Infection and Drug Resistance

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ORIGINAL RESEARCH

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

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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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 (bla_{KPC}, $bla_{CTX-M-14}$, bla_{TEM-1}, bla_{OXA-10}, $bla_{OXA-427}$, bla_{VEB-3} and bla_{MOX-6}), aminoglycosides (*aadA1*), fluoroquinolones (*aac(6')-Ib-cr*), phenicol resistance (*catB3*), sulfonamide (*sul1*), trimethoprim (*dfrA5*) and colistin resistance (*mcr-3.3*).And also, *A. c*aviae ScAc2001 carried 54 putative virulence genes including the type IV pilus, fimbria, flagellarthe, 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*, bla_{KPC-2}, *mcr-3.3*, virulence factors, secondary metabolism products

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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 (bla_{KPC}, bla_{NDM}, bla_{IMP}, and bla_{VIM}), 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 (bla_{KPC}, bla_{NDM}, bla_{IMP}, and bla_{VIM}) 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 (bla_{KPC}, bla_{NDM}, bla_{GES}, bla_{IMP} and bla_{VIM}) 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 SIGI-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 bla_{KPC} 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,

Table 1 The Details of the Representative *A. Caviae* Strains Downloaded from Genbank

Isolate	Access Number	Country	Samples	Resistance Genes[a]
GSH8M-1 chromosomes and plasmids genome	AP019195.1	Japan	Wastewater	*bla*$_{MOX-12}$, *bla*$_{OXA-780}$, mcr-3.18, *bla*$_{OXA-669}$, *aac(6')-Ia, aadA2, sul1*, *bla*$_{KPC-2}$, *mph(A)*
WCW1-2 chromosomes genome	CP039832.1	People's Republic of China	Sewage	*bla*$_{MOX-5}$, *bla*$_{OXA-427}$, *catB3, cmlA1, floR, aac(6')-Ib-cr, qnrVC4, sul1, aac(6')-Ib3, aadA1, aph(3")-Ib, aph(3')-Ia, aph(6)-Id, dfrA14, dfrB4*, *bla*$_{OXA-10}$, *tet(X4)*
T25-39 chromosomes genome	CP025706	People's Republic of China	Wastewater	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$
R25-2 chromosomes genome	CP025777.1	People's Republic of China	Wastewater	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, *aph(3")-Ib, aph(6)-Id, tet(31), sul2, floR*
NCTC12244 Chromosomes genome	LS483441.1	UK	N	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$
Draft genome ZJ17-2	NXBR00000000.1	People's Republic of China	River water	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, *mcr-3, cat, tet(A), aadA1, sul1*
Draft genome A23	LFXO00000000.1	People's Republic of China	Chicken sample	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, *catB3, mph(A)*, *bla*$_{OXA-2}$, *bla*$_{PER-3}$, *aadA1b, aadA16, ac(6')-Ib-Hangzhou, aac(6')-Ib-cr, sul1*
Draft genome ZJ33-3	NXBW00000000.1	People's Republic of China	Human rectal swab	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, *tet(A), mcr-3, cat, aadA1, sul1*
Draft genome AK245	JAAALU000000000.1	USA	Lake water	*bla*$_{MOX-6}$
Draft genome Sch29	CAAKNG000000000.1	UK	Gastroenteritis samples	*bla*$_{MOX-4}$, *bla*$_{OXA-427}$,
Draft genome L12	JWJP00000000.1	Malaysia	Lake water	*bla*$_{MOX-5}$, *mcr-3.15*
Draft genome TCO22	NMSG00000000.1	USA	Gut samples	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, *tetE, mcr3.12, mcr-3.15, aph(3')-Ia, mph(A)*
Draft genome strain D	VZQB00000000.1	South Africa	Seawater	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$,
Draft genome strain CH129	MDSD00000000.1	Brazi	Seawater	*bla*$_{MOX-6}$
Draft genome strain CHZ306	MDSC00000000.1	Brazi	Seawater	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, tetE
Draft genome strain ScAc2001	WUTZ00000000.1 (In this study)	People's Republic of China	Human sample	*bla*$_{MOX-6}$, *bla*$_{OXA-427}$, *aac(6')-Ib3, aadA1*, *bla*$_{OXA-10}$, *catB3, sul1, mcr-3, dfrA5*, *bla*$_{CTX-M-14}$, *bla*$_{KPC-2}$, *bla*$_{TEM-150}$, *bla*$_{VEB-3}$

Notes: [a]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.

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,[28,29] because the *Aeromonas* species have always been identified as enteric pathogens which can lead to severe diarrhoea.[9] Unfortunately, what's more serious is that the carbapenem-

resistant and/or colistin-resistant strains produce emergencies in some countries and pose a serious threat to infectious diseases control and clinical treatment.[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 4487370bp. 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 bla_{KPC-2} co-carried strain GSH8M-1 (AP019195.1)[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 (bla_{KPC}, $bla_{CTX-M-14}$, bla_{TEM-1}, bla_{OXA-10}, $bla_{OXA-427}$, bla_{VEB-3} and bla_{MOX-6}), aminoglycosides (*aadA1*), fluoroquinolones (*aac(6')-Ib-cr*), phenicol resistance (*catB3*), sulfonamide (*sul1*) and trimethoprim (*dfrA5*) and colistin resistance gene (*mcr-3.3*) were detected in the genome of *Aeromonas*

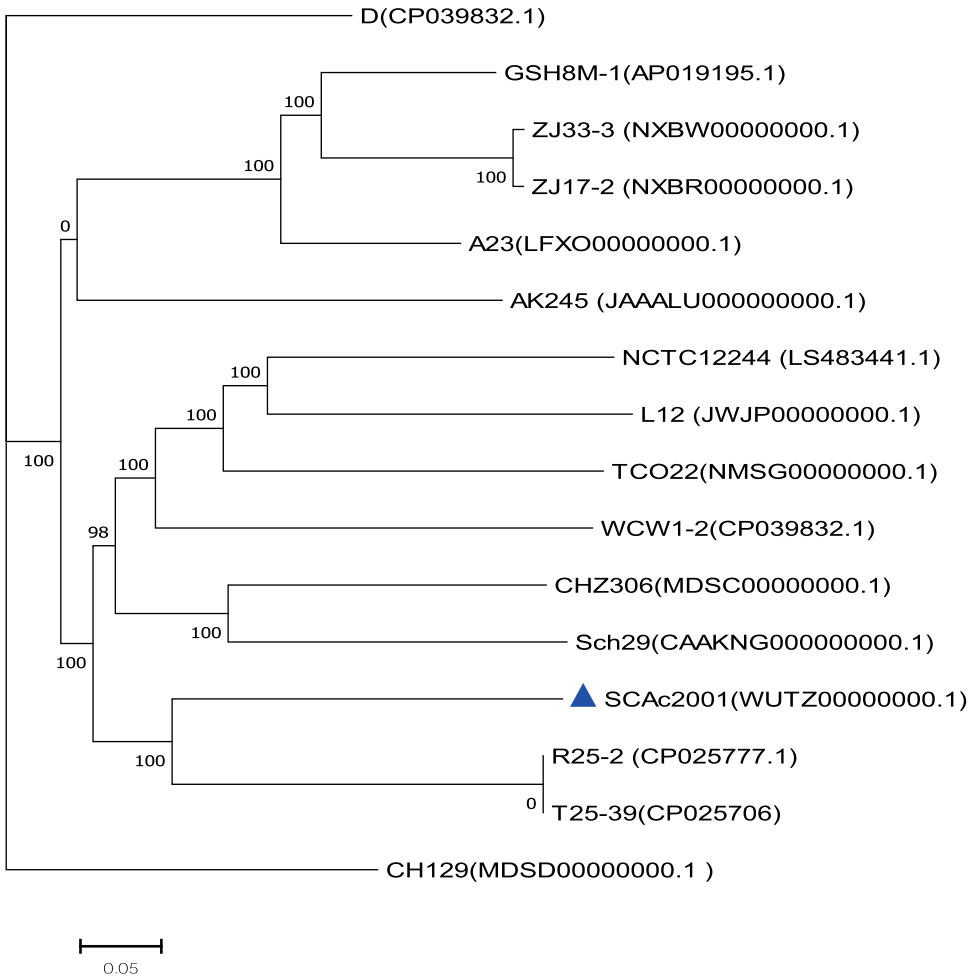

Figure 1 The phylogenetic tree was constructed by aligning the core genome of *Aeromonas caviae* SCAc2001 strain with 15 other representative *A. caviae* strains.

Table 2 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	555/555	Scaffold231	2433-2987	Fluoroquinolone and aminoglycoside resistance	X60321
aadA1	100	792/792	Scaffold231	29-820	Aminoglycoside resistance	JQ414041
bla_{OXA-10}	100	801/801	Scaffold231	837-1637	Beta-lactam resistance Alternate name; PSE-2	J03427
catB3	100	633/633	Scaffold231	1706-2338	Phenicol resistance	U13880
sul1	100	840/840	Scaffold310	25-864	Sulphonamide resistance	U12338
mcr-3.3	100	1623/1623	Scaffold216	1393-3015	Polymyxin resistance	MF495680
dfrA5	100	474/474	Scaffold258	1177-1650	Trimethoprim resistance	X12868
$bla_{CTX-M-14}$	100	876/876	Scaffold180	3068-3943	Beta-lactam resistance	AF252622
bla_{KPC-2}	100	882/882	Scaffold72	15,640-6521	Beta-lactam resistance	AY034847
bla_{MOX-6}	97.83	1153/1152	Scaffold128	10,749–11,900	Beta-lactam resistance AmpC-type	GQ152601
$bla_{OXA-427}$	86.43	796/795	Scaffold165	5530–6324	Beta-lactam resistance	KX827604
$bla_{TEM-150}$	99.82	570/861	Scaffold72	14,849–15,418	Beta-lactam resistance	AM183304
bla_{VEB-3}	100	900/900	Scaffold240	750-1649	Beta-lactam resistance	AY536519

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 *hfq* from the genome (Table 4). It proved that these PHI genes are associated with diseases such as diarrheal, meningitis and urinary tract infections.[33] These

Table 3 Distribution of the Virulence Factors in *Aeromonas caviae* Strain SCAc2001[a]

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
tapY1	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
tapT	Scaffold84	9088–10,044	95.9	Twitching ATPase
hutZ	Scaffold20	28,013–28,570	95.7	Heme iron utilization protein
exeF	Scaffold3	14,698–15,918	95.6	General secretion pathway protein F
tapB	Scaffold45	118–1098	95.3	Type IV-A pilus assembly ATPase PilB
exeI	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
tapB	Scaffold288	85–870	94.4	Type IV pilus assembly protein TapB
fleR/flrC	Scaffold148	2516–3850	93.5	Transcriptional activator
amoA	Scaffold8	60,005–61,177	92.9	Isochorismate synthases
tapW	Scaffold25	28,637–29,758	92.8	Tfp pilus assembly protein, ATPase PilU
tapC	Scaffold45	1211–2467	92.5	Type 4 fimbrial assembly protein PilC
flrB	Scaffold148	1411–2445	92.2	Two-component system flagellar sensor histidine kinase FlrB

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 4 Distribution of the PHI Genes in *Aeromonas caviae* Strain SCAc2001[a]

Gene Name	Scaffold	Position in Scaffold	Identity (%)	PHI-Base Accession	Disease Name	Gene Function
csrA	Scaffold19	1661–1849	96.6	PHI:5061	Diarrheal diseases	Small RNA-binding protein involved in the regulation of a wide range of cellular processes.
lrp	Scaffold106	12,874–13,143	92.1	PHI:6497	No data found	Global transcription factor
crp	Scaffold20	14,048–14,686	89.6	PHI:2684	Food poisoning	Regulators of systemic infection
iscU	Scaffold22	19,716–20,099	85.6	PHI:6892	Salmonellosis	Fe-S cluster sensor
arcA	Scaffold24	11,882–12,601	84.8	PHI:6532	Meningitis	Aerobic respiration control protein
metJ	Scaffold36	1313–1636	83.2	PHI:2695	Blackleg disease	Repressor of the methionine biosynthesis regulon
pykF	Scaffold51	7554–8978	82.8	PHI:3134	Gut-associated diseases; diarrhoea; enteritis; colitis	Part of the pyruvate - tricarboxylic acid cycle node
lon	Scaffold68	14,376–16,730	82.6	PHI:7160	Fire blight	Protease
dksA	Scaffold40	12,676–13,059	81.7	PHI:6508	Salmonellosis	Response Regulator
fur	Scaffold49	144–572	81.7	PHI:4887	Urinary tract infections	global ferric uptake regulator
greB	Scaffold83	192–665	81.5	PHI:7131	Salmonellosis	Transcription elongation factor
hfq	Scaffold27	23,382–23,570	81.1	PHI:4064	Pneumonic plague	RNA-binding protein

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*-*IS*Kpn6-bla_{KPC}-bla_{TEM}-*IS*Kpn27. 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* 17ISAe 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* Vb0624 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 (CP0 25777.1) at nucleotide level, respectively.

Scaffold165 carried one class D β-lactamases (CHDLs) resistance gene $bla_{OXA-427}$. 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 5 Overall Features of the *Aeromonas caviae* SCAc2001 Genomic Islands and Prophages

Genomic Islands and Prophages		Location (Start-End)	Length (bp)	G+C%	Closest Match in Genbank (Query Cover and Identity)
Genomic islands	GI_SCAc2001–1	Scaffold26 (22,433–29,614)	7182	54.58	*Aeromonas sp.* ASNIH7 chromosome genome (74%,91.04%)
	GI_SCAc2001–2	Scaffold42 (14,540–21,489)	6950	63.42	*Aeromonassp.* ASNIH3 chromosome genome (100, 99.07%)
	GI_SCAc2001–3	Scaffold57 (7135–15,483)	8349	62.27	*Aeromonas caviae*FDAARGOS_72 genome (98%, 99.19%)
	GI_SCAc2001–4	Scaffold5 (42,875–50,049)	7175	62.45	*Aeromonas caviae*WCW1-2 genome (100%, 98.27%)
Prophages	Pp_SCAc2001–1	Scaffold159 4884–7973	3090	58.93	plasmid pMCR5_045096 (100%, 100%)
	Pp_SCAc2001–2	Scaffold158 413–7151	6739	63.6	*Aeromonassp.* ASNIH2 chromosome genome (100%, 99.6%)
	Pp_SCAc2001–3	Scaffold209 146–2968	2823	61.18	*Aeromonas caviae*GSH8M-1 genome (100%, 98.87%)
	Pp_SCAc2001–4	Scaffold38 8991–27,083	18,093	65.37	*Aeromonas sp.* ASNIH2 chromosome genome (98%, 97.62%)
	Pp_SCAc2001–5	Scaffold202 221–4124	3904	61.94	*Aeromonas caviae* strain R25-6 genome (100%, 99%)
	Pp_SCAc2001–6	Scaffold201 95–3149	3055	46.94	*Aeromonas media* WS genome (98%, 99.87%)
	Pp_SCAc2001–7	Scaffold128 236–10,450	10,215	63.55	*Aeromonas caviae* FDAARGOS_72 genome (100%, 98.47%)
	Pp_SCAc2001–8	Scaffold129 479–4423	3945	58.93	*Aeromonas hydrophila* strain WCHAH045096 chromosome genome (80%, 94.6%)

$bla_{OXA-427}$ is chromosome borne. Another resistance gene bla_{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 *Int*1-bla_{VEB-3}-*IS*600-*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 54.585%, 63.425%, 62.275% 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-en>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.[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

Table 6 Characterization of Secondary Metabolism Products' Coding Clusters

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
hserlactone	Scaffold70 (246–17,682)	17,438	63.52	13	HldE, LpxL, bifunctional 2′,3′-cyclic-nucleotide 2′-phosphodiesterase/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 IICBA, 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, DoxX family protein, GNAT family N-acetyltransferase, PAS domain S-box protein, DUF3332 domain-containing protein, HD domain-containing protein

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-inflammatories 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]

Conclusion

Isolates of *Aeromonas caviae* ScAc2001 harboring a lot of resistance genes including the carbapenem-resistant gene bla_{KPC}, colistin resistance gene *mcr-3.3* and other β-lactams, aminoglycosides, fluoroquinolones, phenicol resistance (*catB3*), sulfonamide and a variety of virulence factors, PHI genes, four genomic islands and eight prophages may result in a possible risk to public health.

Nucleotide Sequence Accession Numbers

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession WUTZ 00000000.1.

Ethical Statement

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

Funding

This work was supported by the National Natural Science Foundation of China (31500114) and United Funds of Luzhou and Southwest Medical University [2018LZ XNYD-ZK51].

Disclosure

The authors report no conflicts of interest in this work.

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