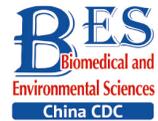


Original Article



Comparative Study of the Genetic Diversity, Antimicrobial Resistance, and Pathogenicity of *Aeromonas* Isolates from Clinical Patients and Healthy Individuals*

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Abstract

Objective This study was performed to compare the genetic diversity, virulence, and antimicrobial resistance of *Aeromonas* strains isolated from patients and healthy individuals.

Methods A total of 38 clinical strains and 19 strains from healthy individuals were isolated from the samples collected in Ma'anshan City, Anhui Province. Their taxonomy was investigated using concatenated *gyrB-cpn60* sequences, and their resistance to 12 antibiotics was evaluated. The pathogenicity of these strains was examined through beta-hemolysis, protease activity, and virulence gene assays.

Results The 57 *Aeromonas* strains were divided into 55 sequence types. Of these types, 21 were novel, suggesting that their genetic diversity was high. These *Aeromonas* isolates could be divided into 7 species, and the positive rates of beta-hemolysis and protease activity were 49.1% and 73.7%, respectively. The detection rate of clinical patients in terms of beta-hemolysis and protease activity was higher than that of healthy individuals. Among the four most common *Aeromonas* strains, *A. dhakensis* had the highest detection rate of virulence genes. The multidrug resistance rate of the clinical isolates was much higher than that of the strains isolated from healthy individuals.

Conclusions The taxonomy, virulence properties, and antibiotic resistance of *Aeromonas* isolates from patients differ from those of the isolates from healthy individuals.

Key words: *Aeromonas*; Genetic diversity; Multidrug resistance; Virulence gene; Antimicrobial resistance gene

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INTRODUCTION

Aeromonas, which belongs to Aeromonadaceae, is a Gram-negative facultative anaerobe positive for oxidase

and catalase. *Aeromonas* spp. are widely distributed in aquatic environments^[1], and more than 28 *Aeromonas* spp. have been identified^[2]. At least 19 of these species are associated with human diseases, and they can cause widespread infection^[3]. The

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Biographical note of the first author: MENG Shuang, female, born in 1981, PhD, majoring in molecular epidemiology and pathogenic mechanism of *Aeromonas*.

common clinical manifestations of *Aeromonas* are diarrhea, localized soft tissue infection, and bacteremia^[4]. Bacteremia primarily occurs in patients with underlying diseases, such as hepatobiliary diseases, cancer, and diabetes^[5].

Phenotypically identifying *Aeromonas* species is considered reasonably difficult because of the limitations of commercially available identification systems for *Aeromonas*, e.g., API20E, API20NE, Vitek, and BioMerieux^[6]. Therefore, molecular methods are widely used to identify *Aeromonas* spp. correctly. The nucleotide sequences of housekeeping genes, such as *gyrB*, *cpn60*, and *rpoB*, have been regarded as reliable markers that can be used to identify *Aeromonas* species^[7-9]. Some reports have shown that strains initially identified as *A. hydrophila* via phenotypic methods can be recognized as *A. dhakensis* through *cpn60* or *gyrB* sequencing^[8,10]. *A. dhakensis* was initially described as an *A. hydrophila* subspecies in 2002 and as *A. aquariorum*; in 2013, it was described as a novel species, namely, *Aeromonas dhakensis*^[11]. *A. dhakensis* has been shown to be a pathogenic bacterium linked to numerous human diseases^[5]. Previous studies on *A. aquariorum* recognized the virulence of *A. dhakensis*, and subsequent evaluations have shown that this strain carries several important virulence genes^[6]. This strain also has the most potent cytotoxic activities against human blood cell lines^[10]. However, the prevalence of *A. dhakensis* infection is likely underestimated because phenotypic identification systems often misidentify *A. dhakensis* as *A. hydrophila*.

Aeromonas sp. pathogenesis involves a series of virulence factors^[12], including hemolytic toxins, such as aerolysin-related cytotoxic enterotoxin (*act*)^[13], heat-labile cytotoxic enterotoxin (*alt*), hemolysin (*hlyA*), heat-stable cytotoxic toxins (*ast*)^[14], and aerolysin (*aerA*)^[15]. In addition, the type III secretion system (*TTSS*), lateral flagella (*laf*), polar flagellum (*fla*)^[16], elastase (*ela*), and lipase (*lip*) contribute to *Aeromonas* pathogenicity^[17]. With these virulence factors, bacteria can colonize, invade, and overcome their hosts' immune response, leading to infection and disease development.

Diarrhea caused by *Aeromonas* is self-limiting, and it is effectively treated with the oral or intravenous antibiotic infusion. However, patients with severe diarrhea or extraintestinal infection should receive antibacterial treatments^[18]. *Aeromonas* is resistant to ampicillin, but it is susceptible to third-generation cephalosporins and aminoglycosides that exhibit potent antibacterial effects against clinical *Aeromonas*

isolates^[19]. However, the extensive use of antibiotics in aquaculture and clinical treatment has caused an increase in antimicrobial resistance^[20]. The development of resistance in *Aeromonas* spp. against several different classes of antibiotics threatens human health because resistant isolates can be transmitted from the aquatic environment to humans via the food chain or direct contact^[21]. Therefore, the antimicrobial resistance of *Aeromonas* should be monitored to guide treatments.

In this study, the diversity and distribution of *Aeromonas* species isolated from healthy individuals and clinical patients in Ma'anshan, Anhui Province, China, were evaluated. Their genetic diversity, pathogenicity, and antimicrobial resistance profiles were compared.

MATERIALS AND METHODS

Aeromonas Isolates

A total of 57 *Aeromonas* isolates (38 from clinical patients and 19 from healthy individuals) were obtained from Ma'anshan, Anhui Province, China, between 2018 and 2019 (Table 1). They were identified using an automatic bacteriological analyzer (Vitek 2 Compact, BioMérieux) and cultured on Luria-Bertani (LB) broth or brain heart infusion agar plates overnight at 37 °C.

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

The *Aeromonas* MLST scheme (<http://pubmlst.org/Aeromonas/>), which relies on the amplification and sequencing of six housekeeping genes, namely, *gyrB*, *groL*, *gltA*, *metG*, *ppsA*, and *recA*, was applied to identify the subtype of each *Aeromonas* isolate^[22]. The total chromosomal DNA from the *Aeromonas* samples was prepared using a DNA purification kit (Promega, Madison, USA) in accordance with the manufacturer's instructions. PCR was performed using previously described primers and protocols^[23], and the sequences of the six loci were compared with those hosted on the *Aeromonas* MLST database and STs. New alleles and STs were submitted to the *Aeromonas* MLST database for naming.

With the limitations related to molecular identification involving 16S rRNA sequencing^[24], the sequences of the housekeeping genes *gyrB* and *cpn60* were included in our species identification assays. Concatenated two-gene phylogenetic trees were constructed and compared with representative species as previously described^[25]. A phylogenetic

tree was also constructed using the neighbor-joining method in Clustal-W^[24] with Bootstrap values calculated using 1,000 replicates.

Beta-hemolysis and Exoprotease Assays

The degree of beta-hemolysis was assessed in isolates cultured in LB agar containing 5% (vol/vol) sheep blood agar (KeMaJia, Shanghai, China). Qualitative assays were performed with LB agar containing 2% (wt/vol) skim milk (KeMaJia, Shanghai, China) to investigate the exoprotease activity. The presence of clear zones surrounding the streaks indicated positive reactions in both tests^[26].

Detection of Virulence-associated Genes

An assay was designed to evaluate the presence of the genes encoding exotoxins or secretion system components, structural components, and extracellular enzymes associated with *Aeromonas* virulence. In this assay, aerolysin (*aerA*), hemolysin (*hlyA*), heat-labile enterotoxin (*act*), heat-stable cytotoxin (*ast*), heat-labile cytotoxin (*alt*), type III secretion system components (*ascV*), ADP-ribosyl transferase toxin (*aexT*), lipase (*lip*), elastase (*ela*), and flagellin (*fla*) were detected using previously described primers and protocols^[25]. PCR was performed in a 50 µL reaction mixture containing 25 µL of Taq PCR MasterMix (Takara Bio, Inc., Japan), 1 µL of the relevant primers (10 µmol/L), 21 µL of ddH₂O, 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; and a final cycle at 72 °C for 5 min. Sequencing was conducted to confirm the positive PCR products.

Antimicrobial Susceptibility

The antimicrobial susceptibility of each *Aeromonas*

isolate was evaluated using the microbroth dilution method in accordance with the current guidelines of the Clinical and Laboratory Standards Institute. The minimum inhibitory concentrations of 12 antibiotics were measured: amoxicillin/clavulanate, cefepime, ceftriaxone, ceftazidime, imipenem, aztreonam, gentamycin, tetracycline, ciprofloxacin, trimethoprim/sulfamethoxazole (SXT), chloramphenicol, and colistin. *Escherichia coli* ATCC 25922 was the strain used for the quality control strain of all the susceptibility tests.

Evaluation of Antimicrobial Resistance Genes

The strains were screened for the presence of various antimicrobial resistance genes, including those for tetracycline resistance (*tetA*, *tetB*, and *tetE*), extended-spectrum b-lactamase (ESBL) resistance (*blaTEM*, *blaSHV*, and *blaCTX*)^[27], aminoglycoside resistance (*armA*, *aphAI-IAB*, *aac(6')-Ib*, and *aac(3)-IIa*)^[28], sulfonamide resistance (*sul1* and *sul2*)^[29], mobile colistin resistance (*mcr-1*, *mcr-2*, *mcr-3*, and *mcr-4*), and PMQR (*qnrA*, *qnrB*, and *qnrS*)^[27], by using previously described primers and protocols^[25]. Sequencing was conducted to confirm the positive PCR products.

RESULTS

MLST of *Aeromonas* Isolates

The 57 *Aeromonas* isolates were divided into 55 STs. Of these isolates, 21 were novel (ST722-ST742), indicating that the degree of genetic diversity was high (Figure 1). No dominant STs were found in either the patient or healthy groups.

Diversity and Distribution of the *Aeromonas* Species Isolated in This Study

gyrB and *cpn60* were sequenced to evaluate the phylogeny of the 57 *Aeromonas* isolates identified in

Table 1. Distribution of *Aeromonas* spp. in isolates collected from clinical patients and healthy individuals

Species	Total no. strains (%)	No. clinical patients (%)	No. healthy people (%)
<i>A. caviae</i>	21 (36.8)	18 (47.3)	3 (15.8)
<i>A. veronii</i>	19 (33.3)	10 (26.3)	9 (47.4)
<i>A. dhakensis</i>	6 (10.5)	5 (13.2)	1 (5.3)
<i>A. jandaei</i>	6 (10.5)	1 (2.6)	5 (26.3)
<i>A. hydrophila</i>	2 (3.5)	1 (2.6)	1 (5.3)
<i>A. media</i>	2 (3.5)	2 (5.3)	0
<i>A. enteropelogenes</i>	1 (1.8)	1 (2.6)	0
Total	57	38	19

this study (Figure 2). This analysis revealed that these isolates could be divided into seven different species. The most common species were *A. caviae* (36.8%), *A. veronii* (33.3%), *A. dhakensis* (10.5%), and *A. jandaei* (10.5%). Notably, the distribution of the *Aeromonas* isolates from the patients and the healthy individuals varied (Table 1). *A. caviae* (47.3%) and *A. veronii* (26.3%) were the most prevalent in the patient samples, whereas *A. veronii* (47.4%) and *A. jandaei* (26.3%) were the most common species in the healthy samples. In addition, the abundance of *A. dhakensis* was higher in the patients than in the healthy individuals.

Beta-hemolysis and Exoprotease Assays

A total of 28 (49.1%) isolates demonstrated beta-hemolysis, and 42 (73.7%) isolates possessed a proteolytic activity (Table 2). *A. media* isolates lacked

hemolytic and proteolytic activities. *A. caviae* isolates (19.0% and 66.7%, respectively) had less hemolytic and exoprotease activities than those of *A. dhakensis* isolates (83.3% and 100%, respectively) or *A. hydrophila* isolates (50% and 100%, respectively). Of the strains isolated from healthy individuals, only 36.8% processed any beta-hemolytic activity, whereas 63.2% exhibited some proteolytic activities. However, 55.2% of the clinical isolates exhibited a hemolytic activity, and 84.2% had a proteolytic activity (Table 2).

Distribution of Virulence Genes

The distribution of the virulence genes in these *Aeromonas* isolates is summarized in Table 3. In particular, *fla* was the most common virulence gene identified in 87.7% of the 57 isolates, and *ela* was the second-most prevalent virulence gene in these

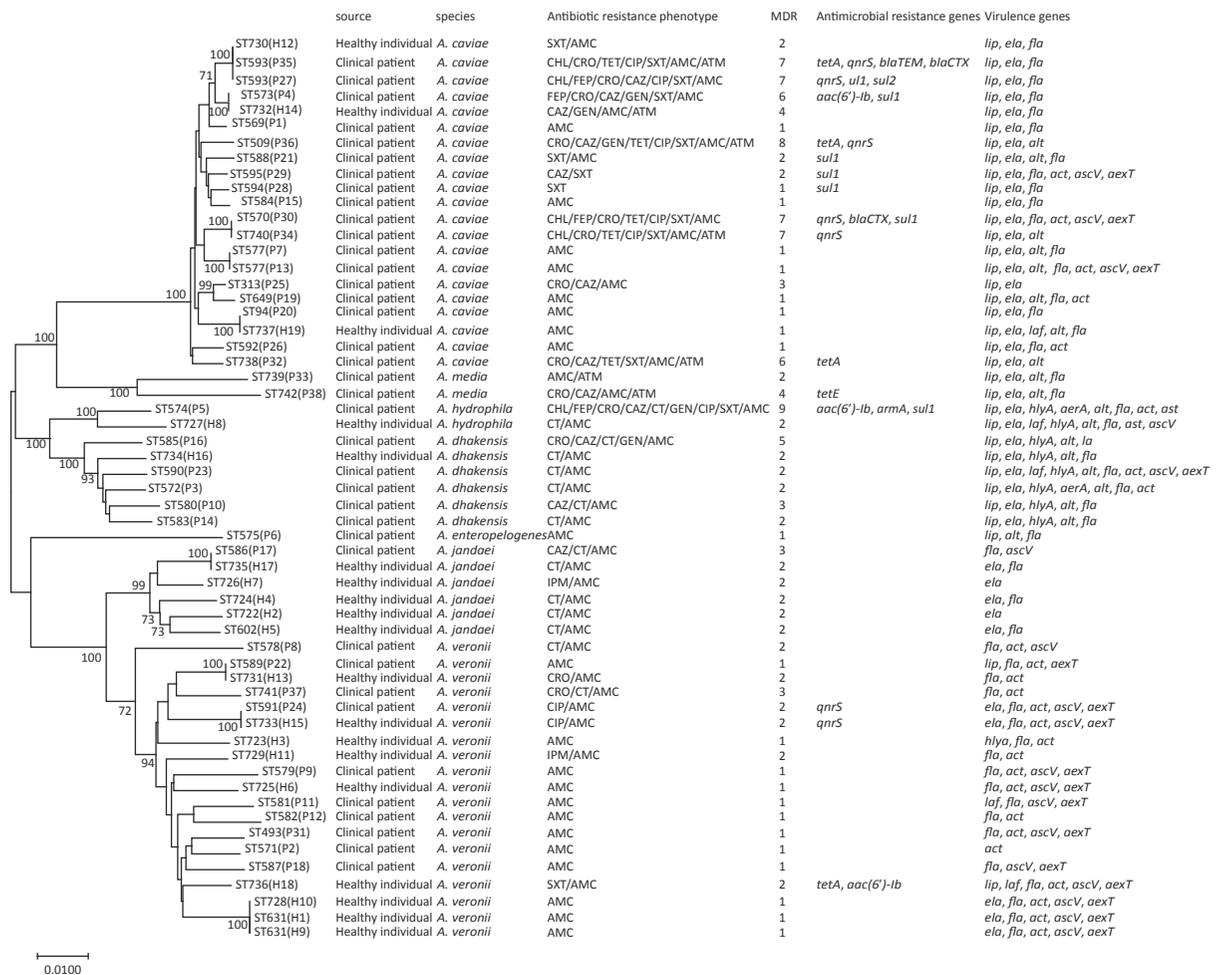


Figure 1. Phylogenetic relationships were established using the concatenated sequences of the six genes included in this study. The source, species, virulence genes, and antibiotic resistance are shown on the right. A phylogenetic tree was determined using a neighbor-joining algorithm. ST: sequence type.

Table 2. Prevalence of hemolytic and proteolytic activity in *Aeromonas* isolates

Species and source	No. (%) of isolates		
	Total	Healthy individual	Clinical patient
<i>A. veronii</i>	19 (100)	9 (47.5)	10 (52.6)
Hemolysis	13 (68.4)	4 (44.4)	9 (90.0)
Proteolysis	15 (78.9)	6 (66.7)	9 (90.0)
<i>A. jandaei</i>	6 (100)	5 (83.3)	1 (16.7)
Hemolysis	4 (66.7)	3 (60.0)	1 (100)
Proteolysis	4 (66.7)	3 (60.0)	1 (100)
<i>A. dhakensis</i>	6 (100)	1 (16.7)	5 (83.3)
Hemolysis	5 (83.3)	0 (0)	5 (100)
Proteolysis	6 (100)	1 (100)	5 (100)
<i>A. caviae</i>	21 (100)	3 (14.4)	18 (85.7)
Hemolysis	4 (19.0)	0 (0)	4 (22.2)
Proteolysis	14 (66.7)	1 (33.3)	13 (72.2)
<i>A. enteropelogenes</i>	1 (100)	0 (0)	1 (100)
Hemolysis	1 (100)	0 (0)	1 (100)
Proteolysis	1 (100)	0 (0)	1 (100)
<i>A. media</i>	2 (100)	0 (0)	2 (100)
Hemolysis	0 (0)	0 (0)	0 (0)
Proteolysis	0 (0)	0 (0)	0 (0)
<i>A. hydrophila</i>	2 (100)	1 (50)	1 (50.0)
Hemolysis	1 (50)	0 (0)	1 (100)
Proteolysis	2 (100)	1 (100)	1 (100)
Total	57 (100)	19 (33.3)	38 (66.7)
Hemolysis	28 (49.1)	7 (36.8)	21 (55.2)
Proteolysis	42 (73.7)	12 (63.2)	32 (84.2)

Table 3. Distribution of virulence-associated genes in *Aeromonas* strains isolated from clinical patients and healthy individuals

Gene	Total no. strains (%)	No. clinical patients (%)	No. healthy individuals (%)
<i>fla</i>	50 (87.7)	33 (86.8)	17 (89.5)
<i>ela</i>	41 (71.9)	27 (71.1)	14 (73.7)
<i>lip</i>	34 (59.6)	28 (73.6)	6 (31.6)
<i>act</i>	25 (43.9)	16 (42.1)	9 (50.0)
<i>ascV</i>	18 (31.6)	11 (28.9)	7 (36.8)
<i>alt</i>	19 (33.3)	16 (42.1)	3 (15.8)
<i>aexT</i>	16 (28.1)	10 (26.3)	6 (31.6)
<i>hlyA</i>	9 (15.8)	6 (15.8)	3 (15.8)
<i>laf</i>	5 (8.8)	2 (5.3)	3 (15.8)
<i>ast</i>	2 (3.5)	1 (2.6)	1 (5.3)
<i>aerA</i>	2 (3.5)	2 (5.3)	0 (0.0)
Total	57 (100)	38 (100)	19 (100)

isolates ($n = 41$, 71.9%). *Laf*, *ast*, and *aerA* were identified in less than 10% of the isolates. The prevalence of *lip* differed significantly between patient- and healthy-derived strains ($P < 0.05$, Fisher's exact test). Only *lip*, *alt*, and *aerA* were more prevalent in patient-derived isolates than in healthy-derived isolates.

In the four most common *Aeromonas* species, the detection rates of *ela*, *lip*, *alt*, *hlyA*, and *aerA* in *A. dhakensis* isolates were significantly higher than those in *A. caviae*, *A. veronii*, and *A. jandaei* ($P < 0.05$, Fisher's exact test; Table 4). Although the total number of *A. jandaei* isolates was low, few of them were positive for any of the evaluated virulence genes. The extracellular protease genes *ela* and *lip* were abundant in *A. caviae* and *A. dhakensis*, and the hemolytic gene *act* was abundant in *A. veronii*. The enterotoxin gene *alt* and hemolytic genes *hlyA* and *aerA* were most commonly identified in *A. dhakensis*. Their rates of occurrence in other species identified in this study were relatively low.

Antimicrobial Susceptibility

The antimicrobial susceptibility rates of 12 common antimicrobials in the *Aeromonas* isolates identified in this study are presented in Table 5. Almost all the *Aeromonas* strains had high amoxicillin/clavulanic acid (96.4%) resistance; however, most of the isolates ($\geq 90\%$) were susceptible to cefepime, imipenem, chloramphenicol, gentamicin, and tetracycline. In general, clinical *A. dhakensis*, *A. hydrophila*, *A. jandaei*, and *A. caviae* isolates were more resistant than their healthy individual counterparts to the

commonly prescribed antibiotics, including ceftriaxone, ceftazidime, ciprofloxacin, and amoxicillin/clavulanic acid. The healthy sample isolates had an increased rate of resistance to colistin and imipenem only (Supplementary Table S1 available in www.besjournal.com). *A. enteropelogenes* and *A. media*, identified only in the patient samples, were susceptible to most of the tested antibiotics except amoxicillin/clavulanic acid, aztreonam, ceftriaxone, and ceftazidime (Supplementary Table S1).

Of the 57 strains identified in this study, 15 (26.3%) were found to have multidrug resistance (MDR), displaying resistance to at least 3 of the antibiotics tested in this assay. The MDR rates of the healthy-individual-derived strains (5.3%) were significantly lower than those of the patient isolates (36.8%). Therefore, these MDR phenotypes were likely acquired after treatment and hospitalization.

Detection of Antimicrobial Resistance Genes

The tetracycline resistance genes *tetA* and *tetE* were found in 4 (6.9%) isolates and 1 (1.7%) isolate, respectively. The *PMQR* gene *qnrS* was present in 7 (12.1%) isolates, and the aminoglycoside resistance genes *aac* (6')-Ib and *armA* were detected in 3 (5.2%) isolates and 1 (1.7%) isolate, respectively. The *ESBL* genes *bla*CTX and *bla*-TEM were observed in 2 (3.4%) isolates and 1 (1.7%) isolate, and the sulfonamide genes *sul1* and *sul2* were found in 7 (12.1%) isolates and 1 (1.7%) isolate, respectively (Supplementary Table S2 available in www.besjournal.com). The mobile colistin resistance gene *mcr-3* was detected in 1 (1.7%) isolate. The following genes were not

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

Gene	No. <i>A. caviae</i> strains (%)	No. <i>A. veronii</i> strains (%)	No. <i>A. dhakensis</i> strains (%)	No. <i>A. jandaei</i> strains (%)
<i>fla</i>	17 (80.9)	18 (94.7)	6 (100)	4 (66.7)
<i>ela</i>	21 (100)	5 (26.3)	6 (100)	5 (83.3)
<i>lip</i>	21 (100)	2 (10.5)	6 (100)	0 (0)
<i>act</i>	5 (23.8)	17 (89.4)	2 (33.3)	0 (0)
<i>ascV</i>	3 (14.3)	12 (63.2)	1 (16.7)	1 (16.7)
<i>alt</i>	8 (38.1)	0 (0)	6 (100)	0 (0)
<i>aexT</i>	3 (14.3)	12 (63.2)	1 (16.7)	0 (0)
<i>hlyA</i>	0 (0)	0 (0)	6 (100)	0 (0)
<i>laf</i>	1 (4.8)	2 (10.5)	1 (16.7)	0 (0)
<i>ast</i>	0 (0)	0 (0)	0 (0)	0 (0)
<i>aerA</i>	0 (0)	0 (0)	1 (16.7)	0 (0)
Total	21 (100)	19 (100)	6 (100)	6 (100)

detected in any isolate: the *ESBL* gene *blaSHV*; the aminoglycoside resistance genes *aphAI-IAB* and *aac(3)-IIa*; the tetracycline resistance gene *tetB*; the colistin resistance genes *mcr-1*, *mcr-2*, and *mcr-4*; and the *PMQR* genes *qnrA* and *qnrB*.

The detection rate of resistance genes in the clinical strains was higher than that in their healthy individual counterparts. The resistance genes of *A. caviae* were higher than that of any of the other species identified in this study (Supplementary Table S2).

DISCUSSION

Aeromonas spp. are ubiquitous in most aquatic environments, and certain species have been reported to be an important cause of acute diarrhea in children and adults^[2]. *Aeromonas* strains were found in 7.6% of patients with diarrhea and 3.8% of a healthy population^[30,31]. These rates may reflect actual differences or may be influenced by seasonal or environmental factors.

In this study, 57 *Aeromonas* isolates were described in detail in terms of species distribution, ST type, pathogenicity, and antimicrobial

susceptibility to evaluate the differences in the epidemiology and etiology of *Aeromonas* strains isolated from clinical patients and healthy individual samples. The results of these analyses were then used to evaluate the significance of *Aeromonas* infections in clinical settings in China. They could be considered as a basis for developing the clinical protocols of treatment and surveillance.

These 57 isolates were separated into 55 STs. Of these isolates, 21 were novel, indicating that the degree of genetic diversity within these sample populations was high. *Aeromonas* is often isolated from patients with diarrhea. However, the role of *Aeromonas* spp. as potential human enteric pathogens remains unclear, possibly because of the lack of systematic analysis of these strains and the inherent difficulty in classifying them.

In this study, *Aeromonas* strains were identified to the species level via *gyrB-cpn60* concatemer sequencing, and their species distribution was compared between the clinical patients and healthy individuals. The distribution of *Aeromonas* species isolated from the clinical patients and healthy individuals varied. Among them, *A. caviae* (47.3%) and *A. veronii* (26.3%) were the most prevalent

Table 5. Prevalence of resistance to different antibiotics

Antibiotics	No and % of resistant isolates		
	Total no. strains (%)	No. clinical patients (%)	No. healthy individuals (%)
Penicillins			
Amoxicillin/clavulanic acid	55 (96.4)	36 (94.7)	19 (100)
Caphems			
Cefepime	4 (7.0)	4 (10.5)	0 (0)
Ceftazidime	12 (21.1)	11 (28.9)	1 (5.3)
Ceftriaxone	13 (22.8)	12 (31.6)	1 (5.3)
Carbapenems			
Imipenem	2 (3.5)	0 (0.0)	2 (10.5)
Monobactams			
Aztreonam	7 (12.3)	6 (15.8)	1 (5.3)
Aminoglycosides			
Gentamicin	5 (8.8)	4 (10.5)	1 (5.3)
Tetracyclines			
Tetracycline	5 (8.8)	5 (13.2)	0 (0.0)
Quinolones			
Ciprofloxacin	8 (14.0)	7 (18.4)	1 (5.3)
Folate pathway inhibitors			
Trimethoprim-sulfamethoxazole	13 (22.8)	11 (28.9)	2 (10.5)
Phenicol			
Chloramphenicol	5 (8.8)	5 (13.2)	0 (0.0)
Polymyxins			
Colistin	15 (26.3)	9 (23.7)	6 (31.6)

clinical isolates, and *A. veronii* (47.4%) and *A. jandaei* (26.3%) were the most common isolates from the healthy individual samples. Wu et al.^[32] reported that the most prevalent species in clinical patients are *A. caviae* and *A. dhakensis*, which account for 29.8%; they also found that *A. veronii* is the second-most prevalent species, which account for 23.4%. In the present study, the isolates were identified as follows: 21 (36.8%) as *A. caviae*, 19 (33.3%) as *A. veronii*, and 6 (10.5%) as *A. dhakensis*. Studies in other countries have shown a slightly different distribution; in particular, the most prevalent clinical strain in India is *A. hydrophila*, while the most prevalent one in France, Italy, and the Netherlands is *A. caviae*^[30]. In addition, most *A. veronii* strains are isolated from human samples, whereas most *A. hydrophila* strains are obtained from water samples. These differences may also depend on sampling season and geographical location. *A. dhakensis*, the third-most prevalent species in our study, is a novel *Aeromonas* species^[33] that has been identified as a potential pathogen in humans^[5]. *A. dhakensis* isolates have been collected from stool, blood, wound, and other extraintestinal samples from humans globally; skin and soft tissue infections (SSTIs) caused by *A. dhakensis* have been reported in Taiwan, China^[6]. In a set of 80 *Aeromonas* wound isolates, 37 (46.3%) samples contain *A. dhakensis*, which can cause severe SSTIs^[34]. Chang et al.^[8] demonstrated that *A. dhakensis* is the most frequently isolated aeromonad in Australia, accounting for 30.7%. In clinical specimens, *A. dhakensis* is the most prevalent in wounds but is less frequently isolated from fecal strains and blood samples. Salas et al.^[35] believed that *A. dhakensis* is the second-most prevalent species in clinical isolates, accounting for 25.5%. Zhou et al.^[31] revealed that the four most prevalent species of *Aeromonas* in clinical isolates are *A. caviae* (41.7%), *A. veronii* (31.3%), *A. dhakensis* (13.9%), and *A. hydrophila* (5.2%). Senderovich et al.^[36] showed that several *Aeromonas* species are recovered from Israeli patients suffering from diarrhea; among the isolates, *A. caviae* (65.0%) and *A. veronii* (29.0%) are the most prevalent species. Therefore, previous studies supported our findings on the most prevalent strains in our samples.

The pathogenic mechanism of *Aeromonas* spp. is complex and multifactorial. In this study, the pathogenic potential was evaluated using beta-hemolytic and proteolytic activity assays, and several virulence genes were detected. A total of 28 (49.1%)

isolates showed beta-hemolysis activities, and 42 (73.7%) had some proteolytic activities. Consistent with previous findings^[32], our results indicated that *A. dhakensis* and *A. hydrophila* isolates were more likely to exhibit hemolytic and proteolytic activities than *A. caviae* isolates. The beta-hemolytic and proteolytic activities of the clinical isolates differed from those of the healthy individual isolates. In particular, 36.8% of the healthy sample strains demonstrated any beta-hemolytic activity, and 63.2% of these strains exhibited a proteolytic activity. However, 55.2% and 84.2% of the clinical isolates had hemolytic and proteolytic activities, respectively. Therefore, the strains from clinical patients were more likely to exhibit beta-hemolytic and proteolytic activities than their healthy sample counterparts (Table 2).

The distribution of the virulence-associated genes in these isolates was determined. The results revealed that *fla*, *ela*, and *lip* were identified in most of the strains, i.e., 50 (87.7%), 41 (71.9%), and 34 (59.6%) of the 57 isolates, respectively. In addition, *fla* was present in most species, while *ela* and *lip* were more prevalent in *A. dhakensis* and *A. caviae* isolates than in other isolates (Table 4). The enterotoxin and hemolysin genes *act*, *aerA*, *alt*, and *ast* were detected in 25 (43.9%), 2 (3.5%), 19 (33.3%), and 2 (3.5%) of the 57 isolates, respectively. *act* was observed in 89.4% of *A. veronii* isolates, and *aerA* was found in 16.7% of *A. dhakensis* isolates. *alt* was detected in 100% of *A. dhakensis* and 38.1% of *A. caviae* isolates. Conversely, *ast* was not present in any of the *A. dhakensis*, *A. caviae*, and *A. veronii* strains (Table 4). The *TTSS* genes *ascV* and *aexT* were present in 18 (31.6%) and 16 (28.1%) of the 57 isolates, respectively, and *ascV* and *aexT* were both present in 63.2% of *A. veronii* strains and 16.7% of *A. dhakensis* strains. The detection rate of *lip* was significantly higher in the clinical isolates than in the healthy individual isolates. By comparison, *ascV* and *laf* were detected more frequently in the strains from the healthy samples than from the clinical samples. Enterotoxins and hemolysins are important virulence factors in *Aeromonas* spp., and many studies have shown that the number of toxin genes harbored by any isolate is positively correlated with its potential virulence^[37,38]. Among the four most common *Aeromonas* strains, *A. dhakensis* had the highest prevalence of virulence genes. This finding was also consistent with previous reports, which described that *A. dhakensis* strains harbor high cytotoxicity and liquid toxicity^[34]. In our study, *fla*, *ela*, *lip*, *alt*, and *hlyA* were detected in all the *A.*

dhakensis isolates, but *aerA* and *hlyA* were detected in *A. dhakensis* isolates only. The abundance of these virulence genes also varies among studies. For example, *ast* was not detected in *A. dhakensis* isolates in our study; however, some researchers have reported that 50.0%–83.3% *ast* is found in *A. dhakensis* isolates from wound and blood infections^[6,10].

Consistent with previous findings^[39], our results suggested that 96.4% of the *Aeromonas* strains exhibited resistance against amoxicillin/clavulanic acid. The resistance rates to most antibiotics were higher in the clinical isolates than in the healthy individual isolates, but the resistance to colistin and imipenem was higher in the strains identified in the healthy individual samples than in the clinical samples. All the *A. dhakensis* isolates exhibited resistance to colistin. This result suggested that *A. dhakensis* should be the focus of future research because it harbors a large number of virulence genes, high rates of drug resistance, and a high degree of beta-hemolytic and proteolytic activities. Some scholars indicated that *Aeromonas* has a high MDR, which is 38.7%, because of the different sources of strains^[40]. In our study, 15 strains (26.3%) exhibited MDR to 12 antimicrobial agents, and this value was consistent with 28.7% as previously reported^[31]. The MDR rates of the healthy individual-derived strains were significantly lower than those of the clinical isolates. Therefore, the extensive use of antimicrobial agents increases the selective pressure on nosocomial infectious bacterial strains to develop resistance. This selective pressure should be addressed in future studies.

Resistance to SXT and quinolone, which are antimicrobials used to treat *Aeromonas* infection, has been widely described. Deng et al.^[40] reported that at least 18.86% of *Aeromonas* isolates collected from cultured freshwater animals are resistant to SXT; they also detected *sul1* and *qnrS* in 18.9% and 4.7% of these samples, respectively. Our study obtained an SXT resistance rate of 22.8%, and 12.1% of these strains were positive for both *sul1* and *qnrS*. The detection rate of antimicrobial resistance genes in the clinical isolates was higher than that in the strains identified in healthy individuals. This observation was consistent with the higher antimicrobial resistance rate in clinical isolates. This increased abundance of antimicrobial resistance genes in *A. caviae* was also consistent with their resistance phenotype, which may explain the increased prevalence of *A. caviae* isolates in clinical samples. However, this finding should be evaluated

in future studies.

CONCLUSIONS

We analyzed 57 *Aeromonas* strains isolated from patients and healthy individuals in Ma'anshan City, Anhui Province, China. These samples exhibited a high degree of genetic diversity with the 57 isolates that produced 55 independent STs. The constructed phylogenetic tree with concatenated *gyrB-cpn60* sequences divided the 57 isolates into 7 species. Beta-hemolysis, proteolytic activity, and virulence gene analyses revealed that these properties were more common in the clinical isolates than in the isolates from the healthy individuals. Furthermore, *A. dhakensis* harbored the highest number of virulence genes. Our results suggested that 26.3% of the strains were MDR (≥ 3) positive, and the MDR status was significantly more common in the clinical isolates than in the isolates from the healthy individuals. *PMQR*, *tetA*, *tetE*, *ESBL*, aminoglycoside resistance, sulfanilamide, and *mcr-3* genes were detected in some of the isolates. Their prevalence increased in the clinical isolates. Therefore, the genetic diversity, antimicrobial resistance, and pathogenicity of *Aeromonas* strains isolated from clinical patients significantly differed from those of the isolates from healthy individuals.

CONFLICT OF INTEREST

The authors have declared that no competing interests exist.

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Supplementary Table S1. Antimicrobial resistance of *Aeromonas* isolates from clinical patient and healthy people*

Drug	No and % of resistant isolates													
	<i>A. veronii</i>		<i>A. jandaei</i>		<i>A. dhakensis</i>		<i>A. enteropelogenes</i>		<i>A. media</i>		<i>A. hydrophila</i>		<i>A. caviae</i>	
	Clinical (n = 10)	Healthy (n = 9)	Clinical (n = 1)	Healthy (n = 5)	Clinical (n = 5)	Healthy (n = 1)	Clinical (n = 1)	Healthy (n = 0)	Clinical (n = 2)	Healthy (n = 0)	Clinical (n = 1)	Healthy (n = 1)	Clinical (n = 17)	Healthy (n = 3)
IPM	0 (0.0)	1 (11.1)	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CHL	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)		1 (100)	0 (0.0)	4 (23.5)	0 (0.0)
FEP	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)		1 (100)	0 (0.0)	3 (17.6)	0 (0.0)
CRO	1 (10)	1 (11.1)	0 (0.0)	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)		1 (50)		1 (100)	0 (0.0)	8 (47.1)	0 (0.0)
CAZ	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	2 (40)	0 (0.0)	0 (0.0)		1 (50)		1 (100)	0 (0.0)	6 (35.3)	0 (0.0)
CT	3 (30)	1 (11.1)	1 (100)	4 (80)	5 (100)	1 (100)	0 (0.0)		0 (0.0)		1 (100)	1 (100)	0 (0.0)	0 (0.0)
GEN	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20)	0 (0.0)	0 (0.0)		0 (0.0)		1 (100)	0 (0.0)	2 (11.8)	1 (33.3)
TET	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)		0 (0.0)	0 (0.0)	5 (29.4)	0 (0.0)
CIP	1 (10)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)		1 (100)	0 (0.0)	5 (29.4)	0
SXT	0 (0.0)	1 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		0 (0.0)		1 (100)	0 (0.0)	9 (52.9)	1 (33.3)
AMC	10 (100)	9 (100)	1 (100)	0 (0.0)	5 (100)	0 (0.0)	1 (100)		2 (100)		1 (100)	0 (0.0)	15 (88.2)	0 (0.0)
ATM	1 (10)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)		2 (100)		0 (0.0)	0 (0.0)	3 (17.6)	1 (33.3)

Note. * IPM, imipenem; CHL, chloramphenicol; FEP, cefepime; CRO, ceftriaxone; CAZ, ceftazidime; CT, colistin; GEN, gentamycin; TET, tetracycline; CIP, ciprofloxacin; SXT, trimethoprim/sulfamethoxazole; AMC, amoxicillin/clavulanate; ATM, aztreonam.

Supplementary Table S2. Prevalence of antimicrobial resistance genes of *Aeromonas* isolates from clinical patient and healthy people

Gene	No and % of resistant genes													
	<i>A. veronii</i>		<i>A. jandaei</i>		<i>A. dhakensis</i>		<i>A. enteropelogenes</i>		<i>A. media</i>		<i>A. hydrophila</i>		<i>A. caviae</i>	
	Clinical (n = 10)	Healthy (n = 9)	Clinical (n = 1)	Healthy (n = 5)	Clinical (n = 5)	Healthy (n = 1)	Clinical (n = 1)	Healthy (n = 0)	Clinical (n = 2)	Healthy (n = 0)	Clinical (n = 1)	Healthy (n = 1)	Clinical (n = 17)	Healthy (n = 3)
<i>tetA</i>	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)	3 (17.6)	0 (0)
<i>tetE</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		1 (50)		0 (0)	0 (0)	0 (0)	0 (0)
<i>qnrS</i>	1	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)	5 (29.4)	0 (0)
<i>aac-6</i>	0 (0)	1 (11.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		1 (100)	0 (0)	1 (5.9)	0 (0)
<i>bla-TEM</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)	1 (5.9)	0 (0)
<i>bla-CTX</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)	2 (11.8)	0 (0)
<i>armA</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		1 (100)	0 (0)	0 (0)	0 (0)
<i>sul1</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		1 (100)	0 (0)	6 (35.3)	0 (0)
<i>sul2</i>	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)	1 (5.9)	0 (0)
<i>mcr-3</i>	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)		0 (0)		0 (0)	0 (0)	0 (0)	0 (0)