Infection and Drug Resistance

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ORIGINAL RESEARCH

Requiring Reconsideration of Differences of *Aeromonas* Infections Between Extra-Intestinal and Intestinal in Hospitalized Patients

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Purpose: The purpose of this study is to examine the variations between extra-intestinal and intestinal infections of *Aeromonas* in terms of strain types, risk factors, drug susceptibility results, and the distribution of drug resistance and virulence genes.

Patients and Methods: A total of 188 *Aeromonas* strains were identified to the species level using housekeeping genes (*rpoD, gyrB*, and *gyrA*). The risk factors for *Aeromonas* extra-intestinal and intestinal infection, as well as mortality, were retrospectively examined in this study. The broth microdilution method was used to investigate the antimicrobial susceptibility profiles. Touchdown polymerase chain reaction (PCR) assays and DNA sequencing were employed to confirm virulence and the presence of drug resistance genes.

Results: The housekeeping genes identified 188 strains into 7 species. Extra-intestinal isolates generally contained *A. caviae* and *A. hydrophila*, while intestinal were *A. veronii* (p=0.0001). Extra-intestinal infections (158/188) were the main type and accounted for 24/27 of all fatalities. Malignant tumors, hepatobiliary diseases, anemia, and hypoproteinemia were linked to infections. Poor results were associated with septic shock. Using the broth microdilution method, over 80% isolates were susceptible to most antimicrobials, except for ceftazidime (79.8%) and ceftriaxone (69.7%). Except for imipenem, intestinal strains were more susceptible to other medications than extra-intestinal. Using touch-down polymerase chain reaction testing and DNA sequencing, 6 strains, 31 strains, and a strain only had bla_{TEM}, bla_{CphA}, and bla_{VIM}, respectively. Two *Aeromonas hydrophila* each possessed bla_{CphA}+ $bla_{CTXM-M-9}$, and bla_{CphA} + $bla_{CTX-M-1}$ + $bla_{CTX-M-15-like}$ + bla_{TEM}; two *Aeromonas caviae* each possessed bla_{NDM} + $bla_{CTX-M-1}$ +$bla_{CTX-M-15-like}$ + bla_{TEM}, and bla_{NDM} + bla_{TEM}. Thirty-four of the 42 strains mentioned above were isolated from extra-intestinal. *Act, aexT*, and *ascF-G*, were in intestinal more frequently, but *alt, hlyA, ela*, and *lip* were in extra-intestinal more frequently.

Conclusion: *Aeromonas* inside and outside intestinal differed in their clinical characteristics, drug susceptibility, drug resistance and virulence genes.

Keywords: intestinal and extra-intestinal infections, *Aeromonas*, risk factor, virulence

Introduction

Aeromonas is a gram-negative, facultative anaerobic oxidase positive bacillus that ranges in size from 0.3 to 1.0 μm.[1] Intestinal infection is the most common type of infectious disease caused by *Aeromonas*,[2] but it can also result in infections of wound, liver and gallbladder, necrotizing fasciitis, sepsis, and other infectious diseases.[3] However, it is important to be aware that there have been more instances of extra-intestinal infections in recent years.[4] As an opportunistic pathogen, *Aeromonas* is more likely to infect and kill patients with low immunity, including those with malignant tumors, hematological tumors, cirrhosis, diabetes, and other diseases.[5–7] In 1986, *Aeromonas* separated from the vibrio family,[8] which it had previously been a part of it. Since then, the group of bacteria known as *Aeromonas* has expanded. However, our capacity to identify them has been constrained by the use of common clinical microbe identification methods, including the VITEK2 Compact system, the VITEK MALDI-TOF system, and even 16S

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rRNA.[2,9,10] For precise identification, housekeeping genes like *rpoD* and *gyrB* can be sequenced.[11,12] It is also possible to use multilocus phylogenetic analysis (MLPA).[3] Drug resistance in *Aeromonas* has been steadily rising in recent years as a result of the expanding use of antibiotics in both aquaculture and human healthcare.[13]

Exotoxin, extracellular protease, adhesion factor, and secretion system are the key components of *Aeromonas*' virulence factors.[14] This makes the harmful elements of this organism complicated and varied. *Aeromonas* is able to thwart the host's immune system and spread infection thanks to a range of virulence factors.[15]

In this work, the characteristics of *Aeromonas* infection and mortality were examined. Comparing the virulence gene and drug susceptibility of intestinal isolates versus extra-intestinal isolates. A preliminary assessment of *Aeromonas*' drug resistance mechanism was made.

Materials and Methods

Material

In the clinical microbiology database of the First Affiliated Hospital of Chongqing Medical University, a 3200-bed facility in southwest China, 188 strains of *Aeromonas* were gathered between January 2013 and September 2020. A total of 158 strains were obtained from patients with parenteral infection (39 strains in skin wound secretion, 28 strains in bile, 25 strains in pleural and peritoneal fluid, 24 strains in blood, 22 strains in urine, and 20 strains in others), while 30 strains were identified from patients with intestinal infection. The VITEK MALDI-TOF technology (bioMerieux, Marcy I 'Etoile, France) was utilized by the microbiology lab to identify microorganisms. And 188 *Aeromonas* strains were kept at 80 °C in 15% glycerol.

Identification of Bacterial Strains

Housekeeping genes (*rpoD, gyrB*, and *gyrA*) sequencing were used to confirm the identity of all isolates. Primers were taken from prior publications.[16–18] The primers used for amplification and sequencing of the housekeeping genes are listed in Table 1.

Table 1 The Primers of Target Genes and PCR Conditions

Gene	Upstream Primer Sequence (5' to 3')	Downstream Primer Sequence (5' to 3')	Annealing Temperature (°C)	Size (bp)	Reference
rpoD	GCAGTGAAAGARTTCTTTGGTT	GTTGCATGTTNGNACCCAT	55	760	[16]
gyrB	TCCGGCGGTCTGCACGGCGT	TTGTCCGGGTTGTACTCGTC	55	1100	[17]
gyrA	ATGAGCGATCTGGCCAGAGA	CGCGCCTFGTTCACCTGATA	55	815	[18]
bla_{CphA}	CCTTGATCAGCGCTTCGTAGTG	GCGGGGATGTCGCTGACGCAG	55	670	[24]
bla_{KPC}	CATTCAAGGGCTTTCTTGCTGC	ACGACGGCATAGTCATTTGC	55	488	[24]
bla_{IMP}	CATGGTTTGGTTGTTCTTGT	ATAATTTAGCGGACTTTGGC	55	488	[24]
bla_{VIM}	TTATGGAGCAGCAACGATGT	CAAAAGTCCCGCTCCAACGA	52	920	[24]
bla_{NDM}	CGGAATGGCTCATCACGATC	GGTTTGGCGATCTGGTTTTC	55	621	[24]
$bla_{OXA-48-like}$	TTGGTGGCATCGATTATCGG	GAGCACTTCTTTTGTGATGGC	55	438	[24]
$bla_{CTX-M-1}$	AAGACTGGGTGTGGCATTGA	AGGCTGGGTGAAGTAAGTGA	60	700	[24]
$bla_{CTX-M-9}$	GCTTTATGCGCAGACGAGTG	GCCAGATCACCGCAATATCA	55	670	[24]
$bla_{CTX-M-15-like}$	TTAGGAAGTGTGCCGCTGCA	CGATATCGTTGGTGGTRCCAT	55	686	[24]
bla_{SHV}	CTTTACTCGCCTTTATCGGC	TTACCGACCGGCATCTTTCC	60	1031	[24]
bla_{TEM}	GTGCGCGGAACCCCTATT	TTACCAATGCTTAATCAGTGAGGC	60	919	[24]
bla_{ACC}	CACCTCCAGCGACTTGTTAC	GTTAGCCAGCATCACGATCC	60	400	[24]
bla_{FOX}	CTACAGTGCGGGTGGTTT	CTATTTGCGGCCAGGTGA	60	200	[24]
act	GAGAAGGTGACCACCAAGAACA	AACTGACATCGGCCTTGAACTC	55	232	[25]
ast	ATGCACGCACGTACCGCCAT	ATCCGGTCGTCGCTCTTGGT	66	260	[26]
alt	CCATCCCCAGCCTTTACGCCAT	TTTCACCGAGGTGACGCCGT	63	338	[26]

(*Continued*)

Table 1 (Continued).

Gene	Upstream Primer Sequence (5' to 3')	Downstream Primer Sequence (5' to 3')	Annealing Temperature (°C)	Size (bp)	Reference
aerA	CCTATGGCCTGAGCGAGAAG	CCAGTTCCAGTCCCACCACT	68	431	[25]
hlyA	GCCGGTGGCCCGAAGATACGGG	GGCGGCGCCGGACGAGACGGG	62	597	[25]
ela	ACACGGTCAAGGAGATCAAC	CGCTGGTGTTGGCCAGCAGG	55	513	[25]
gcaT	CTCCTGGAATCCCAAGTATCAG	GGCAGGTTGAACAGCAGTATCT	55	237	[27]
lip	ATCTTCTCCGACTGGTTCGG	CCGTGCCAGGACTGGGTCTT	63	382	[25]
lafA	GGTCTGCGCATCCAACTC	GCTCCAGACGGTTGATG	60	550	[25]
fla	TCCAACCGTYTGACCTC	GMYTGGTTGCGRATGGT	55	608	[25]
aexT	GGCGCTTGGGCTCTACAC	GAGCCCGCGCATCTTCAG	55	535	[27]
ascF-G	ATGAGGTCATCTGCTCGCGC	GGAGCACAACCATGGCTGAT	55	789	[27]

Data Collection and Definition

In this study, a retrospective analysis was conducted. Only the first episode was considered in patients with more than one positive *Aeromonas* culture. The electronic medical records mainly covered the following contents: characteristics of infection, diagnosis and treatment, auxiliary examination, and prognosis. Sepsis with persistent hypotension needing vasopressors to keep MAP 65 mm Hg and having a blood lactate level >2 mmol/L (18 mg/dL) despite sufficient volume resuscitation are clinical indicators of patients with septic shock.[19] Hemoglobin below 13 g/dL in adult males and 12 g/dL in adult females was considered anemia.[20] Serum albumin components were below 35 g, indicating hypoproteinemia.[21] Serum potassium level <3.5 mmol/L was hypokalemia. Systemic steroid use was defined as oral or intravenous administration of at least 20 mg/day of a steroid (prednisone, hydrocortisone, methylprednisolone, or dexamethasone) within 1 month of infection.[22] The result was death. The inappropriate antimicrobial therapy used improper drugs that are not susceptible to pathogens.

Drug Sensitivity Test

The microbroth dilution method was used to determine the antimicrobial susceptibility pattern of all isolates to some commonly used antimicrobial agents, including ciprofloxacin, levofloxacin, cefuroxime, ceftazidime, ceftriaxone, cefepime, aztreonam, imipenem, and meropenem. The breakpoints were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[23] *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls for antibiotic susceptibility tests.

Polymerase Chain Reaction (PCR) Amplification of Drug Resistance and Virulence Genes

Touchdown PCR assays and sequencing were used to validate the presence of the carbapenemase genes (bla_{CphA}, bla_{KPC}, bla_{IMP}, bla_{VIM}, bla_{NDM}, and $bla_{\text{OXA-48-like}}$), extended-spectrum beta-lactamase (ESBL) genes ($bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CTX-M-15-like}}$, bla_{SHV}, and bla_{TEM}), and Ampc (bla_{ACC} and bla_{FOX}).[24] A total of 12 virulence factor-encoding genes were also found using PCR, including *act, ast, alt, aerA, hlyA, ela, gcaT, lip, lafA, fla, aexT*, and *ascF-G*.[25–27] The amplification primer sequences are listed in Table 1.

Statistical Analysis

The data were analyzed using SPSS statistical software (version 25.0, IBM). Pearson's chi-square test or Fisher's exact test was used to analyze the categorical variables. Student's *t*-test or Mann–Whitney *U*-test was utilized for continuous variables. To assess independent risk factors for *Aeromonas* mortality, univariate analysis was used to evaluate the putative variables, and multivariate logistic regression analysis was carried out for statistically significant variables in univariate analysis. Odds ratios (OR) were calculated with 95% confidence interval (CI). $P<0.05$ was deemed to statistically significant. The positive PCR amplicons were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

(Shanghai, China). Using Seqman (DNAStar), the sequences were assembled, and the data were compared in the NCBI (http://www.ncbi.nlm.nih.gov) using BLAST.

Results

Identification of Bacteria

A total of 188 *Aeromonas* isolates were divided into 7 species based on the results of housekeeping genes. *Aeromonas caviae* (28.7%), *Aeromonas hydrophila* (26.1%), *Aeromonas veronii* (25%), and *Aeromonas dhakensis* (18.1%) were the four species of *Aeromonas* that were most common. Additionally, 2 isolates of *Aeromonas jandaei*, 1 isolate each of *Aeromonas sobria* and *Aeromonas media* were identified. Figure 1 depicts the spread of *Aeromonas* isolated from infections of the extra-intestinal and intestinal. Overall, intestinal isolates typically contained *A. veronii* (p=0.0001); and extra-intestinal isolates were mainly contained *A. caviae* and *A. hydrophila* (p=0.0001).

Characteristics of Infected Patients

A total of 188 patients with *Aeromonas* infection were found between 2013 and 2020. The patients were on average 53.9 years old, with a male-to-female ratio of 1.5 (114/74). Malignant neoplasms (solid tumors and hematologic malignancies), hepatobiliary diseases (posthepatitic cirrhosis and cholelithiasis), anemia, and hypoproteinemia were the most prevalent underlying illnesses among infected people (Table 2). Different comorbidities were seen in patients with infections both inside and outside the intestinal. Patients with age >80, hematological malignancies, and gastrointestinal diseases were more likely to have an intestine infection with *Aeromonas*.

Twenty-seven individuals (14.4%) died from *Aeromonas* infection, 24 of whom had extra-intestinal infection. Hematological malignancies (P=0.006), cholelithiasis (P=0.028), septic shock (P=0.002), and surgical history within 6 months (P=0.011), were all risk factors for the nosocomial death of *Aeromonas*, according to a univariate analysis. Septic shock was the only independent risk factor for *Aeromonas* death (P=0.020), according to the multivariate logistic regression model's results, which are displayed in Table 3. A connection between intestinal infection and death was not discovered. Most patients with intestinal infections had hematological malignancies, cholelithiasis, septic shock, chemoradiotherapy, systemic steroid use, and surgical history within 6 months (Table 4).

Drug Sensitivity

According to Table 5, which summarizes the results of the antimicrobial susceptibility tests, more than 83% of the bacterial strains were susceptible to ciprofloxacin (83.5%), levofloxacin (89.4%), cefepime (87.2%), aztreonam (86.2%), imipenem (87.2%), and meropenem (93.6%). Ceftazidime and ceftriaxone each had a 79.8% and a 69.7% sensitivity rate for *Aeromonas*, respectively.

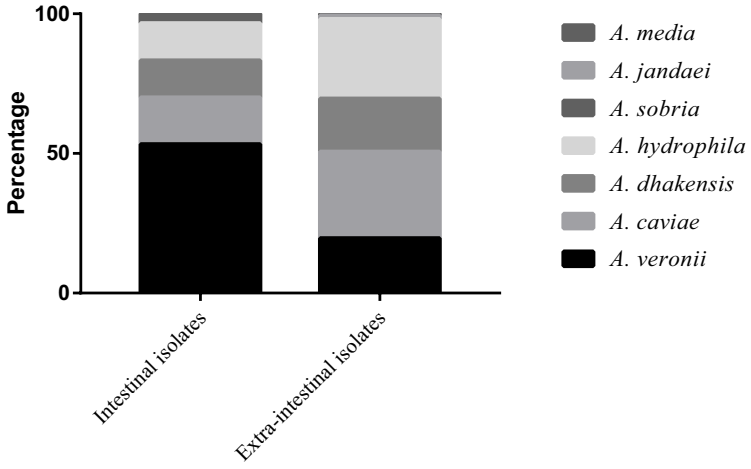

Figure 1 Distribution of various *Aeromonas* that have been isolated in and outside of the intestinal.

Table 2 Clinical Characteristics of Patients with *Aeromonas* Infection

Clinical Characteristics	Total (n=188)	Intestinal Isolates (n=30)	Extra-Intestinal Isolates (n=158)	P value
Male	114	20	94	0.461
Female	74	10	64	0.461
Age				
<30	21	6	15	0.174
30–49	55	7	48	0.437
50–79	103	12	91	0.076
>80	9	5	4	**0.004**
Hypertension	35	8	27	0.217
Coronary artery diseases	6	2	4	0.539
Diabetes	27	5	22	0.913
Solid malignancy	33	5	28	0.889
Hematologic malignancy	18	7	11	**0.014**
Posthepatitic cirrhosis	6	2	4	0.539
Cholelithiasis	40	6	34	0.852
Peripheral vascular diseases	12	4	8	0.197
Gastrointestinal diseases	27	10	17	**0.003**
Renal diseases	9	3	6	0.321
Chronic obstructive pulmonary diseases	2	1	1	0.294
Anemia	53	8	45	0.840
Hypoproteinemia	98	15	83	0.799
Hypokalemia	8	2	6	0.826
Chemoradiotherapy	13	5	8	0.057

Note: Bold face indicate values that are significant (P < 0.05).

Table 3 Analysis of Risk Factors Associated with Hospital Death Caused by *Aeromonas* Infection

Variable	Death (n=27)	Survival (n=161)	Univariable		Multivariable	
			OR (95% CI)	P value	OR (95% CI)	P value
Chronic obstructive pulmonary diseases	1	5	1.200(0.135–10.688)	1.000		
Hypertension	5	30	0.992(0.348–2.833)	0.989		
Coronary artery diseases	0	5	0.969(0.943–0.996)	0.456		
Anemia	10	43	1.614(0.686–3.798)	0.270		
Hematologic malignancy	7	11	4.773(1.660–13.724)	**0.006**	2.086(0.550–7.919)	0.280
Solid malignancy	7	27	1.737(0.669–4.513)	0.382		
Diabetes	3	24	0.714(0.199–2.557)	0.823		
Cholelithiasis	1	35	0.138(0.018–1.056)	**0.028**	0.271(0.026–2.777)	0.272
Hepatitis	1	12	0.478(0.060–3.831)	0.764		
Hepatic cirrhosis	0	5	0.969(0.943–0.996)	0.456		
Hepatic carcinoma	1	6	0.994(0.115–8.593)	1.000		
Gastrointestinal diseases	3	27	0.620(0.174–2.208)	0.646		
Renal diseases	2	9	1.351(0.276–6.622)	1.000		
Hypokalemia	12	47	1.940(0.845–4.457)	0.114		
Septic shock	7	9	5.911(1.983–17.620)	**0.002**	5.088(1.290–20.070)	**0.020**
Surgery in the past 6 months	8	90	0.332(0.137–0.803)	**0.011**	0.351(0.054–2.263)	0.271
ICU admission	4	19	1.300(0.406–4.166)	0.901		
Drainage tube	8	56	0.789(0.325–1.918)	0.601		
Nasogastric tube insertion	3	15	1.217(0.327–4.521)	1.000		
Central venous catheterization	4	9	2.937(0.836–10.322)	0.181		
Urinary catheter	11	50	1.526(0.661–3.525)	0.320		
Mechanical ventilation	4	13	1.980(0.594–6.598)	0.443		

(*Continued*)

Table 3 (Continued).

Variable	Death (n=27)	Survival (n=161)	Univariable		Multivariable	
			OR (95% CI)	P value	OR (95% CI)	P value
Chemoradiotherapy	4	9	2.937(0.836–10.322)	0.181		
Systemic steroid use	6	15	2.781(0.972–7.959)	0.101		
The inappropriate antimicrobial therapy	2	7	1.760 (0.346–8.959)	0.840		

Note: Bold face indicate values that are significant (P < 0.05).
Abbreviations: OR, odds ratio; ICU, intensive care unit.

With the exception of imipenem, intestinal infection strains were more susceptible to other medications than extra-intestinal infection strains, and ceftazidime, ceftriaxone, and ciprofloxacin all demonstrated statistically significant sensitivity. However, compared to intestinal strains, extra-intestinal strains had a considerably higher susceptibility to imipenem (Table 5).

Drug Resistance and Virulence Genes

By using PCR, it was discovered that 42 *Aeromonas* strains solely carried the ESBLs and carbapenemase genes: 6 strains only carried bla_{TEM}; 31 strains only carried bla_{CphA}, including *A. dhakensis, A. veronii, A. hydrophila* and *A. jandaei*; and one *A. caviae* with bla_{VIM}. The remaining four *Aeromonas* strains possessed the following combinations of drug

Table 4 Analysis of Risk Factors for Death from Intestinal and Extra-Intestinal *Aeromonas* Infection

Variable	Intestinal Infection			Extra-Intestinal Infection		
	Death (n=3)	Survival (n=27)	P value	Death (n=24)	Survival (n=134)	P value
Chronic obstructive pulmonary diseases	1	0	1.000	0	5	1.000
Hypertension	1	7	1.000	4	23	1.000
Coronary artery diseases	0	2	1.000	0	3	0.608
Anemia	2	6	1.660	8	37	0.567
Hematologic malignancy	0	7	1.000	7	4	**0.000**
Solid malignancy	2	3	0.064	5	24	0.776
Diabetes	0	5	1.000	3	19	1.000
Cholelithiasis	0	2	1.000	1	33	**0.025**
Hepatitis	0	5	1.000	1	7	1.000
Hepatic cirrhosis	0	2	1.000	0	3	1.000
Hepatic carcinoma	1	1	1.000	0	5	1.000
Renal diseases	1	4	1.000	1	5	1.000
Hypokalemia	1	11	1.000	11	36	0.061
Septic shock	0	1	1.000	7	8	**0.001**
Surgery in the past 6 months	1	5	0.501	7	85	**0.002**
Drainage tube	0	1	1.000	8	55	0.477
Nasogastric tube insertion	0	1	1.000	3	14	1.000
Central venous catheterization	0	0	-	4	9	0.219
Urinary catheter	0	1	1.000	11	49	0.389
Mechanical ventilation	0	0	-	4	13	0.512
Chemoradiotherapy	0	5	0.194	4	4	**0.021**
Systemic steroid use	0	10	0.132	6	5	**0.001**

Note: Bold face indicate values that are significant (P < 0.05).

Table 5 Antimicrobial Susceptibilities of *Aeromonas* Isolates Determined by the Broth Microdilution Method and *Aeromonas* Isolates from Inside and Outside the Intestinal Were Compared for Drug Sensitivity

Antimicrobial Agents	Breakpoint (μg/mL)		MIC (μg/mL)			Susceptibility (%)			Susceptible (%)		
	Susceptible	Resistant	Range	MIC 50%	MIC 90%	Susceptible	Intermediate	Resistant	Intestinal Isolates (n=30)	Extra-Intestinal Isolates (n=158)	P value
Ceftazidime	≤4	≥16	0.25–256	0.25	16	79.8	2.1	18.1	93.3	77.2	**0.044**
Ceftriaxone	≤1	≥4	0.25–256	0.25	4	69.7	0.5	29.8	93.3	65.2	**0.002**
Cefepime	≤2	≥16	0.25–256	0.25	16	87.2	2.2	10.6	96.7	85.4	0.164
Aztreonam	≤4	≥16	0.25–256	0.25	16	86.2	1.6	12.2	93.3	84.8	0.341
Imipenem	≤1	≥4	0.25–256	0.25	4	87.2	0.6	12.2	76.7	96.8	**0.000**
Meropenem	≤1	≥4	0.25–256	0.25	0.25	93.6	1.6	4.8	90	86.7	0.844
Levofloxacin	≤2	≥8	0.25–16	0.25	4	89.4	6.3	4.3	93.3	88.6	0.655
Ciprofloxacin	≤1	≥4	0.25–16	0.25	4	83.5	2.6	14.9	96.7	79.8	**0.026**

Note: Bold face indicate values that are significant ($P < 0.05$).
Abbreviation: MIC, minimum inhibitory concentration.

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Table 6 Intestinal and Extra-Intestinal Infections Caused by *Aeromonas* Were Compared in Terms of Their Virulence Genes

Virulence Genes	Intestinal (n=30)	Extra-Intestinal (n=158)	P value
act	63.3% (19/30)	34.8% (55/158)	**0.003**
ast	30.% (9/30)	24.7% (39/158)	0.540
alt	43.3% (13/30)	82.3% (130/158)	**0.000**
aerA	73.3% (22/30)	54.4% (86/158)	0.055
hlyA	30.% (9/30)	51.9% (82/158)	**0.028**
ela	50.% (15/30)	69% (109/158)	**0.044**
gcaT	83.3% (25/30)	89.2% (141/158)	0.540
lip	53.3% (16/30)	74.7% (118/158)	**0.018**
lafA	13.3% (4/30)	23.4% (37/158)	0.220
fla	83.3% (25/30)	70.3% (111/158)	0.142
aexT	36.7% (11/30)	17.1% (27/158)	**0.014**
ascF-G	33.3% (10/30)	15.8% (25/158)	**0.024**

Note: Bold face indicate values that are significant ($P < 0.05$).

resistance genes: two *A. hydrophila* each had bla_{CphA} + $bla_{\text{CTXM-M-9}}$, and bla_{CphA} + $bla_{\text{CTX-M-1}}$ + $bla_{\text{CTX-M-15-like}}$ + bla_{TEM}; two *A. caviae* each carried bla_{NDM} + $bla_{\text{CTX-M-1}}$ + $bla_{\text{CTX-M-15-like}}$ + bla_{TEM}, and bla_{NDM} + bla_{TEM}. All of them were generated from extra-intestinal isolates, except for 7 strains with bla_{CphA}, and one with bla_{VIM}. 21 strains (63.64%) of the 33 strains with a positive *CphA* gene were discovered to be incompatible with the drug sensitivity of carbapenems. The ratios of *act, ast, alt, aerA, hlyA, ela, lip*, and *gcaT* were found to be 39.4%, 25.5%, 76.1%, 58%, 48.4%, 66%, 71.3%, and 88.3% respectively. *Fla, lafA, aexT*, and *ascF-G* were found in ratios of 72.3%, 21.8%, 20.2%, and 18.6%. The virulence genes *act, aexT*, and *ascF-G* were found in intestinal infection more frequently than extra-intestinal. Additionally, the detection rate of the virulence genes *alt, hlyA, ela*, and lip from extra-intestinal infection was considerably higher than that from intestinal infection (Table 6).

Discussion

Aeromonas are easy to isolate; however, because of its heterogeneous phenotypic characteristics, species identification is difficult. In contrast to the use of the 16S rRNA gene and the VITEK MALDI-TOF method, nucleotide sequencing of housekeeping genes, such as *rpoD* and *gyrB*,[6,28] or multilocus phylogenetic analysis (MLPA),[3] can enable a more precise identification of the species.

Despite the fact that *Aeromonas* infections are primarily intestinal infections,[29] there have been an increasing number of reports in recent years of extra-intestinal infections with *Aeromonas*.[30,31] While Qu et al[32] found that *Aeromonas* extra-intestinal infection primarily came from blood, Fu et al[4] reported that skin and soft tissue infection was the primary cause of *Aeromonas* extra-intestinal infection. In this study, extra-intestinal infection was the primary type of *Aeromonas* infection, while skin wound infection was the primary extra-intestinal infection type. There were statistically different *Aeromonas* species isolated from different infection sites. *A. veronii* was the most prevalent species of intestinal infection in the paper, despite earlier research[32] suggesting that *A. dhakensis* was most frequently found in patients with intestinal infection. This study and that of Chen et al[33] are identical. In both cases, the extra-intestinal infections were mainly caused by *A. caviae and A. hydrophila.*

Similar to the findings of earlier studies,[2,6] the work revealed that men made up the majority of those infected with *Aeromonas*, and it was noted that people in their middle and later years made up the majority of those infected, which may be related to their pre-existing illnesses and lowered immune systems.[7] According to this study, the majority of patients with *Aeromonas* infection also had underlying illnesses like malignancies (solid tumors, hematological malignancies), hepatobiliary diseases (posthepatitic cirrhosis, cholelithiasis), anemia and hypoproteinemia. Su et al[6] reported that patients with liver cirrhosis have significant morbidity and mortality from *Aeromonas* infection, which may be

caused by abnormal performance of the mononuclear macrophage system in patients with liver cirrhosis, and a decrease in the body's immune defense mechanism.

In contrast to the 14.9% to 63% described in the literature,[5,34] the mortality rate with an *Aeromonas* infection (14.4%) was relatively low. According to Ji et al,[5] the use of appropriate antimicrobial medicines, metastatic cancer and shock were linked to *Aeromonas* mortality (P<0.05). In the study, the following conditions increased the probability of death in patients with *Aeromonas* infection: hematological malignancy, cholelithiasis, septic shock, recent surgery, and transfusion. Immunosuppressive medications and intensive chemotherapy accelerate the immune system's degeneration in hematological malignancy patients, making them more vulnerable to infection.[35] In a clinical study by Xu et al,[35] 42 people with leukemia complicated by an *Aeromonas* infection took part. Most *Aeromonas* infections were found to occur in leukemia patients who were experiencing granulocyte shortage, and granulocyte insufficiency lasting longer than 7 days was associated with patient mortality.

The 188 strains had higher susceptibility rates to ciprofloxacin, levofloxacin, cefepime, amtraenem, imipenem and meropenem, both of which were over 83%, similar to those reported in other regions.[6,29] And then, there are differences in the drug resistance rates of *Aeromonas* in different infection sites, and the drug resistance rate of strains with extra-intestinal infection is significantly higher than that of strains with intestinal infection, which is in line with the study results of Zhou et al.[29] However, intestinal infection strains were much less susceptible to imipenem than extra-intestinal strains, with a susceptibility rate of only 76.7%. Imipenem susceptibility ranged from 89.8% to 95% in earlier reports[29,36] using intestinal isolates. In order to do future research, we must increase the sample size.

The three primary lactamases found in *Aeromonas* are Ambler C cephalosporin enzymes, D penicillinases, and B metallic lactamases (MBLs).[2] The metalloenzyme *CphA* gene mainly exists in *A. hydrophila, A. veronii*, and *A. jandaei*, but not in *A. caviae*.[37] This experiment's discovery of the *CphA* gene in *A. dhakensis, A. veronii, A. hydrophila*, and *A. jandaei* supported this assertion. In addition, imipenem and meropenem sensitivity was discovered in 22 of 33 *CphA*-positive *Aeromonas* strains. According to earlier research, the genotype and drug sensitivity phenotype of *CphA* were discordant because gene mutation altered the gene expression of the protein.[38] The research team will continue to look into what specific mechanism triggered this outcome. Two *Aeromonas* strains harboring the *CphA* gene and ESBLs were identified in this study, and the findings of the drug sensitivity tests were in line with the genotype expression. We need to pay more attention to the combined drug resistance of the *CphA* gene and the *Aeromonas* ESBLs, which has not yet been extensively explored. Additionally, two strains with both NDM and ESBLs were found, and NDM frequently co-existed with other drug resistance genes, which were transferred horizontally through plasmids and integrons in strains,[39] leading to its spread in different strains, which presented serious challenges to the prudent use of antibiotics.

The diversity and complexity of *Aeromonas*'s harmful nature were caused by a wide range of virulence factors. When relevant virulence factors were screened, it was discovered that 188 *Aeromonas* strains had detection rates of *alt, aerA, ela, lip, gcaT* and *fla* that were higher than 58%, comparable to those documented in the literature.[40] Extracellular proteases have the ability to break down different types of proteins, supply amino acids to bacteria, and directly cause significant tissue damage. This is a key pathogenic mechanism used by infected bacteria to get past the host's defenses and propagate throughout the body.[2,41] In the study, it has been discovered that the extracellular protease detection rate of strains isolated from the intestinal was higher than that from the extra-intestinal, indicating a difference in pathogenicity between strains infected from the intestinal and those infected from the extra-intestinal.

Conclusion

In a word, *Aeromonas* in this region was mainly infected by extra-intestinal. Compared with intestinal strains, it was found that there were differences in species distribution, drug susceptibility, drug resistance and virulence gene distribution between the two types of infection.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. The collection of culture isolates and collation of anonymous clinical data was in accordance with the approved clinical practice guidelines. Approval was

granted by the Institutional Review Board and Ethics Committee of Chongqing Medical University approved this study (approval number: KY2021-557).

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Disclosure

The authors report no conflicts of interest in this work.

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