	0 (0.0)	2 (8.7)	0 (0.0)	2 (28.6)	6 (100.0)
aerA	0 (0.0)	2 (8.7)	1 (8.3)	3 (42.9)	5 (83.3)
hlyA	1 (3.4)	2 (8.7)	0 (0.0)	7 (100.0)	6 (100.0)
ascV	18 (62.1)	12 (52.2)	2 (16.7)	2 (28.6)	1 (16.7)
aexT	3 (10.3)	13 (56.5)	2 (16.7)	1 (14.3)	0 (0.0)
fla	18 (62.1)	18 (78.3)	10 (83.3)	7 (100.0)	6 (100.0)
lip	1 (3.4)	1 (4.3)	11 (91.7)	7 (100)	6 (100.0)
ela	3 (10.3)	5 (100.0)	12 (100)	7 (100.0)	6 (100.0)
laf	4 (13.8)	2 (8.7)	0 (0.0)	1 (14.3)	1 (16.7)

veronii and 16.7% of *A. caviae*. Enterotoxins and hemolysins are very important virulence factors in *Aeromonas spp.*[35] and many studies have shown a positive correlation between the number of toxin genes harbored by an isolate and its potential virulence[35,13]. The virulence genes detected in this study indicate the potential pathogenicity of the isolates from clinical, food, and environmental sources, as well as their possible risk to human health.

In this study, the majority of *Aeromonas* strains displayed MDR phenotypes, with 100.0% resistance against amoxicillin and 86.7% resistance against amoxicillin/ clavulanic acid, consistent with previous studies[36,37]. Due to their chromosomal β-lactamase expression, *Aeromonas spp*. are naturally resistant to β-lactams. As such, the resistance rate of *Aeromonas* strains derived from patients was significantly higher than those from tap water systems or food, with the exception of colistin. Moreover, the drug resistance rate of strains isolated from tap water systems was significantly higher than that of clinical and food strains. Colistin is a last-resort antibacterial used to treat clinically serious infections caused by MDR gram-negative bacteria[38]. A new mobile colistin resistance gene, *mcr-3*, has been detected in MDR bacteria isolated from severely ill patients in many countries. It is particularly important to determine the presence of these strains in meat products and drinking water due to their direct impact on public health[31]. In this study, three of the *Aeromonas* strains that were resistant to colistin harbored *mcr-3* genes and were derived from the feces of patients with diarrhea, tap water, and fresh pork from the supermarket.

The existence of these *mcr-3* genes is of great importance to global public health because obtaining an *mcr-3* gene may lead to high levels of colistin resistance in *Aeromonas*, particularly since it is ubiquitous in soil and water systems and has the opportunity to interact with bacteria from a variety of different sources. *Aeromonas* species may therefore be a reservoir for *mcr-3* and contribute toward its potential spread. In China, *mcr* genes have not only been detected in a large number of human pathogens, but also have a high positive test rate in

Table 5. Prevalence of resistance to different antibiotics

Antibiotics	Resistant isolates (*n*, %)			
	Total strains (*n*, %)	Clinical strains (*n*, %)	Environmental strains (*n*, %)	Food strains (*n*, %)
Penicillins				
Amoxicillin/clavulanic acid	78 (86.7)	31 (96.9)	29 (80.6)	18 (85.7)
Ampicillin	90 (100.0)	33 (100.0)	36 (100.0)	21 (100.0)
Caphems				
Cefepime	5 (5.6)	5 (15.6)	0 (0.0)	0 (0.0)
Ceftazidime	16 (17.8)	9 (28.1)	3 (8.3)	4 (19.0)
Ceftriaxone	11 (12.2)	6 (18.8)	1 (2.8)	4 (19.0)
Carbapenems				
Imipenem	3 (3.3)	1 (3.0)	2 (5.6)	0 (0.0)
Monobactams				
Aztreonam	2 (2.2)	2 (6.1)	0 (0.0)	0 (0.0)
Aminoglycosides				
Gentamicin	3 (3.3)	3 (9.4)	0 (0.0)	0 (0.0)
Tetracyclines				
Tetracycline	1 (1.1)	1 (3.1)	0 (0.0)	0 (0.0)
Quinolones				
Ciprofloxacin	5 (5.6)	5 (15.6)	0 (0.0)	0 (0.0)
Folate pathway inhibitors				
Trimethoprim-sulfamethoxazole	15 (16.7)	8 (25.0)	2 (5.6)	5 (23.8)
Phenicols				
Chloramphenicol	3 (3.3)	3 (9.4)	0 (0.0)	0 (0.0)
Polymyxins				
Colistin	38 (42.2)	10 (31.2)	21 (58.3)	7 (33.3)

animals (livestock, pets, and even wildlife) and the environment (soil and water)[39]. Since colistin is being used at increasingly high frequencies in veterinary and human medicine, it is essential to continuously monitor *mcr* genes in both clinical and environmental settings.

Resistance to SXT and quinolone, which are antimicrobials used to treat *Aeromonas* infection, has been widely documented. Deng et al.[40] reported that *Aeromonas* isolates collected from cultured freshwater animals have the 5% resistance for Ciprofloxacin and 18.86% resistance for SXT, at the same time, the detection rate of *sul1* gene was 18.86% and that of *qnrS* gene was 4.7%. The researchers noted[33] that *Aeromonas* isolated from clinical patients have the 6.1% resistance rates of Ciprofloxacin, as well as 5.2% resistance rates of SXT. A total of 186 *Aeromonas*, collected from commercially reared fish and ornamental fish, were evaluated for their antimicrobial susceptibilities. The researchers[28] found that the resistance rate of SXT was 9.4%, and the detection rate of *sul1* was 9.4%. In our study, the resistance rate of SXT was 16.7% and the detection rate of *sul1* was 3.3%. As reported, the SXT resistance and its determinants are highly prevalent in *Aeromonas*[28,41,42], most likely due to the overuse of sulfonamide drugs in animal farms and fish ponds. PMQR genes have recently been characterized in *Aeromonas* strains[22,43]; Chenia[20] reported that *qnrS* was found to be present in 21% of *Aeromonas* isolates from freshwater fish in South Africa. In the present study, the detection rate of *qnrS* was 4.4%. however, when we screened the 90 *Aeromonas* isolates for the three PMQR genes *qnrA, qnrB,* and *qnrS*, only *qnrS* was detected in strains isolated from clinical specimens. It is thought that *Aeromonas* may act as a carrier of these resistance genes via horizontal transfer[44]; therefore, the prevalence of MDR in *Aeromonas* species could be considered a threat to public health.

CONCLUSIONS

We obtained 90 *Aeromonas* isolates from clinical patients, tap water systems, and food in Ma'anshan, Anhui Province, China. High genetic diversity was observed in these isolates, which belonged to 80 novel STs. Concatenated *gyrB-cpn60* gene sequences classified 82 (91.1%) of the *Aeromonas* isolates into eight different species as well as several new species. Virulence genes were examined by PCR, indicating that the isolates may be pathogenic and pose a risk to human health. When measuring antibiotic resistance to ten distinct antibiotic classes, 21.1% of the strains were found to be MDR (≥ 3). The PMQR, ESBL, aminoglycoside resistance, sulphonamide, and *mcr-3* genes were detected in the isolates, as well as a new *mcr-3* gene variant. Thus, this study sheds light on the genetic diversity, antibiotic resistance, and pathogenicity of *Aeromonas* species identified from a variety of sources.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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Supplementary Table S1. The *gyrB* and *cpn60* genes of twenty-eight representative *Aeromonas* species available in GenBank

Strains	Species name	GenBank locus	
		gyrB	*cpn60*
A.allosaccharophila-CECT4200	*A. allosaccharophila*	AY101823	EU741624
A.bestiarum-112A	*A. bestiarum*	JN711733	EU741625
A.bestiarum-628A	*A. bestiarum*	JN711738	EU306797
A.bivalvium-665N	*A. bivalvium*	EF465524	EU306798
A.bivalvium-868E	*A. bivalvium*	EF465525	EU306799
A.caviae-CECT838	*A. caviae*	JN829497	EU306800
A.encheleia-CECT4342	*A. encheleia*	JN829499	EU306801
A.enteropelogenes-CECT4487	*A. enteropelogenes*	EF465526	EU306837
A.eucrenophila-CECT4224	*A. eucrenophila*	JN829501	EU306803
A.eucrenophila-CECT4854	*A. eucrenophila*	AY101813	EU741634
A.hydrophila-CECT5236	*A. hydrophila*	JN711791	EU741635
A.allosaccharophila-CECT4199	*A. allosaccharophila*	JN829495	EU306795
A.aquariorum-MDC317	*A. aquariorum*	HQ442717	JN711581
A.aquariorum-MDC573	*A. aquariorum*	HQ442715	JN711582
A.aquariorum-MDC47	*A. aquariorum*	EU268444	FJ936120
A.caviae-A4EL5	*A. caviae*	JF938610	JF920575
A.caviae-E7EL42	*A. caviae*	JF938613	JF920578
A.diversa-CECT4254	*A. diversa*	JN829523	EU306835
A.diversa-CECT5178	*A. diversa*	GU062401	GQ365713
A.encheleia-CECT4253	*A. encheleia*	JN829522	EU306802
A.enteropelogenes-CECT4255	*A. enteropelogenes*	JN829517	EU306836
A.fluvialis-717	*A. fluvialis*	FJ603455	GU062398
A.hydrophila-AP60	*A. hydrophila*	JF938654	JF920619
A.hydrophila-CECT839	*A. hydrophila*	JN711776	EU306804
A.hydrophila-CF38	*A. hydrophila*	JF938658	JF920623
A.jandaei-ATCC49568	*A. jandaei*	FN706559	AY922357
A.jandaei-CECT4228	*A. jandaei*	JN829507	EU306807
A.media-CECT4234	*A. media*	KP400958	EU741641
A.media-CECT4232	*A. media*	JN829508	EU306808
A.molluscorum-431E	*A. molluscorum*	EF465520	EU306810
A.molluscorum-848T	*A. molluscorum*	AM179827	EU306811
A.piscicola-R94	*A. piscicola*	JN711768	JN711540
A.piscicola-S1.2	*A. piscicola*	JN711765	GU062399
A.popoffii-LMG17541	*A. popoffii*	JN711769	EU306814
A.salmonicida-CECT5173	*A. salmonicida*	JN711837	EU741642
A.salmonicida-621A	*A. salmonicida*	JN711829	EU306819
A.salmonicida-856T	*A. salmonicida*	JN711833	EU306823
A.sanarellii-A2-67	*A. sanarellii*	FJ807277	JN215527

Continued

Strains	Species name	GenBank locus	
		gyrB	*cpn60*
A.sanarellii-E4P29	*A. sanarellii*	JF938619	JF920584
A.sharmana-DSM17445	*A. sharmana*	EF465528	EU306831
A.simiae-CIP107797	*A. simiae*	AJ632225	EU306832
A.simiae-CIP107798	*A. simiae*	JN829555	EU306833
A.sobria-CECT4245	*A. sobria*	JN829516	EU306834
A.taiwanensis-A2-50	*A. taiwanensis*	FJ807272	JN215528
A.veronii-AT46	*A. veronii*	JF938687	JF920652
A.veronii-AT48	*A. veronii*	JF938688	JF920653
A.veronii-CECT4257	*A. veronii*	HQ442728	EU306838
A.veronii-CECT4486	*A. veronii*	EF465527	EU306841
A.rivuli-CECT7518	*A. rivuli*	CDBJ01000001	JN215526
A.schubertii-BT3-772	*A. schubertii*	LC003078	LC003165
A.schubertii-BT3-777	*A. schubertii*	LC003081	LC003168
A.tecta-CECT7082	*A. tecta*	JN829521	NZ_CDCA01000043
A.cavernicola-MDC2508	*A. cavernicola*	PGGC01000001	PGGC01000001
A.lusitana-MDC2473	*A. lusitana*	PGCP01000001	PGCP01000001