Taxonomy, virulence determinants and antimicrobial susceptibility of *Aeromonas* spp. isolated from bacteremia in southeastern China

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Research

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Abstract

Background: The study was aimed to elucidate the species taxonomy, clinical manifestations, virulence gene profiles and antimicrobial susceptibilities of *Aeromonas* strains isolated from life-threatening bacteremia in southeastern China.

Methods: Clinical samples of *Aeromonas* causing bacteremia were isolated from a teaching hospital in Wenzhou during 2013 to 2018 and retrospective cohort study was performed. *Aeromonas* strains were identified to species level by housekeeping gene *gyrB*. Six virulence-associated genes (*aer*, *lip*, *hlyA*, *alt*, *ast*, and *act*) were screened by polymerase chain reaction (PCR) and antibiotics susceptibility testing (AST) was performed by VITEK 2 Compact system.

Results: A total of 58 patients with bacteremia caused by *Aeromonas* species were collected during 6 years (2013-2018). 58 isolates were identified to five different species, where *Aeromonas dhakensis* appeared to be predominant (26/58), followed by *Aeromonas veronii* (13/58), *Aeromonas caviae* (10/58), *Aeromonas hydrophila* (7/58) and *Aeromonas jandaei* (2/58). 16 of 58 patients had poor prognosis. Poor prognosis was significantly associated with community-acquired infections and liver cirrhosis. The progression of bacteremia caused by *Aeromonas* was extremely fast, especially in *A. dhakensis* infections. Virulence genes *aer*, *lip*, *hlyA*, *alt*, *ast*, and *act*, were detected at ratios of 24.1% (14/58), 62.1% (36/58), 65.5% (38/58), 58.6% (34/58), 15.5% (9/58) and 65.5% (38/58), respectively. Antimicrobials susceptibility exhibited that 9 out of 58 isolates were identified as multi-drug resistant (MDR) organism. The majority of *Aeromonas* strains maintained susceptible to 3rd generation cephalosporins, aminoglycosides, fluoroquinolones and furantoin.

Conclusions: The prevalence and dangerousness of *Aeromonas* infections, especially *A. dhakensis*, are underestimated in clinic. Continuous monitoring is essential to keep track of MDR *Aeromonas* due to the increasing prevalence recently and more effective measure is required to control the spread of resistance determinants.

Backgroud

Aeromonas species are Gram-negative and rod-shaped bacteria, which are ubiquitous in aquatic environment, foodstuffs, and soil. *Aeromonas* are responsible for a variety of human infectious diseases, such as gastroenteritis, wound infections, hepatobiliary infections, necrotizing fasciitis and septicemia [1]. Humans carry *Aeromonas* species in their gastrointestinal tract. The carrying rate of *Aeromonas* in the feces of healthy people ranges from 0–4% [2]. Many infections caused by *Aeromonas* are self-limiting. While, in patients who have severe underlying diseases or immunocompromised individuals, invasiveness infections can be urgent and rapid-developed [3].

The *Aeromonas* taxonomy is complex. Nowadays, accurate laboratory identification is still a great challenge. Conventional biochemical tests, 16sRNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) analysis is unreliable enough in identifying

Aeromonans to the species level. Accurate identification can be achieved by housekeeping genes sequencing, including *rpoD* and *gyrB*, or multilocus phylogenetic analysis (MLPA) [1]. *Aeromonas dhakensis* (formerly known as *Aeromonas aquariorum*), which often misidentified as *Aeromonas hydrophila* by traditional biochemical methods [4].

Virulence factors produced by *Aeromonas* species are multifactorial, including adhesins, cytotoxins, hemolysins, lipases, and proteases as well as the capacity to form biofilms, use specific metabolic pathways, and mediate virulence factor expression through quorum sensing [5]. The reported mortality rate among patients with *Aeromonas* bacteremia range from 24–63% [3]. *A. dhakensis* has been found to be prevalent in human infections and probably more lethal than other *Ameomonas* species in recent years. The pathogenicity of *Aeromonas* seems to be varied among species levels. Moreover, along with the overuse of antimicrobials in agriculture, fish farming and clinical settings, increasing resistance has been noted in *Aeromonas* [6]. The antibiotic susceptibility varies with the geographical area and the species of *Aeromonas* tested [2]. Appropriate antimicrobials treatment is necessary to control the development of infections.

The prevalence and dangerousness of *Aeromonas* infections seems to be underestimated, as they vary among different geographic regions and type of infections [7], but there are not enough fundamental reports in many countries. Wenzhou, a coastal city located in southeast China with subtropical climate, is prone to *Aeromonas* infection due to the humid weather. Incidences of bacteremia due to *Aeromonas* was increasing observed in Wenzhou with high morbidity and mortality in clinic. The present study was aimed to investigate the clinical manifestations of bacteremia due to *Aeromonas* species over a 6-year period in a teaching hospital in southern China, and assessed the risk factors associated with mortality. Virulence gene determinants and antimicrobial susceptibility were also analyzed for the sake of advancing the understanding of *Aeromonas* causing bacteremia and establishing appropriate therapy strategy.

Methods

Bacterial strains and identification

This study was conducted at the First Affiliated Hospital of Wenzhou Medical University, a 4100-bed teaching hospital located in southeast China. A total of 58 isolates were obtained from patient with positive blood cultures for *Aeromonas* species during January 2013 and December 2018. The isolates were primarily identified using the VITEK 2 Compact System (bioMe ´rieux, Marcy l' Etoile, France) or MALDI-TOF MS. Strains were further identified by housekeeping gene sequencing (*gyr*B). Strains used in this study was stored in 20% glycerol at -80^{O}C.

Data Collection

Retrospective cohort study was performed. The medical records of all patients with *Aeromonas* bacteremia were retrospectively reviewed and the following information was collected: demographics (age, gender), laboratory data (monomicrobial or polymicrobial infection), antimicrobial susceptibility test

results, inpatient records (history of hospitalization, length of stay), underlying diseases, and patient outcome. Nosocomial infections were defined as the bacteremia episodes detected at least 48 hours after admission. Patients died or discharged from hospital without further treatment under therapy failure were defined as prognosis poor, and who got better or be cured were considered to be prognosis well.

Detection Of Genetic Determinants Related To Virulence

The identified strains were recovered by streaking on nutrient agar plate and incubating for 24 h at 35 ^{o}C. Total DNAs of *Aeromonas* isolated from bacteremia were obtained with an AxyPrep Bacterial Genomic DNA Miniprep kit (Axygen Scientific, Union City, CA, USA) and were used as polymerase chain reaction (PCR) templates. Six virulence associated genes were selected as potential markers, including aerolysin (*aerA*), heat-stable cytotonic enterotoxin (*ast*), heat-labile cytotonic enterotoxin (*alt*), cytotoxic enterotoxin (*act*), hemolysin (*hlyA*), and phospholipase (*lip*). Primer sequences for the amplification were as previously described [8]. The positive PCR amplicons were sequenced by Shanghai MajorbioBioPharm Technology Co. (Shanghai, China). The sequences were blasted using BLAST at NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Antimicrobial Susceptibility Testing (AST)

The antimicrobial susceptibility patterns of all isolates to a panel of antimicrobials were determined using the VITEK 2 Compact System, including ampicillin (AMP), ampicillin/sulbactam (SAM), ceftriaxone (CRO), ceftazidime (CAZ), cefotetan (CTT), cefazolin (CZO), cefepime (FEP), piperacillin/tazobactam (TZP), aztreonam (ATM), imipenem (IPM), levofloxacin (LEV), ciprofloxacin (CIP), Trimethoprim/sulfamethoxazole (SXT), amikacin (AMK), gentamicin (GEN), tobramycin (TOB) and furantoin (NIT). The breakpoints were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2018 guidelines.

Phylogenetic And Statistical Analysis

The positive PCR amplicons (*gyrB* and virulence determinants) were sequenced by Shanghai MajorbioBioPharm Technology Co. (Shanghai, China). Nucleotide sequences were analyzed and compared using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). A phylogenetic tree was generated using the unrooted neighbor-joining method with the Kimura's 2-parameter method by Mega 5.0 software. Bootstrap values calculated by 1000 replicates [9]. Statistical analyses were performed using SPSS, version 17.0 (SPSS Inc., Chicago, IL, USA). Pearson’s Chi-square test was used to examine categorical variables and Student t test or Mann-Whitney U test was used for continuous variables. Risk factors for prognosis of *Aeromonas* bacteremia were analyzed with binary logistic regression models. Odds ratios (OR) were calculated with 95% confidence interval. A p value of ≤ 0.05 was regarded as statistically significant.

Results

Aeromonas diversity

Phylogenetic tree based on housekeeping gene *gyrB* exhibited that all 58 isolates were divided into 5 different species, with the predominant specie being *A. dhakensis* (26/58). Besides, 13 isolates of *Aeromona*s veronii, 10 isolates of *Aeromonas caviae*, 7 isolates of *A.s hydrophila* and 2 isolates of *Aeromonas jandaei* were identified to the species level (Fig. 1). Vitek 2 Compact system and the MALDI-TOF MS system showed poor coincidence with housekeeping gene sequencing analysis at the species level. *A. dhakensis* was incorrectly identified as *A. hydrophila* by Vitek 2 Compact system or MALDI-TOF MS. Moreover, two *A. jandaei* strains were misidentified as *A. hydrophila* and *Aeromonas veronii*, respectively.

Characteristics Of Investigated Patients

During the investigated period, 58 patients were detected with positive blood culture of *Aeromonas*. 16 patients had poor prognosis (death or therapy failure), where *A. dhakensis* (12/26) to be predominant, followed by *A. veronii* (2/13), *A. caviae* (1/10), *A. hydrophila* (1/7) and *A. jandaei* (0/2). The mean age was 61.1 ± 16.7 and the percentage of male patients were up to 70% (40/58). Polymicrobial infections were detected in nine cases, which co-infected with *Klebsiella pneumoniae* (3 cases), *Escherichia coli* (3 cases). *Proteus vulgaris* (1 case), *Klebsiella oxytoca* (1 case) and *Enterobacter cloacae* (1 case). The exact characteristics of patients were listed in the Table 1. Logistic regression analysis showed that poor prognosis was only significantly associated with community-acquired infections (OR = 11.027, 95% confidence interval (CI), 1.646–73.867, $P < 0.05$), and liver cirrhosis (OR = 16.854, 95% CI, 1.755-161.844, $P < 0.05$). Age, Gender, monomicrobial or polymicrobial infection, antimicrobial susceptibility and other underlying diseases didn't have the predictive ability of bacteremia-related prognosis. Length of hospital stay of community-acquired infections with poor prognosis range from 1 to 7 days (median 2 days), which indicated that the progression of bacteremia caused by *Aeromonas* was extremely fast. The outcomes of *A. dhakensis* bacteremia were worsen than other species ($p < 0.05$).

Table 1
Clinical characteristics of 58 patients with bacteremia caused by *Aeromonas* species

Clinical characteristic	No (%) of all patients (n = 58)	Prognosis		*p*
		Poor (n = 16)	Well (n = 42)	
Gender				
Male	40 (70.0%)	12 (75.0%)	28 (66.7%)	o.752
Female	18 (30.0%)	4 (25.0%)	14 (33.3%)	
Age, years (means ± SD)	61.1 ± 16.7	56.56 ± 14.45	62.76 ± 17.30	0.208
Age				
65	30 (51.7%)	10 (62.5%)	20 (47.6%)	0.311
≥ 65	28 (48.3%)	6 (37.5%)	22 (52.4%)	
Microbial findings				
Monomicrobial	49 (84.5%)	16 (100.0%)	33 (78.6%)	0.051
Polymicrobial	9 (15.5%)	0 (0.0%)	9 (21.4%)	
Antimicrobial susceptibility				
MDR	9 (15.5%)	4 (25.0%)	5 (11.9%)	0.243
Non MDR	49 (84.5%)	12 (75.0%)	37 (88.1%)	
Source of infection				
Community acquired	34 (58.6%)	13 (81.3%)	21 (50%)	0.031*
Nosocomial infection	24 (41.4%)	3 (18.8%)	21 (50%)	
Underlying disease				
liver cirrhosis	26 (44.8%)	11 (68.8%)	15 (35.7%)	0.024*
Diabetes mellitus	7 (12.1%)	2 (12.5%)	5 (11.9%)	1.000
Malignancy	18 (31.0%)	6 (37.5%)	12 (28.6%)	0.538
Leukemia	8 (13.8%)	3 (18.8%)	5 (1.9%)	0.672
Clinical outcomes				
Length of stay in hospital, days	17 (6-24.75)	2.5 (1–6)	19 (11.25-30)	0.000*

Values are presented as No. (%), mean ± SD or median (25th – 75th percentile) of patients. Poor prognosis cases include in-hospital deaths and deaths after discharge from hospital without treatment. * significant.

Distribution Of Virulence Determinants

Virulence encoding genes, including *aer*, *lip*, *hlyA*, *alt*, *ast*, and *act*, were detected at ratios of 24.1% (14/58), 62.1% (36/58), 65.5% (38/58), 58.6% (34/58), 15.5% (9/58) and 65.5% (38/58), respectively. Virulence genes profile of 58 *Aeromonas* isolates was showed in Fig. 2. At least one virulence determinants were found in all 58 isolates. The gene *hlyA* and *act* were most prevalent in these isolates. Single virulence gene was detected in 12.1% (7/58) of isolates, and more than two virulence genes were found in remaining strains. There was no significant difference in virulence genes between strains isolated from patients with poor prognosis and well prognosis. Additionally, no statistical significance was observed in the prevalence of all the studied virulence genes between isolates separated from community acquired and nosocomial infection. We found 27 different combination patterns (PTs) of six examined genes. The two most prevalent PT ($n \geq 5$) were PT1 *(lip/ hlyA/ alt/ act*, n = 6) and PT2 (*lip/ alt*, n = 5). Only one isolate of *A. hydrophila* carried all the investigated virulence genes, and the patient was cured after 32 days of hospitalization. Notably, 4 of 6 isolates grouped into PT1 were *A. hydrophila*, among which 3 lead to poor prognosis.

Antimicrobial Susceptibility

Antimicrobials susceptibility test exhibited that the majority of 58 isolates maintained susceptible to aminoglycosides, fluoroquinolones and furantoin (Table 2). Resistance to ceftazidime, cefotetan, ceftriaxone, cefepime, piperacillin/tazobactam, aztreonam were 10.3%, 13.8%, 15.5%, 1.7%, 10.3% and 5.2%, respectively. No significant increase in resistance during six years was observed. 9 out of 58 isolates was identified as multi-drug resistant (MDR) organism, including four isolates of *A. dhakensis*, 3 *A. hydrophila*, 1 *A. veronii*, and 1 *A. caviae*. Among which, six MDR strains were isolated in 2017 and 2018. The first MDR strain was recovered from a 78-years old woman with community-acquired infection in 2013. 24.1% (14/58) isolates were non-susceptible to imipenem.

Table 2
Antimicrobial susceptibility patterns of 58 Aeromonads separated from bacteremia.

Antimicrobial agent	CLSI breakpoint interpretation (%)			MIC50	MIC range
	S	I	R		
ceftazidime	88	1.7	10.3	≤ 1	≤ 1 ~ ≥ 64
cefotetan	86.2	0	13.8	≤ 4	≤ 4 ~ ≥ 64
ceftriaxone	79.3	5.2	15.5	≤ 1	≤ 1 ~ ≥ 64
cefepime	98.3	0	1.7	≤ 1	≤ 1 ~ 32
piperacillin/tazobactam	88	1.7	10.3	≤ 4	≤ 4 ~ ≥ 128
aztreonam	91.4	3.4	5.2	≤ 1	≤ 1 ~ ≥ 64
imipenem	75.9	10.3	13.8	≤ 1	≤ 1 ~ ≥ 16
levofloxacin	96.6	1.7	1.7	≤ 0.25	≤ 0.25 ~ ≥ 8
ciprofloxacin	96.6	0	3.4	≤ 0.25	≤ 0.25 ~ ≥ 4
Trimethoprim/sulfamethoxazole	87.9	0	12.1	≤ 20	≤ 20 ~ ≥ 320
amikacin	100	0	0	≤ 2	≤ 2
gentamicin	100	0	0	≤ 1	≤ 1
tobramycin	93.1	5.2	1.7	≤ 1	≤ 1 ~ ≥ 16
furantoin	100	0	0	≤ 16	≤ 16

Discussion

Aeromonas spp. are of increasing importance that causing multiple of clinical infections, including diarrhea, soft tissue infection, and bacteremia. *Aeromonas* bacteremia is an urgent, rapid-developing disease with high mortality [10]. Moreover, according to similar clinical manifestations, *Aeromonas* infections often be misdiagnosed as Vibrio infections before microbiology identification by laboratory, which may lead to improperly use of antimicrobials and ineffective treatment [10]. *Aeromonas* infections are reported to be prevalent in regions with a high prevalence of chronic hepatitis and warm climate, like Taiwan, which is regarded as one of the endemic areas [11]. However, in mainland China, the incidence of *Aeromonas* bacteremia in human beings remains to be elucidated. Wenzhou is in the southeastern coastal area with subtropical climate. Increasing prevalence of *Aeromonas* bacteremia is found in the studied hospital with high morbidity and mortality.

Aeromonas are not difficult to isolate, but identification to species level is challenging due to its phenotypic heterogeneity. Compared with the use of 16 s rRNA gene, nucleotide sequencing of housekeeping genes, such as *gyrB*, *rpoB* and *rpoD*, can provided a more definitive identification of the genus [12]. Several researches have shown that MALDI-TOF MS could efficiently identify *A. dhakensis*, which is often clinically misidentified as *A. hydrophila* by phenotypic methods [4]. While, *A. dhakensis* couldn't be identified by MALDI-TOF MS in this study, possibly because it hasn't been included in the commercial database of BioMerienx system. Housekeeping gene *gyrB* sequencing exhibited that *A. dhakensis* was the most common *Aeromonas* species, followed by *A. veronii*. In contrast to previous reports that *A. hydrophila* and *A. caviae* were most frequent *Aeromonas* species causing bacteremia in Taiwan, and *A. caviae* was the most common pathogen contributing to *Aeromonas* bacteremia in Japan [13]. Notably, *A. dhakensis* and *A. jandaei* were misidentified as *A. hydrophila* or *A. veronii* by biochemistry methods and MALDI-TOF MS. The patients with *A. dhakensis* bacteremia are reported to have a higher sepsis-related mortality rate than those with other species in recent years, with the applying of molecular biological method [14]. Similarly, bacteremia caused by *A. dhakensis* is more lethal than other species in our research. Notably, the importance of *A. dhakensis* in human infections might be seriously underrated and should be re-valuated along with the changing taxonomy, and more accurate epidemiological researches are needs to establish the bacteriology distribution of *Aeromonas* bacteremia in different regions.

Retrospective analysis of patients with positive blood culture exhibited that older people was more susceptible than younger individuals with an average of 61.1 ± 16.7 years old. 40 out of 58 patients were male, which may attribute to that alcoholic cirrhosis was more prevalent in male than female in our study. Similar with previous researches [15] that the majority of patients had a variety of underlying diseases, including liver cirrhosis, diabetes mellitus, under immunosuppressed conditions, leukemia and other kinds of malignancy. Nearly half of patients in this study were diagnosed as liver cirrhosis. In accordance with previous research that *Aeromonas* bacteremia accounted for significant morbidity and mortality in cirrhotic patients [10], suggesting that patients with liver cirrhosis are at risk of developing *Aeromonas* bacteremia. The epidemiology and high mortality rate of *Aeromonas* bloodstream infections in cirrhotic patients might be a consequence of dysregulated intestinal bacterial translocation and cirrhosis associated immune dysfunction (CAID) [16]. Among 58 patients with *Aeromonas* bacteremia in this study, four patients were claimed to be dead in hospital, and 12 have dismal prognosis and then discharged without treatment. Polymicrobial infection didn't result in worsen prognosis than monomicrobial ($P > 0.05$). We found that consumption of sea food, trauma exposed or contact with water contaminated with *Aeromonas* [17], people with liver cirrhosis were the potential risk factor of *Aeromonas* infections or even lead to more rapid infections progresses. Additionally, length of hospital stays of community-acquired infections with poor prognosis range from 1 to 7 days (median 2 days), indicating that community-acquired infections developed more rapidly and lethally. No statistical significance in prognosis was observed between MDR and non-MDR strains. Compared to antimicrobial susceptibility, the pathogenicity of pathogens and health status of patients probably to be more critical to the prognosis of patients.

Pathogenicity of *Aeromonas* is multi-factorial, complex and may be associated with different interaction of various virulence factors acting either synergy or alone. The majority of *Aeromonas* isolates investigated in this study possess more than two virulence genes and seven strains harbor only one single gene. Isolates carrying more virulence genes didn't mean higher pathogenicity. One patient was died of an *A. veronii* strain which only possess lipase encoding gene *lip* after six day admission to ICU. However, another one infected by *A. hydrophila* carrying all the studied virulence determinants was cured after 32 day of hospitalization. The most obvious difference between those two patients was that the former one suffered from liver cirrhosis. However, it may be explained by different expression level of the genes or interaction with other virulence factor not included in this study. Inconsistent with previous study [7], no particular pattern of virulence genes was observed in this study.

Expect for ceftriaxone (79.3%) and imipenem (75.9%), more than 80% of the isolates were susceptible to all remaining antimicrobials studied. In spite of intrinsically resistant to many antimicrobials, *Aeromonas* maintained well susceptibility to most antimicrobials generally used in clinic. Relatively high carbapenem resistant rate may due to *Aeromonas* spp. specific "Carbapenem hydrolysing *Aeromonas*" metallo-betalactamase (CphA) [17], which remind us that carbapenem should avoid to be empirical therapy of *Aeromonas* infection. 9 isolates were identified as MDR due to resistance to more than three classes of antimicrobials, which composed of 4 *A. dhakensis*, 3 *A. hydrophila*, 1 *A. veronii* and 1 *A. caviae*. Six MDR strains were isolated in 2017 and 2018. The first MDR strain was recovered from a 78-years old woman with community-acquired infection in 2013. Moreover, the resistance of bacteria associated with food animals and enviroments to antimicrobial agents represents a potential health threat [18]. It raises an alert for the developing of multidrug resistant strains in *Aeromonas* spp. isolated from clinic.

Conclusions

Considering the high morbidity and mortality, people should attach great importance to bacteremia caused by *Aeromonas* spp., especially in those immunocompromised patients with severe underlying diseases. Identification of *Aeromonas* to the species level is important for predicating clinical severity and outcome. The increasing emergence of MDR strains in recent years requires more attention and monitoring.

Abbreviations

AMK: Amikacin; AMP: Ampicillin; AST: Antibiotics Susceptibility Testing; ATM: aztreonam; CAID: Cirrhosis Associated Immune Dysfunction; CAZ: Ceftazidime; CLSI: the Clinical and Laboratory Standards Institute; CIP: Ciprofloxacin; CRO: Ceftriaxone; CTT: Cefotetan; CZO: cefazolin; FEP: cefepime; GEN: Gentamicin; ICU: Intensive Care Unit; IPM: imipenem; LEV: levofloxacin; MALDI-TOF MS: Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry; MDR: Multi-Drug Resistant; MLPA: Multilocus Phylogenetic Analysis; NIT: Furantoin; OR: Odds ratios; PCR: Polymerase Chain Reaction; SAM: Ampicillin/Sulbactam; SXT: Trimethoprim/sulfamethoxazole; TOB: Tobramycin; TZP: Piperacillin/Tazobactam.

Declarations

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

The need for ethics approval and consent is deemed unnecessary in this research according to the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Contributions

YS, YJZ and WYX carried out experiments. YS, YJZ, RCF and WQ analyzed the data. YS wrote the manuscript. HKH and CQX performed the results analysis and CZ directed the drawing. JMC, LJC and TLZ designed the study and revised the manuscript. All authors reviewed and approved the final version of the manuscript.

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Figures

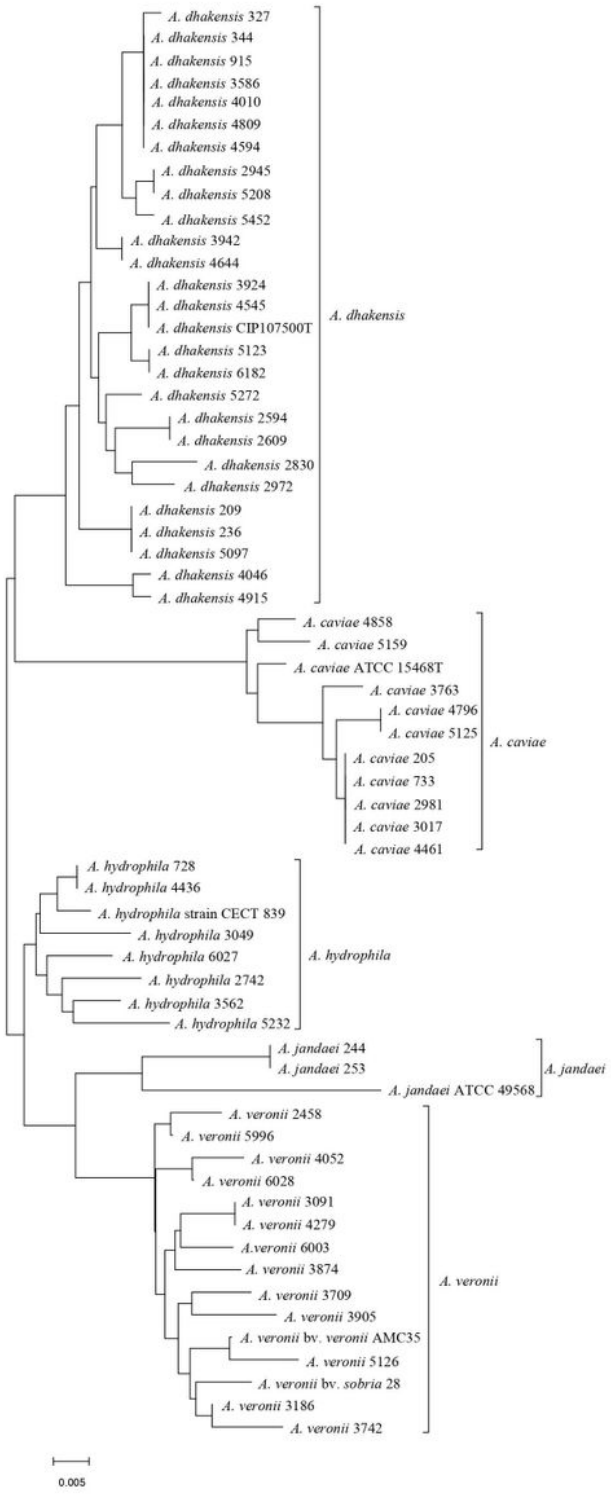
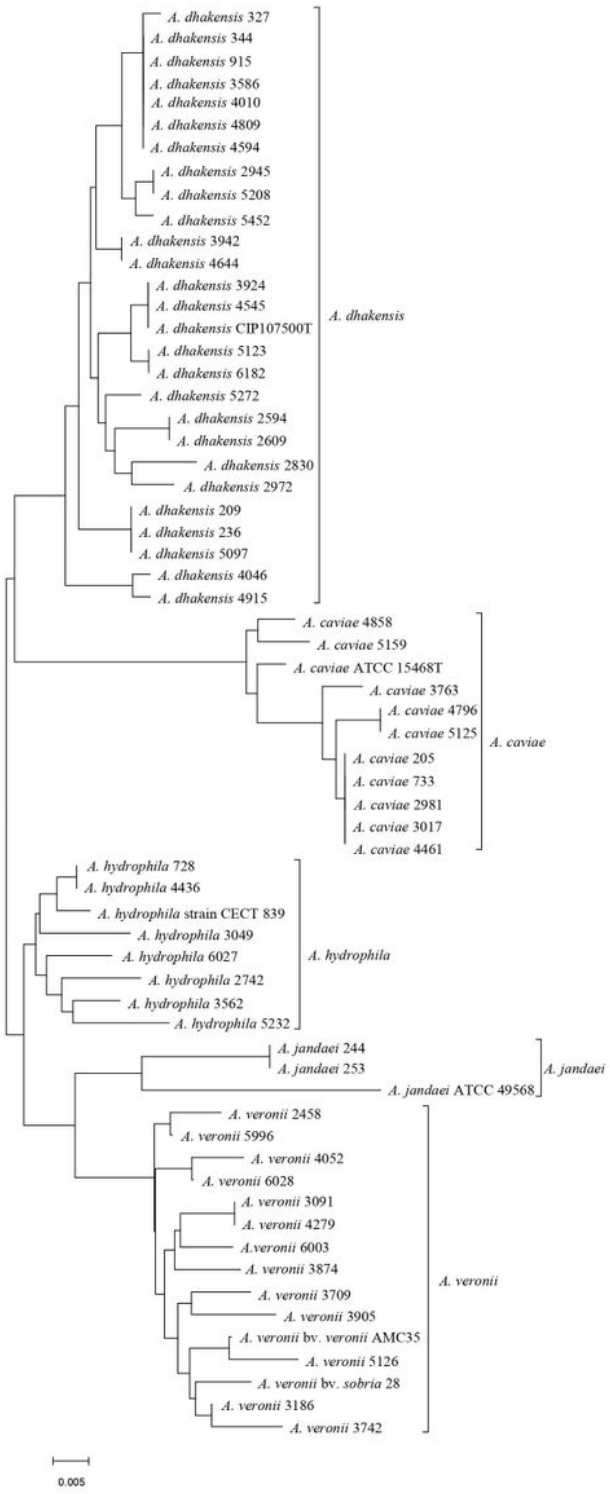


Figure 1

Phylogenetic tree based on gyrB gene sequences. Phylogenetic tree was constructed by the neighbor-jioning methed with Kimura's 2-parameter methed. Scale bar represents 0.05 substitutions per site. Bootstrap values above 50% are shown (n=1000 bootstrap replicates).



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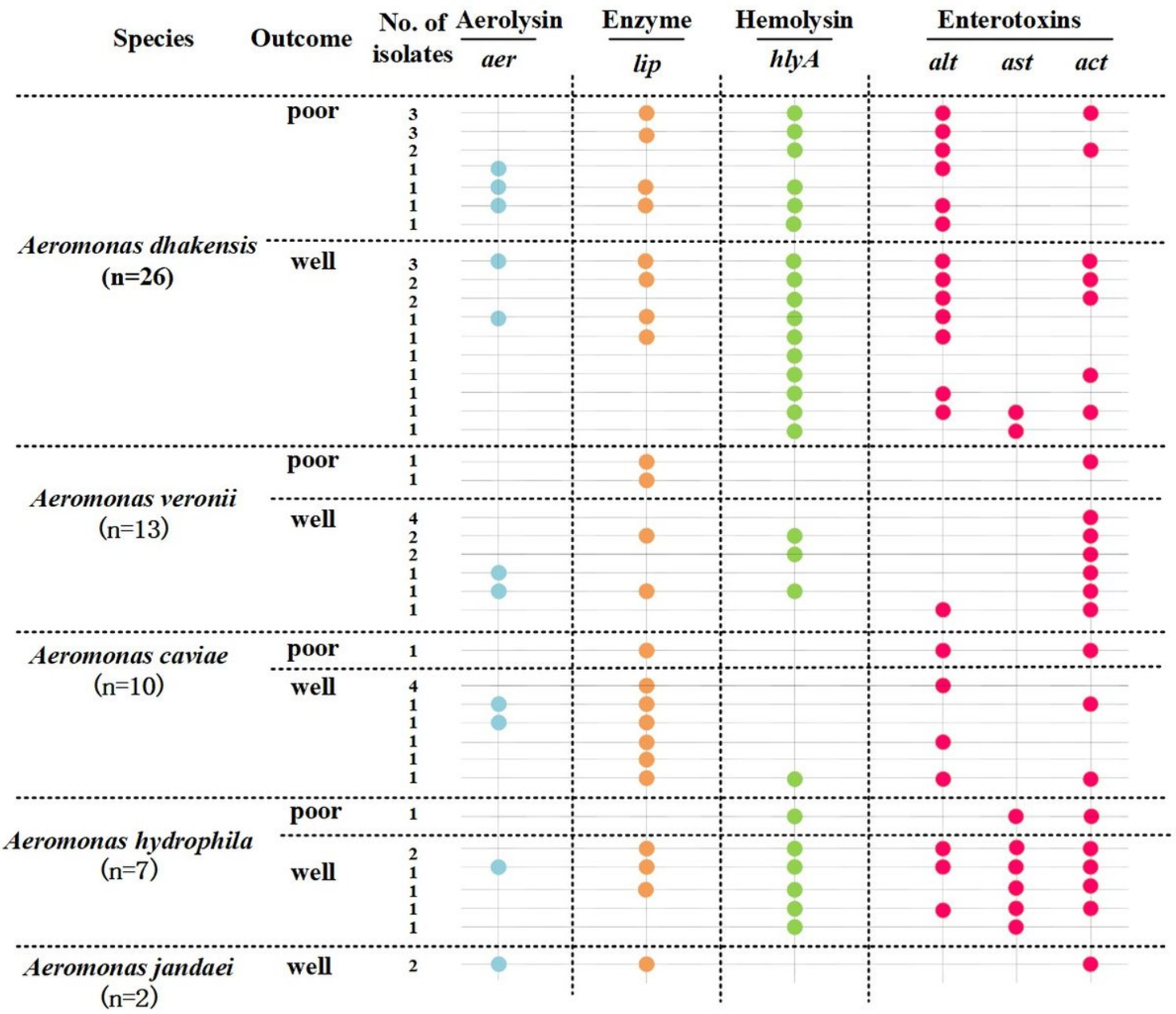
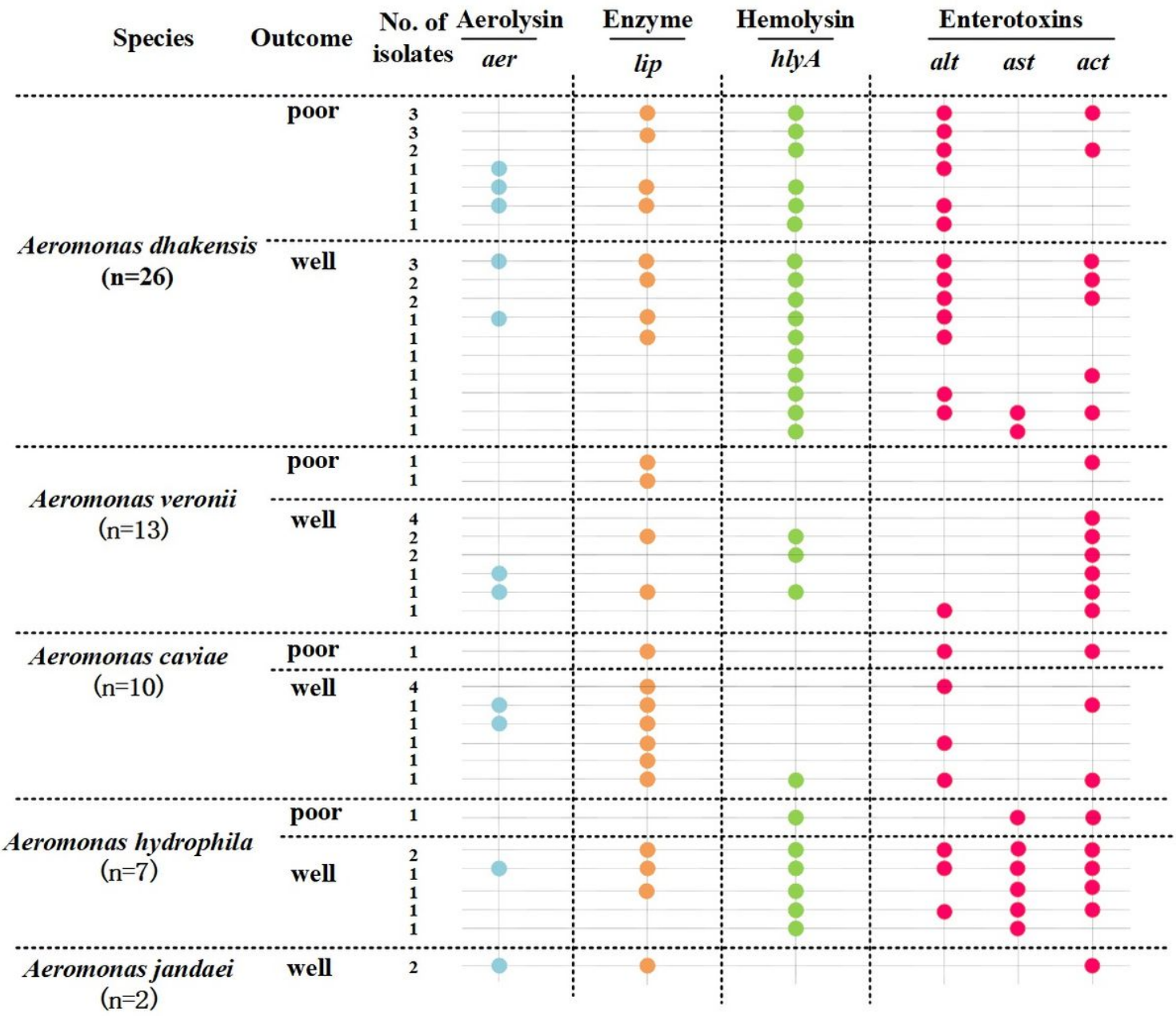


Figure 2

Virulence genes profile of 58 Aeromonas isolates. Virulence genes identified by PCR-based profiling are shown by colored dots.



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