Co-Occurrence of the $\text{bla}_{KPC-2}$ and $\text{Mcr}-3.3$ Gene in *Aeromonas caviae* SCAc2001 Isolated from Patients with Diarrheal Disease

Lingtong Tang,$^{1,2}$*\#*  
Jianlian Huang$^3,4$*\#*  
Junping She$^1$  
Kelei Zhao$^4$  
Yingshun Zhou$^1$

$^1$Department of Pathogenic Biology, School of Basic Medicine, Southwest Medical University, Luzhou 646000, Sichuan, People’s Republic of China; $^2$Department of Clinical Laboratory, People’s Hospital of Gao County, Yibing 644000, Sichuan, People’s Republic of China; $^3$Department of Clinical Laboratory, The Second Affiliated Hospital of Xiamen Medical College, Xiamen 361600, People’s Republic of China; $^4$Antibiotics Research and Re-Evaluation Key Laboratory of Sichuan Province, Sichuan Industrial Institute of Antibiotics, Chengdu University, Chengdu 610052, Sichuan, People’s Republic of China.

*These authors contributed equally to this work.

Correspondence: Yingshun Zhou  
Department of Pathogenic Biology, School of Basic Medicine, Southwest Medical University, Luzhou, Sichuan 646000, People’s Republic of China, No. 319, Zhongshan Road, Tel +86-0830-3160073  
Email yingshunzhou@swmu.edu.cn

Kelei Zhao  
Antibiotics Research and Re-Evaluation Key Laboratory of Sichuan Province, Sichuan Industrial Institute of Antibiotics, No. 168, Huaguan Road, Chengdu 610052, Sichuan, People’s Republic of China  
Tel +86-028-84216035  
Email zhaokelei@cdu.edu.cn

Purpose: To characterize the genetic feature of a multi-drug-resistant *Aeromonas caviae* strain isolated from the diarrhea sample of a 45-year-old male patient with acute diarrhea.

Materials and Methods: Whole-genome of the *A. caviae* strain SCAc2001 was sequenced via the Illumina system, followed by a series of bioinformatic analyses to describe the genetic feature.

Results: The genome sequence of *A. caviae* SCAc2001 was assembled into 340 scaffolds (305 of them were > 1000 bp in length and 4,487,370 bp in total) with an average G+C content of 61.09%. Phylogenetic analysis showed that the *A. caviae* SCAc2001 strain was highly similar to the *A. caviae* strain R25-2 and T25-39. Resistome analysis identified that *A. caviae* SCAc2001 carried 13 antimicrobial resistance genes, including β-lactams (\text{bla}_{KPC}, \text{bla}_{CTX-M-14}, \text{bla}_{TEM-1}), \text{bla}_{OXA-10}, \text{bla}_{OXA-427}, \text{bla}_{VEB-3} and \text{bla}_{MOX-\alpha}), \text{aminoglycosides (aadA1), fluoroquinolones (aac{\text{6}{\prime}})-Ib-cr}, \text{phenicol resistance (catB3), sulfonamide (sul1), trimethoprim (dfrA5) and colistin resistance (mcr-3.3)}. And also, *A. caviae* SCAc2001 carried 54 putative virulence genes including the type IV pilus, fimbia, flagellarhete, and hemolysin A encoding genes, and 12 pathogen-host interactions (PHI) genes. There were also four genomic islands and eight prophages in the genome of *A. caviae* SCAc2001. In addition, *A. caviae* SCAc2001 also carried three secondary metabolism products coding clusters including nonribosomal peptide synthetases (nrps), hserlactone and bacteriocin.

Conclusion: *A. caviae* SCAc2001 carries many resistance genes, a variety of virulence factors, PHI genes and four genomic islands and eight prophages, which poses a severe threat to infectious diseases control strategies, diagnosis methods and clinical treatment.

Keywords: *Aeromonas caviae*, \text{bla}_{KPC-2}, \text{mcr}-3.3, virulence factors, secondary metabolism products

Introduction

Colistin is the last resort for the treatment of infections caused by multidrug-resistant bacteria, particularly the carbapenem-resistant microorganisms.$^{1,2}$ However, the mobile colistin-resistant gene *mcr-1*, was first reported in the *Enterobacteriaceae* by Liu et al$^3$ in 2015, which led the *mcr-1* carried bacteria resistant to colistin. Since then, *mcr-1* or the *mcr* gene family-carrying bacteria have been reported in different species (such as *E. coli*, *klebsiella pneumoniae*, *Acinetobacter*, *Pseudomonas* and other gram-negative bacteria) isolated from food, animals, the environment and clinical samples worldwide.$^{4-6}$ To our knowledge, co-carriers of the colistin-resistant gene *mcr* and carbapenemase-resistant...
genes (blaKPC, blaNDM, blaIMP, and blavIM), microorganisms have potentially evolved into extensively drug-resistant or pan-drug-resistant isolates. Infections caused by these clinical isolates co-harboring the colistin-resistant gene (mcr-1) and carbapenem-resistant genes (blaKPC, blaNDM, blaIMP, and blavIM) pose a serious threat because the antibiotic options would be much fewer.\(^4\,7,8\) Aeromonas species, one kind of the gram-negative bacteria, was identified in the 1980s as an enteric pathogen which can lead to severe diarrhea.\(^9\) In addition, the carbapenem-resistant and/or colistin-resistant Aeromonas species strains have been reported increasingly in recent years to pose a serious threat in infection control.\(^10\,12\) In this study, we recovered a colistin and carbapenem-resistant Aeromonas strain from the diarrhea sample of a 45-year-old male patient with acute diarrhea in the affiliated hospital of Southwest Medical University, and the genomic information of this strain was characterized to gain insight into further infection control.

Materials and Methods
Isolation and Identification of Aeromonas caviae SCAc2001
A. caviae SCAc2001 was recovered from the diarrhea sample of a 45-year-old male patient with acute diarrhea in a hospital in Sichuan, China, in May, 2019. It was identified as Aeromonas caviae using the Vitek-2 compact system (bioMérieux, Marcy-l’Étoile, France). The presence of the acquired carbapenemase genes (blaKPC, blaNDM, blaGES, blavMP, and blavIM) and mcr genes in SCAc2001 was determined by PCR amplification as described previously.\(^13\,15\)

Antimicrobial Susceptibility Testing
In vitro susceptibility tests of A. caviae SCAc2001 against 17 antimicrobial agents (Solarbio, China) including meropenem, imipenem, cefepime, cefotaxime, ceftazidime, piperacillin-tazobactam, amoxicillin-clavulanic acid, gentamicin, amikacin, aztreonam, erythromycin, chloramphenicol, colistin, tigecycline, fosfomycin and ciprofloxacin and trimethoprim-sulfamethoxazole determined by broth microdilution method according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2013, M100-S23), and the breakpoints of colistin were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (http://www.eucast.org); E.coli J53 was used as quality control.

Genome Sequencing and Analysis
The genomic DNA of A. caviae SCAc2001 was extracted using the Axygen® DNA Gel Extraction Kits (Axygen, People's Republic of China) according to the manufacturer’s protocol. Purified DNA was subjected to whole genomic sequencing on the Illumina system with the 150-bp paired-end approach and >180× coverage (Novogene, People’s Republic of China). The reads were assembled using the software SOAP denovo (version 2.04).\(^16\) Gene prediction was performed with GeneMarkS (version 4.17).\(^17\) Gene annotation was achieved using the NCBI Prokaryotic Genome Annotation Pipeline. The pairwise alignment was performed by blastn search (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The resistome was identified using ResFinder (https://cge.cbs.dtu.dk/services/ResFinder)\(^18\) (minimum threshold for identity, 80%; minimum coverage, 60%) and Comprehensive Antibiotic Resistance Database (CARD). The virulence factors were identified by the VFAnalyzer (http://www.mgc.ac.cn/VFs/main.htm). The pathogen–host interactions (PHI) genes were identified by comparison with the pathogen–host interactions database (minimum threshold for identity, 80%).\(^19\) To determine the phylogenetic groups of the A. caviae SCAc2001 strain, the phylogenetic tree was constructed by aligning the core genome of A. caviae SCAc2001 strain with other representative A. caviae strains available in the genbank (Table 1). All the sequences were aligned using Mugsy and thereafter a maximum-likelihood phylogeny tree was generated using RAxML version 8 and MEGA7.0.\(^20\,22\) The genomic island sequences were predicted based on three different genomic islands (GIs) prediction softwares (IslandPATH-DIMOB, IslandPick, and SGI-HMM)\(^23\,25\) and the prophages were predicted by using phiSpy.\(^26\) The secondary metabolism products coding clusters were identified by the antiSMASH.\(^27\)

Results and Discussion
Characteristics of the Isolate SCAc2001
A carbapenem-resistant gene blakPC and a colistin-resistant gene mcr-3.3 co-carried by A. caviae strain SCAc2001 was isolated and identified by the Vitek-2 compact system and resistance genes PCR detection from the diarrhea sample. The results of antimicrobial susceptibility testing showed that A. caviae SCAc2001 strain was resistant to meropenem, imipenem, cefepime, cefotaxime, ceftazidime, piperacillin-tazobactam, amoxicillin-clavulanic acid, gentamicin,
amikacin, aztreonam, erythromycin, chloramphenicol, colistin, ciprofloxacin and trimethoprim-sulfamethoxazole and sensitive to the tigecycline and fosfomycin. While the negative control *E. coli* J53 was sensitive to all the test antibiotics.

To the best of our knowledge, the *Aeromonas* species isolated from environmental water samples received widespread attention several years ago. However, there have been more reports of the *Aeromonas* species infection in humans worldwide in recent years, because the *Aeromonas* species have always been identified as enteric pathogens which can lead to severe diarrhoea. Unfortunately, what’s more serious is that the carbapenem-

| Isolate | Access Number | Country | Samples | Resistance Genes |
|---------|---------------|---------|---------|------------------|
| GSH8M-1 chromosomes and plasmids genome | AP019195.1 | Japan | Wastewater | *bla*<sub>MOX-12</sub>, *bla*<sub>OKRA-780</sub>, mcr-3.18, *bla*<sub>OKRA-449</sub>, acr(6')-la, aadA2, sul1, *bla*KPC-2, mph(A) |
| WCW1-2 chromosomes genome | CP039832.1 | People’s Republic of China | Sewage | *bla*<sub>MOX-5</sub>, *bla*<sub>OKRA-427</sub>, catB3, ermA1, floR, acr(6')-ib-cr, qnrVC4, sul1, acr(6')-ib3, aadA1, aph(3')-ib, aph(3')-la, ahpC3, dfrA14, dfrB4, *bla*OKRA-10, tet(X4) |
| T2S-39 chromosomes genome | CP025706 | People’s Republic of China | Wastewater | *bla*<sub>MOX-4</sub>, *bla*OKRA-427 |
| R2S-2 chromosomes genome | CP025777.1 | People’s Republic of China | Wastewater | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, aph(3')-ib, aph(3')-la, tet(3I), sul2, floR |
| NCTC12244 Chromosomes genome | LS483441.1 | UK | N | *bla*<sub>MOX-4</sub>, *bla*OKRA-427 |
| Draft genome Zj17-2 | NXBR00000000.1 | People’s Republic of China | River water | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, mcr-3, cat, tet(A), aadA1, sul1 |
| Draft genome A23 | LFKO00000000.1 | People’s Republic of China | Chicken sample | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, catB3, mph(A), *bla*OKRA-2, *bla*OKRA-3, aadA1b, aadA16, acr(6')-ib-Hangzhou, acr(6')-ib-cr, sul1 |
| Draft genome Zj33-3 | NXBW00000000.1 | People’s Republic of China | Human rectal swab | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, tet(A), mcr-3, cat, aadA1, sul1 |
| Draft genome AK245 | JAAALU00000000.1 | USA | Lake water | *bla*<sub>MOX-4</sub> |
| Draft genome Sch29 | CAAKN00000000.01 | UK | Gastroenteritis samples | *bla*<sub>MOX-4</sub>, *bla*OKRA-427 |
| Draft genome L12 | JWJP00000000.1 | Malaysia | Lake water | *bla*<sub>MOX-4</sub>, mcr-3.15 |
| Draft genome TCO22 | NMSG00000000.01 | USA | Gut samples | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, tetE, mcr3.12, mcr3.15, aph(3')-la, mph(A) |
| Draft genome strain D | VZQB00000000.1 | South Africa | Seawater | *bla*<sub>MOX-4</sub>, *bla*OKRA-427 |
| Draft genome strain CH129 | MDSO00000000.1 | Brazil | Seawater | *bla*<sub>MOX-4</sub> |
| Draft genome strain CHZ306 | MDCO00000000.1 | Brazil | Seawater | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, tetE |
| Draft genome strain SCAc2001 | WUTZ00000000.1 (In this study) | People’s Republic of China | Human sample | *bla*<sub>MOX-4</sub>, *bla*OKRA-427, acr(6')-ib3, aadA1, *bla*OKRA-10, catB3, sul1, mcr-3, dfrA5, *bla*CTX-M-14, *bla*KPC-2, *bla*TEM-150, *bla*OBE-3 |

Notes: *The resistance genes were identified by the ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) (minimum threshold for identity, 80%; minimum coverage, 60%). Abbreviations: N, Not shown.*
resistant and/or colistin-resistant strains produce emergencies in some countries and pose a serious threat to infectious diseases control and clinical treatment.\textsuperscript{30,31}

**Draft Genome Characterization of SCAc2001 and Phylogenetic Analysis**

A total of 1 Gigabases pairs (Gbp) of raw genome data was obtained from the Illumina system. Thereafter, we got about 800 million bases (Mbp) of clean data from the 1Gbp raw genome data by using the readfq (version 10). The 800Mbp clean data was assembled into a 340 scaffolds draft genome sequence of *Aeromonas caviae* strain SCAc2001 by the SOAP denovo, with a G+C content of 61.09\%, for a total of 448730bp. We predicted 4265 protein-coding sequences (CDS) in the draft genome. The genome encodes 103 tRNAs and 14 rRNAs, which contains five copies of the 5S rRNA gene, five copies of the 16S rRNA gene, and four copies of the 23S rRNA genes. The core genome-based phylogenetic analysis showed that the *A. caviae* SCAc2001 strain was highly similar to the *A. caviae* strain R25-2 (Genbank accession number: CP025777.1) and T25-39 (Genbank accession number: CP025706), but distant from the clade grouped by the *mcr-3* carrying strain ZJ33-3 (NXBW00000000.1) and ZJ17-2 (NXBR00000000.1), and the *mcr-3* and \textit{bla}KPC-2 co-carried strain GSH8M-1 (AP019195.1)\textsuperscript{32} (Figure 1).

**Identification of the Resistance Genes, Virulence Factors and PHI Genes**

A total of 13 antibiotic drug resistance genes including the β-lactams (\textit{bla}KPC, \textit{bla}CTX-M-14, \textit{bla}TEM-1, \textit{bla}OXA-10, \textit{bla}OXA-427, \textit{bla}VEB-3 and \textit{bla}MOX-6), aminoglycosides (\textit{aadA}1), fluoroquinolones (\textit{aac(6')-Ib-cr}), phenicol resistance (\textit{catB}3), sulfonamide (\textit{sul}l) and trimethoprim (\textit{dfr}A5) and colistin resistance gene (\textit{mcr}-3.3) were detected in the genome of *Aeromonas*

![Figure 1](image-url)
**Aeromonas caviae** strain ScAc2001c (Table 2). To the best of our knowledge, many reports are showing that the multi-drug-resistant *Aeromonas* species have been isolated from clinical, animal, food, and environmental water samples (Table 1). These isolates carried many more types of antibiotic resistance genes, especially the co-carried carbapenem- and colistin- resistance gene may be a huge risk for infectious disease control. Also, as shown in Table 3, *Aeromonas caviae* strain ScAc2001 carries 54 putative virulence factors including type IV pilus, fimbria, flagellarthe and hemolysin A. What’s more, we also identified 12 PHI genes including csrA, lrp, crp, iscU, arcA, metJ, pykF, lon, dksA, fur, greB and hQq from the genome (Table 4). It proved that these PHI genes are associated with diseases such as diarrheal, meningitis and urinary tract infections.33

![Table 2](http://www.mgc.ac.cn/VFs/main.htm)  Distribution of the Resistance Genes in *Aeromonas caviae* SCAc2001

| Resistance Gene | Identity (%) | Query/Length | Scaffold | Position in Scaffold | Predicted Phenotype | Accession Number |
|-----------------|--------------|--------------|----------|---------------------|---------------------|------------------|
| aac(6’)-Ib3     | 100          | Scaffold231  | 2433-2987| Fluoroquinolone and aminoglycoside resistance | X60321             |
| aadA1           | 100          | Scaffold231  | 29-820   | Aminoglycoside resistance | JQ414041           |
| blaOXA-10       | 100          | Scaffold231  | 837-1637 | Beta-lactam resistance Alternate name; PSE-2 | J03427             |
| catB3           | 100          | Scaffold231  | 1706-2338| Phenicol resistance | U13880             |
| sul1            | 100          | Scaffold310  | 25-864   | Sulphonamide resistance | U12338             |
| mcr-3.3         | 100          | Scaffold231  | 1393-3015| Polymyxin resistance | MF495680           |
| dfiA5           | 100          | Scaffold258  | 1177-1650| Trimethoprim resistance | X12868             |
| blaCTX-M-14     | 100          | Scaffold180  | 3068-3943| Beta-lactam resistance | AF252622           |
| blaKPC-2        | 100          | Scaffold72   | 15,640-6521| Beta-lactam resistance | AY034847           |
| blaMOX-6        | 97.83        | Scaffold128  | 10,749-11,900| Beta-lactam resistance AmpC-type | GQ152601           |
| blaOXA-427      | 86.43        | Scaffold165  | 5530-6324| Beta-lactam resistance | KX827604           |
| blaTEM-150      | 99.82        | Scaffold72   | 14,849-15,418| Beta-lactam resistance | AM183304           |

| Notes: | a The resistance genes were identified by the virulence factors which were identified by the VFAnalyzer (http://www.mgc.ac.cn/VFs/main.htm).

![Table 3](http://www.mgc.ac.cn/VFs/main.htm)  Distribution of the Virulence Factors in *Aeromonas caviae* Strain SCAc2001

| Virulence Factor | Scaffold | Position in Scaffold | Identity(%) | Characteristic |
|-----------------|----------|----------------------|-------------|----------------|
| tppE            | Scaffold14 | 20,853-21,356        | 99.4        | Type IV pilus pseudopilin |
| tppB            | Scaffold14 | 20,443-20,856        | 99.3        | Type IV pilus modification protein PilV |
| tppA            | Scaffold14 | 15,090-15,491        | 99.2        | Type IV pilin |
| topY1           | Scaffold14 | 15,500-18,853        | 99.1        | Type IV pilus biogenesis protein |
| cheW-2         | Scaffold65 | 270-758              | 98.1        | Chemotaxis protein CheW |
| pomA2           | Scaffold18 | 20,514-21,245        | 97.9        | Chemotaxis protein PomA |
| hlyA            | Scaffold96 | 11,532-12,851        | 97.5        | Hemolysin A |
| exeG            | Scaffold3  | 14,115-14,546        | 97.2        | General secretion pathway protein G |
| exeE            | Scaffold3  | 15,920-17,425        | 96.2        | General secretory pathway protein E |
| topT            | Scaffold84 | 9088-10,044          | 95.9        | Twitching ATPase |
| hutZ            | Scaffold20 | 28013-28,570         | 95.7        | Heme iron utilization protein |
| exeF            | Scaffold3  | 14,698-15,918        | 95.6        | General secretion pathway protein F |
| topB            | Scaffold45 | 118-1098             | 95.3        | Type IV-A pilus assembly ATPase PilB |
| exel            | Scaffold3  | 13,085-13,429        | 94.7        | General secretion pathway protein I |
| hutC            | Scaffold20 | 30,092-31,120        | 94.4        | ABC-type hemin transporter, permease protein |
| topB            | Scaffold288| 85-870               | 94.4        | Type IV pilus assembly protein TapB |
| filR/filC       | Scaffold148| 2516-3850            | 93.5        | Transcriptional activator |
| amoA            | Scaffold8  | 60,005-61,177        | 92.9        | Isochorismate synthases |
| topW            | Scaffold25 | 28,637-29,758        | 92.8        | Tlp pilus assembly protein, ATPase PilU |
| topC            | Scaffold45 | 1211-2467            | 92.5        | Type 4 fimbrial assembly protein PilC |
| flrB            | Scaffold148| 141-2445             | 92.2        | Two-component system flagellar sensor histidine kinase FlrB |
results indicate the emergence of the co-location of a large number of resistance genes, a variety of virulence factors, and the PHI genes carrying *Aeromonas caviae* ScAc2001-like strain is a serious issue for public health.

### The Genetic Context of the Resistance Genes

Silicon analysis showed that the resistance gene *bla*KPC-2 was located in scaffold72 and that it also carried the ESBL gene *bla*TEM-1. Sequence analysis showed that scaffold72 had 68%, 59% and 59% query cover and 99.95%, 99.98% and 99.98% sequence similarities with plasmid pGSH8M-1-2 (AP019197), \(^{32}\) plasmid p1713-KPC (MH624132) and plasmid p198-KPC (MH624131) at nucleotide level, respectively. The linear structure of this genetic context is repA-orf-klcA-korC-IJKpn6-bla*KPC*-bla*TEM*-ISKpn27. However, the other sequence of scaffold72 was unique to *Aeromonas caviae* strain ScAc2001. The colistin resistance gene *mcr*-3.3 was located in the scaffold216. Scaffold216 had 81%, 81% and 78% query cover and 99.89%, 99.06%, and 98.29% sequence similarities with the plasmid pGSH8M-1-2 (AP019197), *Aeromonas* ASNIH7 chromosome genome (CP026226), *Aeromonas veronii* 1718Se chromosome genome (CP028133) at nucleotide level, respectively. The result showed the *mcr*-3.3 carrying scaffold maybe derive from the plasmid and *Aeromonas* chromosome genome. In any case, the plasmid or chromosome borne colistin resistance gene *mcr* carried by the carbapenem-resistant -microorganisms is a risk factor in clinical control.

The ESBLs gene *bla*CTX-M-14 carrying scaffold180 had 100%, 98% and 98% query cover and 100%, 99.96% and 99.95% sequence similarities with the *Vibrio* Vib0624 chromosome genome (CP041202), plasmid pKP96 (EU195449)\(^{34}\) and plasmid pEC224_4 (CP018944) at nucleotide level, respectively. Scaffold231 carried four resistance genes (*catB3*, *bla*OXA-10, *aadA1* and *aac(6’)-Ib3*). Sequence analysis showed that scaffold231 had 99%, 99% and 99% query cover and 99.97%, 99.97% and 99.97% sequence similarities with the plasmid pC45_002 (CP042553), plasmid pE33_002 (CP042519) and plasmid pEC224_4 (CP042480) at nucleotide level, respectively. It speculated that scaffold231 was derived from the plasmid.

The ESBLs gene *bla*MOX-6 was carried by the scaffold128. Sequence analysis showed that scaffold128 had 99%, 98% and 98% query cover and 98.3%, 98.17% and 98.15% sequence similarities with the *Aeromonas caviae* GSH8M-1 complete genome (AP019195.1).\(^{32}\) *Aeromonas caviae* strain T25-39 chromosome genome (CP025706.1) and *Aeromonas* strain R25-2 chromosome genome (CP025777.1) at nucleotide level, respectively.

Scaffold165 carried one class D β-lactamases (CHDLs) resistance gene *bla*OXA-42. Sequence analysis showed that scaffold165 had 98%, 98%, and 95% query cover and 95.83%, 95.83% and 95.67% sequence similarities with the *Aeromonas caviae* R25-2 genome (CP025777.1), *Aeromonas caviae* strain T25-39 genome (CP025706) and *Aeromonas caviae* GSH8M-1 genome (AP019195.1) at nucleotide level, respectively. This result showed that the resistance gene

| Table 4 Distribution of the PHI Genes in *Aeromonas caviae* Strain SCAc2001*\(^{a}\)* |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| **Gene Name**                  | **Scaffold**    | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Scaffold Position** | **Identity (%)** | **PHI-Base Accession** |
| Diarrheal diseases         | Small RNA-binding protein involved in the regulation of a wide range of cellular processes. |
| Global transcription factor | Regulators of systemic infection |
| Fe-S cluster sensor        | Aerobic respiration control protein |
| Repressor of the methionine biosynthesis regulon | Part of the pyruvate - tricarboxylic acid cycle |
| node                        | Transcript elongation factor |
| node                        | RNA-binding protein |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
| **Scaffold** | **Position in Scaffold** | **Identity (%)** | **PHI-Base Accession** |
| **Disease Name**              | **Gene Function** |
| **Accession**                     |                     |
| **Name**                       |                     |
bla\textsubscript{OXA-427} is chromosome borne. Another resistance gene \(\text{bla}_{\text{VEB-3}}\) was carried by the scaffold240. Sequence analysis showed that scaffold240 had 90% and 90% query cover and 99.96% and 99.95% sequence similarities with Aeromonas hydrophila strain MX16A genome (CP018201) and JM45 plasmid p1 (CP006657.1) at nucleotide level, respectively. The context genetic of this resistance gene is the \text{Int}1-\text{bla}_{\text{VEB-3}}-\text{IS}600-\text{IS}26. These results indicated co-carriage of a large number of resistance genes in genome making Aeromonas caviae strain highly resistant to almost all kinds of commonly used antibiotics, and brings a serious challenge for resistance control and clinical treatment.

Characterization of the Genomic Islands and Prophages

As shown in Table 5, four genomic islands, named GI_SCAc2001-1 to GI_SCAc2001-4, were identified by the software IslandPATH-DIMOB, IslandPick, and SIGI-HMM. Silicon analysis showed that the length of the four genomic islands were 7,182, 6,950, 8,349 and 7,175bp with the G+C context of 54.58%, 63.42%, 62.27% and 62.45%, respectively. The sequences of four genomic islands are all the closest match to the Aeromonas sp. chromosome genome sequence in genbank. A total of eight prophages (length>2kbp), named Pp_SCAc2001-1 to Pp_SCAc2001-8, were identified by phiSpy. The size of the eight prophages ranged from 2823bp to 18,093bp with the average G+C content of 46.94%-65.37%, respectively. Among them, one of the prophage sequences' closest match was the corresponding region of plasmid pMCR5_045096 and seven of the prophages were the closest match to the Aeromonas sp. chromosome genome in genbank. This indicated that the mobile genetic elements (genomic islands and prophages) can be excised and integrated from the chromosome and mobile genetic elements into each other. However, no resistance genes or virulence genes were found in the genomic islands and prophages. To the best of our knowledge, the mobile genetic elements (including the genomic islands and prophages) are effective integrative elements in bacterial evolution including the resistance, virulence and some function genes.\textsuperscript{35-37}

Characterization of Secondary Metabolism Products Coding Clusters

In this study, three secondary metabolism products coding clusters (nonribosomal peptide synthetases (nrps), hserlactone and bacteriocin), which are responsible for the biosynthesis of
secondary metabolic products, were predicted using the search tool antiSMASH. The silicon analysis showed that the length of the three secondary metabolism coding clusters were 38,514, 17,438, and 9887 bp with the G+C context of 65.27%, 63.52% and 64.36%, respectively. Sequence analysis showed that the three putative gene clusters carrying 27, 13 and 12 ORFs, respectively (Table 6). It's proved that a large number of pharmaceutical agents, microbial natural products including the sterigmatocystin (carcinogen), penicillin vancomycin and (antibiotic), lovastatin (antihypercholesterolemic agent), and cyclosporin A (anti-inflammatory and immunosuppressants) are synthesized by the diverse array of the secondary metabolism products coding clusters. Researching the characterization of secondary metabolism products coding clusters can be seen as one of the potential ways to research the new drugs.38

### Nucleotide Sequence Accession Numbers

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WUTZ00000000.1.

### Ethical Statement

This study was approved by the Experimentation Ethics Committee of Southwest Medical University.

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### Disclosure

The authors report no conflicts of interest in this work.

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| Cluster Name | Location (Start-End) | Length (bp) | G+C% | ORF Number | Predicted Protein |
|--------------|----------------------|-------------|------|------------|------------------|
| nrps         | Scaffold8 (25,698–64,211) | 38,514      | 65.27 | 27         | TerC family protein, AroG, AroA, SerC, FixB, FixA, FAD-binding protein, CorA, DUF2919 family protein, hypothetical protein, amonabactin ABC transporter permease subunit 2, Amonabactin ABC transporter permease subunit 1, Amonabactin ABC transporter ATP-binding protein, 4'-phosphopantetheinyl transferase superfamily protein, ABC transporter substrate-binding protein, AmoH, AmoG, DhBA, AmoF, Isochorismatase family protein, EntE isochorismate synthase, Lipoprotein, hypothetical protein |
| hserlacitone | Scaffold70 (246–17,682) | 17,438      | 63.32 | 13         | HldE, LpxL, bifunctional 2'3'-cyclic-nucleotide 2'-phosphoesterase/3'-nucleotidase, LysE family transporter, LuxR family transcriptional regulator, GNAT family N-acetyltransferase, ArgP/LysG family DNA-binding transcriptional, exoribonuclease II, PTS mannitol transporter subunit IIICBA, mannitol-1-phosphate 5-dehydrogenase, MltR family transcriptional regulator, sodium:proton antiporter, hypothetical protein |
| bacteriocin   | Scaffold139 (89–9975) | 9887        | 64.36 | 12         | Hypothetical protein, BCCT family transporter, DUF2282 domain-containing protein, DUF692 family protein, DUF2063 domain-containing protein, DsoX family protein, GNAT family N-acetyltransferase, PAS domain S-box protein, DUF3332 domain-containing protein, HD domain-containing protein |

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*Table 6 Characterization of Secondary Metabolism Products’ Coding Clusters*
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