Journal of King Saud University - Science 36 (2024) 103402

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Journal of King Saud University - Science

journal homepage: www.sciencedirect.com

# Comprehensive analysis of antibiotic and heavy metal resistance, and virulence factors in *Aeromonas veronii* CTe-01: Implications for global antimicrobial resistance

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ARTICLE INFO

*Keywords:*
*Aeromonas veronii*
Heavy metal resistance
Antibiotic resistance
Genomics

ABSTRACT

*Objectives:* This study aimed to characterize *Aeromonas veronii* CTe-01 focusing on its resistance to heavy metal and antibiotics.
*Methods: A. veronii* CTe-01 was characterized using standard microbiological and molecular techniques. Antibiotic susceptibility and heavy metal resistance were tested per standard protocols. Genomic analysis, including plasmid characterization, was conducted in silico.
*Results: A. veronii* CTe-01 showed resistance to various heavy metals and antibiotics. Multiple resistance genes were identified, including those for beta-lactamases, heavy metal resistance, and type III secretion system components. The bacterium carries a 9 kb plasmid with *repA/repB* replication genes, *parA/parB* partitioning genes, and a type II toxin-antitoxin system for stability.
*Conclusions: A. veronii* CTe-01 is a genetic reservoir for antibiotic resistance, heavy metal resistance genes, and virulence factors. The study offers insights into its dual role as a pathogen and heavy metal remediator in aquatic environments.

## 1. Introduction

The Aeromonads group includes about 36 species (Fernández-Bravo and Figueras, 2020), with *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas dhakensis*, *Aeromonas veronii*, and *Aeromonas salmonicida* being economically and pathologically significant (Fernández-Bravo and Figueras, 2020; Skwor et al., 2014). These species are primary causes of infections and mortality in fish (Janda and Abbott, 2010; Walczak et al., 2017), and other aquatic animals (Hu et al., 2023). Besides, some are emerging pathogenic bacteria in humans (Janda and Abbott, 2010; Zhou et al., 2019). The pathogenicity factors related to these bacteria are: *aer, hly, act, ast, alt* (hemolysin), *fla* (flagellin), *ser* (serine protease), *exu* (DNase) (Fernández-Bravo and Figueras, 2020), and type III secretion systems (T3SS), which inject toxins and effectors into host cells (Silver et al., 2007).

Members of *Aeromonas* genus are resistant to multiple antibiotics and the related genes include β-lactams ($bla_{TEM}$, $bla_{SHV}$, $bla_{CepH}$) (Sun et al., 2021), quinolone (*qnrAB*), tetracycline (*tetACE*) (Nawaz et al., 2006), sulfonamide (*sul1*), macrolide (*mphA-mrx-mphR*), aminoglycosides (*aadA2*, *aac(30)-IIa*), chloramphenicol (*catB3*), among others (Dahanayake et al., 2020). Similarly, heavy metal-resistant *Aeromonas* strains possess genes for resistance to copper (*copA*), mercury (*merA*), cobalt/zinc/cadmium efflux protein (*czcA*), and chromium (*chrR*) (De Silva et al., 2018).

In the environment, virulence factors and resistance genes can be transferred between bacteria through horizontal gene transfer (HGT).

*Abbreviations:* bp, base pairs; kb, Kilobase pair; kDa, Kilodaltons; $Te^R$, tellurium resistance; $Hg^R$, mercury resistance; $Ag^R$, silver resistance; $Cr^R$, chromium resistance; $Co^R$, cobalt resistance; PGAP, Prokaryotic Genome Annotation Pipeline; NODE, contiguous sequence of bases in an assembly graph; Locus Tag, specific genomic region within an organism's genome.

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https://doi.org/10.1016/j.jksus.2024.103402
Received 10 June 2024; Received in revised form 17 August 2024; Accepted 18 August 2024
Available online 19 August 2024

*Aeromonas* spp. carry mobile genetic elements (MGEs) like plasmids, with segregation mechanisms involving *repA/repB* genes (Dobiasova et al., 2016), transposons, bacteriophages, integrons, and insertion sequences (Piotrowska and Popowska, 2015). These elements drive bacterial evolution and adaptation to adverse conditions (Aminov, 2011).

*A. veronii* has been isolated from aquatic sources, soil, food, warm-blooded, and cold-blooded animals (Fernández-Bravo and Figueras, 2020; Janda and Abbott, 2010). Strains from different sources exhibit varied profiles of virulence, heavy metal resistance, and antimicrobial resistance (Hu et al., 2023). In wastewater, the coexistence with diverse bacterial species enhances the potential for gene exchange between clinical and environmental origins (Aminov, 2011).

Genomic sequencing and in silico analysis are powerful tools for predicting genes related to metabolism, pathogenicity, resistance, and more, aiding our understanding of microbes roles in nature (Elarabi et al., 2023; El-Beltagi et al., 2023; Halema et al., 2024). Genomic analysis of *A. veronii* MS-18–37 predicted genes for heavy metal, fluoroquinolone, and multidrug resistance (Abdelhamed et al., 2019). Similarly, the draft genome of *A. veronii* CTe-01 revealed genes for heavy metal and antibiotic resistance (Tataje-Lavanda et al., 2019).

This study aimed to characterize the antibiotic and heavy metal resistance, along with the pathogenic properties of *A. veronii* CTe-01. We also investigated potential MGEs on its genome, an aspect not yet widely explored in *Aeromonas* spp. Our findings suggest that *A. veronii* serves as a reservoir of virulence, heavy metal, and antibiotic resistance genes that could spread to other bacteria in aquatic sources as wastewater.

## 2. Material and methods

### 2.1. Bacterial strains and growth conditions

*A. veronii* CTe-01 and *E. coli* K-12 BW25113 were grown in LB medium at 37 °C for 24 h. Strain stocks were stored at −20 °C in LB medium with 30 % glycerol.

### 2.2. Molecular identification

The identity of *A. veronii* CTe-01 was confirmed through sequence analysis of the 16S rRNA, *rpoD*, and *gyrB* genes from the draft genome (**VATZ00000000.2**), compared with the GenBank database using BLASTn for nucleotide collection and highly similar sequences (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch). The sequences are available under accession codes **MK876839.1, VATZ01000092.1:14350–16215,** and **VATZ01000001.1:16817–19228**, respectively.

### 2.3. Biochemical and physiological characterization

In addition to biochemical characteristics assessed with the VITEK® 2 Compact system (Tataje-Lavanda et al., 2019), further tests included citrate utilization; fermentation of glucose, lactose, and sucrose; catalase and oxidase tests; lysine decarboxylation and deamination; acetoin production; urease, gelatinase, and ornithine decarboxylase activities; indole production; motility; and esculin hydrolysis.

### 2.4. Determination of minimal inhibitory concentration (MIC) for heavy metals and antibiotics

The MICs (μg/mL) of various metal(loid) salts for *A. veronii* CTe-01 were determined as follows: $HgCl_2$, $AgNO_3$, $K_2Cr_2O_7$, and $K_2CrO_4$ (0–100); $K_2TeO_3$ (0–200); $CdCl_2$ and $ZnSO_4{\cdot}7H_2O$ (0–300); $CoCl_2{\cdot}6H_2O$ (0–400); $NiSO_4{\cdot}6H_2O$ and $CuSO_4$ (0–600). Triplicate assays were conducted at 37 °C in 2 mL LB medium, without agitation. Antibiotic susceptibility and MICs were assessed using the MicroScan WalkAway 96 Plus system (Siemens).

### 2.5. Molecular characterization of plasmid pCTe-01

The plasmid pCTe-01 was purified with the QIAprep Spin Miniprep kit (QIAGEN) and its size estimated by agarose gel electrophoresis (1 %) in TAE buffer, using a Supercoiled DNA ladder (New England Biolabs) as a standard. The plasmid was then digested with *Bam*HI, *Bs*TNI, *Mbo*I, and *Pst*I, according to the manufacturer's instructions (New England Biolabs). Fragment sizes were determined by 1.5 % agarose gel electrophoresis using a 1 kb Plus DNA ladder (Thermo Fisher Scientific) as a standard.

### 2.6. Sequencing plasmid pCTe-01 and improving the draft genome of *A. veronii* CTe-01

Draft genome of *A. veronii* CTe-01 [**VATZ00000000.1**], and plasmid pCTe-01 were additionally sequencing at Macrogen, Inc. (South Korea) using the Illumina platform with 101-bp paired-end reads (TruSeq Nano DNA Kit) [BioSample: **SAMN11620977**]. Quality control included FastQC v0.11.9 and Trimmomatic v0.39 (Bolger et al., 2014) for the reads of three libraries. De novo genome assembly using SPAdes v3.13.1 improved metrics compared to the previous version (N50: 103,789; L50: 12). Contig identity was verified with KmerFinder v3.2, showing similarity to *A. veronii* strain X12 (**NZ_CP024933.1**). Contiguator2 (Galardini et al., 2008) organized the contigs into 60 mapped and 809 unmapped contigs. Plasmid pCTe-01 was manually assembled and circularized, incorporating unmapped contigs with coverage over 10 000 bp. The updated draft genome has been deposited in GenBank for annotation with NCBI PGAP v5.1 after automatically removing small contigs and contaminants (**VATZ00000000.2**).

### 2.7. In silico predictions

Resistome analysis was performed using ARIBA v.2.146 with default parameters and databases including ARG-ANNOT (Gupta et al., 2014), CARD (McArthur et al., 2013), MEGARes (Doster et al., 2020), ResFinder (Zankari et al., 2012), and VFDB (Chen et al., 2016), using unfiltered reads, and KmerFinder software. The RASTtk (Aziz et al., 2008), KmerResistance v2.2, and Phaster (Arndt et al., 2016) were used to identify antimicrobial and heavy metal resistance genes, and phage and prophage sequences in contigs, using default parameters. Prophage DNA sequences were classified as intact (>90), questionable (70–90), or incomplete (<70). The pCTe-01 sequence was analyzed in silico with PGAP v5.1 and RASTtk, and subjected to virtual digestion using SnapGene 1.1.3. We also examined *A. veronii* CTe-01 genome for the presence of *aer*, *hlyA*, *alt*, and *ast* virulence genes.

## 3. Results

### 3.1. Characterization of *A. veronii* CTe-01

The identity of *A. veronii* was confirmed by 16S rRNA, *rpoD*, and *gyrB* sequence similarity. This strain is motile, non-lactose fermenting, catalase-negative, oxidase-negative, and indole-positive. It utilizes citrate and succinate, and efficiently ferments D-glucose, D-maltose, D-mannitol, D-mannose, and D-trehalose, with less efficiency for sucrose. Additionally, *A. veronii* CTe-01 exhibits L-proline-arylamidase, tyrosine-arylamidase, β-N-acetyl-glucosaminidase, and lysine decarboxylase activities.

This bacterium harbors the hemolytic *aer* gene, which is 1467 bp long and located between nucleotides 14 313 and 15 779, on NODE 18 (**VATZ00000000.2**). The Aer protein is predicted to be a 54 kDa polypeptide (https://web.expasy.org/compute_pi/). No similar sequences to other hemolytic genes as *hlyA*, *alt*, and *ast* genes were found on the gDNA sequence.

### 3.2. Characterization of plasmid pCTe-01

We identified a 9059 bp plasmid in *A. veronii* CTe-01 with a GC content of 54.8 %, confirmed by electrophoresis and in silico analysis (Fig. 1, Fig. 2). Gel electrophoresis fragment sizes (Fig. 1), matched predictions from in silico digestion using SnapGene v1.1.3. Digestion with *Bam*HI and *Pst*I enzymes yielded fragments of approximately 5586, 2752, 721 bp; and 2813, 2216, 1941, 1696, 393 bp, respectively. *Bs*TNI produced a 2000 bp fragment and several smaller than 700 bp, while *Mbo*I yielded no fragments.

In silico predictions indicated that plasmid pCTe-01 replicates with RepA/RepB proteins and segregates using ParA-like and ParB proteins. Stabilization proteins identified include a type II toxin-antitoxin (TA) system (RelE/ParE, RelB/StbD, and RelB/DinJ) (Table 1). Additionally, a secretory immunoglobulin A-binding protein, EsiB, and five hypothetical proteins also were detected.

### 3.3. Heavy metal and antibiotic resistance of A. veronii CTe-01

*A. veronii* CTe-01 demonstrated resistance to various toxic metal (loid) salts, including $K_2TeO_3$, $HgCl_2$, $AgNO_3$, $K_2CrO_4$, $CdCl_2$, $ZnSO_4{\cdot}7H_2O$, and $NiSO_4{\cdot}6H_2O$ In silico analysis revealed potential heavy metal (loid) resistance genes on the chromosome (Table 2). Antibiotic MIC results showed intrinsic resistance to penicillin, ampicillin, ampicillin/sulbactam, amoxicillin/clavulanic acid, erythromycin, and cefazolin (Table 3). Both strains tested were resistant to clindamycin, oxacillin, linezolid, daptomycin, and synercid; showed intermediate resistance to piperacillin/tazobactam and rifampicin; and were susceptible to cefepime, ceftazidime, gentamycin, levofloxacin, moxifloxacin, ertapenem, nitrofurantoin, ticarcillin/clavulanic acid, trimethoprim/sulfamethoxazole, and tobramycin.

### 3.4. Improved genomic annotation and identification of resistance-associated genes, pathogenicity factors, and phage-related sequences on A. veronii CTe-01 genome

PGAP annotations of the *A. veronii* CTe-01 draft genome revealed significant improvements: contigs decreased from 272 to 200, pseudogenes from 162 to 145, rRNAs increased from 4 to 14, and tRNAs from 40 to 107.

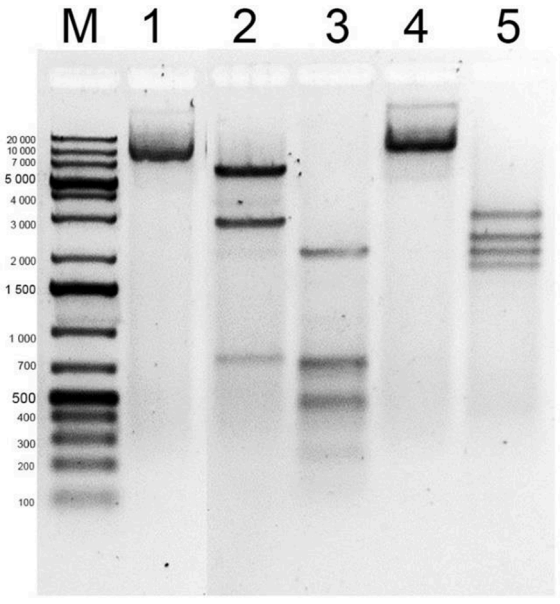


**Fig. 1.** Agarose gel electrophoresis (1.5 % in TAE buffer) of plasmid pCTe-01 after restriction endonuclease digestion. Lane M, molecular weight standard (1 kb plus); lane 1, undigested pCTe-01; lanes 2–5, digestions carried out using *Bam*HI, *Bs*TNI, *Mbo*I, and *Pst*I enzymes, respectively.

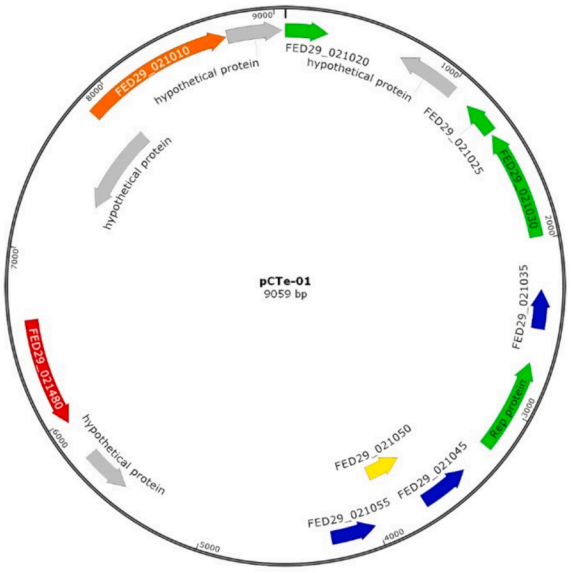


**Fig. 2.** Circular diagram of plasmid pCTe-01. Colored arrows indicate coding sequences (CDS): green, ParAB and RepAB; blue, Rel; yellow, antitoxins; red, Sel1 family proteins; orange, integrase domain proteins; and gray, hypotheticals.

**Table 1**
Products of CDS in plasmid pCTe-01.

| Product | Length (bp) | Locus Tag |
|---|---|---|
| Integrase domain-containing protein | 876 | FED29_021010* |
| RepA, replication initiation | 534 | **, *** |
| RepB, plasmid replication | 243 | FED29_021020* |
| ParA-like, ATPase chromosome-partitioning | 630 | FED29_021030* |
| ParB, chromosome partitioning | 198 | FED29_021025* |
| RelB/StbD antitoxin to RelE/StbE (replicon stabilization) | 243 | FED29_021050* |
| RelB/DinJ antitoxin (Type II toxin-antitoxin) | 261 | FED29_021055* |
| RelE/ParE toxin (Type II toxin-antitoxin) | 222 | FED29_021035* |
| EsiB, immunoglobulin A-binding protein | 624 | FED29_021480* |

Annotated by, *: PGAP v5.1, **: RASTtk, ***: BLASTp.

**Table 2**
Minimal inhibitory concentrations (MICs) and associated genes to heavy metal (loid) resistance for *A. veronii* CTe-01.

| Heavy metal | *A. veronii* CTe-01 MIC (μg/mL) | *E. coli* BW25113 | Genomic analysis | |
|---|---|---|---|---|
| | | | Gene detected | Function |
| $K_2TeO_3$ | 150 | 1 | *terABD* | $Te^R$ |
| $HgCl_2$ | 20 | 3 | *merA* | $Hg^R$ |
| $AgNO_3$ | 50 | 7 | *cusAB* | $Ag^R$ |
| $K_2CrO_4$ | 60 | 40 | *chrA* | $Cr^R$ |
| $CdCl_2$ | 100 | 75 | *czcD* | Cd transport |
| $ZnSO_4{\cdot}7H_2O$ | 250 | 130 | *czcD* | Zn transport |
| $NiSO_4{\cdot}6H_2O$ | 450 | 250 | *nikACE2* | Ni transport |
| $CoCl_2{\cdot}6H_2O$ | 150 | 250 | *czcD* | $Co^R$ |
| $CuSO_4$ | 250 | 550 | *cutCE, corC, scsABCD, cpxAR* | Cu homeostasis |

Analysis of the raw data revealed several resistance genes, including β-lactamases like *amp*H/*amp*S, $bla_{OXA12}$, $bla_{TEM}$, and *cph*A4, among others. Additionally, type III secretion systems (*acr, asc, exs*) and motility genes, key pathogenicity factors, were predicted (Table 4). A search for heavy metal resistance genes identified candidates for Cu, Co, Zn, Cd, As, Ni, Hg, and Te (Table 5). The draft genome of *A. veronii* CTe-

**Table 3**
Antibiotic MICs for *A. veronii* CTe-01 and *E. coli* BW25113.

| Antibiotic | MIC (μg/mL) | | | |
|---|---|---|---|---|
| | ***A. veronii* CTe-01** | | ***E. coli* BW25113** | |
| Penicillin | > 8 | R | < 2 | S |
| Ampicillin | >8 | R | ≤ 8 | S |
| Ampicillin/Sulbactam | ≥ 8/4 | R | ≤ 8/4 | S |
| Amoxicillin/Clavulanic acid | ≤4/2 | R | ≤ 4/4 | S |
| Erythromycin | > 4 | R | 2 | I |
| Cefazolin | ≥ 4 | R | ≤ 2 | S |
| Ceftriaxone | ≤ 8 | S | ≤ 8 | S |
| Cefuroxime | ≤ 4 | S | ≤ 4 | S |
| Ciprofloxacin | ≤ 1 | S | ≤ 1 | S |
| Aztreonam | ≤ 8 | S | ≤ 8 | S |
| Amikacin | ≤ 8 | S | ≤ 8 | S |
| Imipenem | ≤ 1 | S | ≤ 1 | S |
| Piperacillin | 16 | I | ≤ 16 | S |

S: sensitive, I: intermediate, R: resistant.

**Table 4**
Resistome analysis of raw data using ARIBA and KmerFinder.

| Gene | Product/Description | Database |
|---|---|---|
| *Acr, aop, asc* | associated to Type III secretion system (T3SS) | VFDB_CORE |
| *acr1, acr2* | Acr1, gatekeeper; Acr2 chaperone | |
| *acrGHRV* | AcrG, AcrH, AcrR, chaperones; AcrV, V-tip protein | |
| *aopN* | AopN, gatekeeper | |
| *ascBY* | AscB, AscY chaperones | |
| *ascCD* | AscC outer membrane, AscD inner membrane rings | |
| *ascRSTU* | AscR, AscS, AscT, AscU, export apparatus | |
| *ascEHIJKLNOQVX* | AscEHIJKLNOQVX proteins | |
| *ampHS* | β-lactamases | RESFINDER/ KMERFINDER |
| $bla_{OXA-12}$ | Associated to cefazolin inactivation | CARD |
| $bla_{OXA-72}$ | β-lactamase OXA-72, carbapenem-hydrolyzing | MEGARES |
| $bla_{TEM-1}$ | Antibiotic inactivation | CARD/MEGARES |
| $bla_{TEM-10,}$ | β-lactamase TEM-10 | RESFINDER/ KMERFINDER |
| $bla_{TEM-101}$ | β-lactamase TEM-101 | ARGANNOT |
| *cphA4* | β-lactamase, carbapenem resistance | KMERFINDER |
| *exeG* | ExeG, pseudopilin | VFDB_CORE |
| *exsABCD* | ExsABCD proteins | |
| *fimACD* | FimACD proteins | |
| *fliGM* | FliG, FliM, flagellar motor switches | |
| *tapTW* | TapT, TapW, pilus ATPases | |

01 showed four regions with phage-associated genes related to *Aliivibrio fischeri*, *Bacillus cereus*, *Acinetobacter baumannii*, and *E. coli* (Table 6). Predicted phage and prophage proteins, included recombinase/integrase sequences and proteins related to phage DNA synthesis and structure, were found. Components of the *psp* operon, such as *pspABC*, were also detected using RASTtk (Table 7).

## 4. Discussion

### 4.1. Characterization of A. veronii CTe-01

*A. veronii* CTe-01 was isolated from wastewater containing domestic, hospital, and industrial effluents, rich in various microorganisms and chemicals (Tataje-Lavanda et al., 2019). This bacterium can utilize various carbohydrate sources and shows diverse enzymatic activities, which could contribute to its survival in the complex environment. Its β-hemolytic property may be related to aerolysin (Aer), encoded by the *aer* gene, which was the only gene detected among those investigated. This property may contribute to degrade animal or organic compounds in the environment, similar to processes in domestic wastewater (Tataje-Lavanda et al., 2019). β-hemolysis from aerolysin is a common feature in *Aeromonas* isolates from fish, clinical, and food samples (Janda and Abbott, 2010). In contrast, other strains have additional hemolytic genes like *act*, *hly*, and *ast* (Sun et al., 2021).

**Table 5**
Resistance analysis for heavy metal and antimicrobial compounds using RASTtk.

| Resistance subsystem | Role | Locus Tag |
|---|---|---|
| Arsenic-antimonite | ArsA, arsenite/antimonite pump-driving ATPase | FED29_015035 |
| Arsenic | ArsBCDR proteins | FED29_015040, FED29_015045, FED29_015030, FED29_000690 |
| Cobalt-magnesium-zinc homeostasis | CorA, magnesium/cobalt transporter; ZntB, zinc transporter | FED29_018305 |
| Cobalt-zinc-cadmium | CzcD, cobalt/zinc/cadmium resistance | FED29_006865 |
| Cu, Pb, Cd, Zn, Hg homeostasis | Copper-translocating P-type ATPase; lead-cadmium-zinc-mercury transporting ATPase | FED29_006880, FED29_018810 FED29_013500 |
| Copper | CutACE, Cu resistance proteins | FED29_016755, FED29_009335, FED29_008035 |
| | ScsABCD, suppression copper sensitivity proteins | FED29_015795, FED29_015800, FED29_015805, FED29_015810 |
| Copper-silver homeostasis | CusAB, copper/silver efflux RND transporters | FED29_006475 FED29_006480 |
| Mercury | MerA, mercuric reductase | FED29_001585 |
| | MerR family, transcriptional regulator | FED29_011840 |
| Nickel homeostasis | HypA, HypB, [NiFe] hydrogenase nickel | FED29_003565, FED29_003560 |
| | NikA2, substrate-binding protein; NikC2, permease; NikE2, ATP-binding protein | FED29_003535, FED29_003545, FED29_003555 |
| Chromium | ChrA, chromate transport | FED29_019065 |
| Tellurite | TerABC, tellurite resistance | FED29_020300, FED29_020305, FED29_020310 |
| Fluoroquinolones | LiuR, regulator; DNA gyrase subunit B | FED29_008890, FED29_012790 |
| Multidrug | MacA, macrolide-specific efflux protein | FED29_002090 |
| | Multi antimicrobial extrusion protein | FED29_007815 |

### 4.2. Characterization of plasmid pCTe-01

The plasmid pCTe-01 is about 9 kb in size. Analysis of the intact plasmid and fragments from *Bam*HI, *Bs*TNI, and *Pst*I digestion matched in silico predictions using SnapGene v1.1.3. The *Mbo*I enzyme did not cut the DNA, likely due to absent recognition sites or methylation (Fig. 1). These results confirm the plasmid size and Illumina sequence, though the plasmid sequence remains incomplete.

In silico analysis predicted that pCTe-01 replicates using *repA* and *repB* genes, which encode for DNA replication initiation and transcriptional regulation. These genes, part of the *repABC* operon, are found in plasmids with low copy number, as seen in pCTe-01. They belong to several incompatibility groups (Pérez-Oseguera and Cevallos, 2013). Additionally, also predicted it use ParAB partitioning proteins for segregation, and type II toxin-antitoxins (TA) for stabilization (Kamruzzaman et al., 2021), but not antibiotic resistance genes (Table 1). The absence of resistance genes on pCTe-01 suggests that

**Table 6**
Prediction of phages and prophages in contigs using PHASTER.

| Characteristic | Region (N°) | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| Region position (NODE) | 32 | 49 | 51 | 62 |
| Start and end position | 804–35 093 | 1970–16 680 | 275–11 348 | 32–6624 |
| Completeness (score) | Questionable (90) | Incomplete (60) | Incomplete (50) | Questionable (70) |
| Phage hit proteins | 29 | 10 | 9 | 6 |
| Specific keywords | integrase, tail, portal | transposase, tail,terminase | transposase, tail | transposase |
| Different phage species similar | 27 | 9 | 6 | 3 |
| Most common phage | Douglas 12A4(NC_021068) | Shanette (NC_028983.1) | vB_AbaM_ME3 (NC_041884.1) | SH2026Stx1 (NC_049919.1) |
| Host of most common phage | *A. fischeri* | *B. cereus* | *A. baumannii* | *E. coli* |
| GC % | 59.25 | 45.91 | 52.10 | 54.91 |

**Table 7**
Prediction of phages and prophages in contigs using RASTtk.

| Subsystem | Role | Locus Tag |
|---|---|---|
| Integrase | Phage antirepressor protein | FED29_017795 |
| | Rha family transcriptional regulator | FED29_017950 |
| | DNA replication protein O | FED29_017920 |
| | Phage immunity repressor protein C | FED29_009230 |
| | Phage integrase | FED29_017780 |
| | Tyrosine-type recombinase/integrase | FED29_004490 |
| | Phage regulatory protein/antirepressor Ant | FED29_017900 |
| Phage DNA synthesis | Abi family protein | FED29_012710 |
| | Adenine DNA methyltransferase | FED29_017835 |
| | Peptidylprolyl isomerase | FED29_014880 |
| | DNA invertase | FED29_015945 |
| | GpQ, capsid protein | FED29_010635 |
| | Prohead core protein | * |
| | Ogr protein | FED29_010640 |
| | AAA family ATPase | FED29_004150 |
| | AlpA, transcriptional regulator | FED29_012715 |
| | Site-specific recombinase | FED29_010770 |
| Phage shock *psp* operon | PspABC, phage shock proteins | FED29_015785 FED29_010340 FED29_010345 |

*: not annotate with PGAP.

*A. veronii* CTe-01 carries these genes on its chromosome, and the observed resistance genes in this bacterium may have been acquired through phages or other mobile elements integrated into the genome (Table 6), rather than plasmids, as no mobilization genes were detected. The plasmid pCTe-01 also encodes EsiB, a protein that interacts with secretory immunoglobulin A, potentially aiding the bacterium in evading the neutrophil response during infections in fish and aquatic animals (Pastorello et al., 2013). Additionally, also contains a gene for an integrase domain-containing protein (Table 1), which phages use to integrate into the bacterial genome, suggesting exposure to MGEs similar to those in *A. veronii* C198 (Hatrongjit et al., 2020). Several hypothetical proteins were also identified and warrant further investigation.

### 4.3. Heavy metals and antibiotic MICs

*A. veronii* CTe-01 showed resistance to various heavy metals (loids) and the in silico analysis suggest genes related (Table 2). This is the first demonstration of tellurite resistance and the *terABD* operon in *A. veronii*, suggesting a possible link. However, similar traits and genes have been reported in *A. caviae* (Arenas et al., 2014). The bacterium forms black colonies, indicating tellurite reduction, but the mechanism in this species is unknown. In *A. caviae*, the dihydrolipoamide dehydrogenase enzyme (LpdA) reduces tellurite to elemental tellurium (Arenas et al., 2014). The *copA* and *czcA* genes, involved in copper and cadmium-zinc resistance, respectively, were not found in *A. veronii* CTe-01. However, they have been detected in 25 % and 61 % of 36 *Aeromonas* spp. isolates from shellfish *Ruditapes philippinarum*, respectively (Dahanayake et al., 2019). Similar genes for Cu, Pb, Cr, Hg, and Cd resistance have been observed in other *Aeromonas* species, supporting the idea of natural bioremediation of heavy metals (De Silva et al., 2018).

*A. veronii* CTe-01 showed resistance to β-lactams like penicillin, ampicillin, and cefazolin, as well as to the macrolide erythromycin, while remaining sensitive to several other antibiotics (Table 3). These resistances may be related to the presence of β-lactamases and extended-spectrum β-lactamases (Table 4), which are common in these bacteria (De Silva et al., 2018). Additionally, Aeromonads are known for resistance to tetracyclines, quinolones, and cephalosporins (Zhou et al., 2019). The presence of multiple antibiotic resistance genes in environmental bacteria represents a global public health concern, particularly with the rising prevalence of resistances.

### 4.4. Genome analysis of *A. veronii* CTe-01: Identification of antibiotic and heavy metal resistance, virulence genes, potential phages, and prophages through in silico analysis

The resistome analysis suggest that resistance to penicillin and ampicillin (Table 3) may be associated with extended-spectrum β-lactamase genes such as $bla_{\text{TEM-10}}$, $bla_{\text{TEM-101}}$, $bla_{\text{OXA-12}}$, and others (Table 4). Some of these genes, such as *blacphA3* and $bla_{\text{OXA-12}}$, have been observed in *A. veronii* C198 (Hatrongjit et al., 2020), and $bla_{\text{OXA-12}}$ and *cphA3*, in *A. veronii* XhG1.2 (Das et al., 2021). These genes, known as mobile, are widely disseminated among enteric bacteria. They can be acquired horizontally through MGEs (Piotrowska and Popowska, 2015). The resistance to cefazolin displayed could be related to $bla_{\text{OXA-12}}$ (Table 4), an antibiotic-inactivation gene detected in *Aeromonas* (Hatrongjit et al., 2020). The resistance to erythromycin is likely associated with the presence of the macrolide-specific efflux protein MacA (Table 5).

The heavy metal resistances observed in *A. veronii* CTe-01 could be associated with various genes (Table 2, Table 5), similar to other members of its genus (De Silva et al., 2018). However, further investigation is needed to explore its potential as a bioremediator.

Several genes related to the *asc* family type III secretion system (T3SS) have been identified on the chromosome of *A. veronii* CTe-01 (Table 4). This virulence system is also found in other bacteria such as *Salmonella enterica*, *Shigella* spp., *Citrobacter rodentium*, and pathogenic *E. coli* (Sanchez-Garrido et al., 2022). In *A. salmonicida*, the T3SS is recognized as the primary virulence system (Frey and Origgi, 2016). A comparison between the T3SS components of *A. veronii* CTe-01 and *A. salmonicida* reveals significant similarity, indicating that these genes might contribute to the pathogenic traits and resistance profile in *A. veronii*. However, no previous reports were found on the presence of T3SS or the RelBE toxin-antitoxin system in this species. Other pathogenicity factors detected were the *fim* and *fli* genes, related to fimbria and the flagellar motor (Table 4), which may contribute to virulence, by aiding cell surface adherence (Fernández-Bravo and Figueras, 2020).

Phage and prophage-related sequences on *A. veronii* CTe-01 gDNA (Table 6) suggest these MGEs originate from water-associated species, such as *A. fischeri*, *B. cereus*, *A. baumannii*, and *E. coli*. Additionally, several components, including integrase, phage DNA synthesis elements, and the phage shock *pspABC* operon, have been identified (Table 7). This operon is critical in phage stress response, PspA protein helps maintain membrane integrity where damage is perceived by PspBC, as reported in *Yersinia enterocolitica* (Flores-Kim and Darwin, 2016).

The specific compounds produced by *A. veronii* during heavy metal activity –such as volatile mercury, reduced tellurium, and nanodots- have not yet been identified. Consequently, the functional roles of genes and proteins remain undemonstrated. Future research will focus on completing the *A. veronii* CTe-01 genome using PacBio or nanopore technologies, exploring its evolutionary relationships with other bacteria and plasmids, analyzing its transcriptome in the presence of specific heavy metals, and identifying the resulting compounds for potential applications.

## 5. Conclusions

The findings suggest that *A. veronii* CTe-01 may serve as a genetic reservoir for antibiotic and heavy metal resistance genes, as well as virulence factors. These genetic elements could potentially be transferred horizontally to other bacteria in aquatic environments, contributing to the emergence of new bacterial strains with altered characteristics and ecological roles.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

## CRediT authorship contribution statement

**Luis Tataje-Lavanda:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Phillip Ormeño-Vásquez:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ricardo Choque-Guevara:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Rosa Altamirano-Díaz:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Manolo Fernández-Díaz:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Juan C. Tantaleán:** Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

To the Laboratory BioSLab EIRL and FARVET SAC for their support in conducting the microbiological assays and DNA sequencing, respectively.

## References

Abdelhamed, H., Lawrence, M.L., Waldbieser, G., 2019. Complete genome sequence data of multidrug-resistant *Aeromonas veronii* strain MS-18-37. Data Brief 23, 10–13. https://doi.org/10.1016/j.dib.2019.01.037.

Aminov, R.I., 2011. Horizontal gene exchange in environmental microbiota. Front. Microbiol. 2, 1–19. https://doi.org/10.3389/fmicb.2011.00158.

Arenas, F.A., Leal, C.A., Pinto, C.A., Arenas-Salinas, M.A., Morales, W.A., Cornejo, F.A., Díaz-Vásquez, W.A., Vásquez, C.C., 2014. On the mechanism underlying tellurite reduction by *Aeromonas caviae* ST dihydrolipoamide dehydrogenase. Biochimie 102, 174–182. https://doi.org/10.1016/j.biochi.2014.03.008.

Arndt, D., Grant, J.R., Marcu, A., Sajed, T., Pon, A., Liang, Y., Wishart, D.S., 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucl. Acids Res. 44, W16–W21. https://doi.org/10.1093/nar/gkw387.

Aziz, R.K., Bartels, D., Best, A., DeJongh, M., Disz, T., Edwards, R.A., et al., 2008. The RAST Server: rapid annotations using subsystems technology. BMC Genomics 9, 1–15. https://doi.org/10.1186/1471-2164-9-75.

Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. Bioinformatics 30, 2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

Chen, L., Zheng, D., Liu, B., Yang, J., Jin, Q., 2016. VFDB 2016: Hierarchical and refined dataset for big data analysis - 10 years on. Nucl. Acids Res. 44, D694–D697. https://doi.org/10.1093/nar/gkv1239.

Dahanayake, P.S., Hossain, S., Wickramanayake, M.V.K.S., Heo, G.J., 2019. Antibiotic and heavy metal resistance genes in *Aeromonas spp*. isolated from marketed Manila Clam (*Ruditapes philippinarum*) in Korea. J Appl Microbiol 127, 941–952. https://doi.org/10.1111/jam.14355.

Dahanayake, P.S., Hossain, S., Wickramanayake, M.V.K.S., Heo, G.J., 2020. Prevalence of virulence and antimicrobial resistance genes in *Aeromonas* species isolated from marketed cockles (*Tegillarca granosa*) in Korea. Lett Appl Microbiol 71, 94–101. https://doi.org/10.1111/lam.13261.

Das, S., Sreejith, S., Babu, J., Francis, C., Midhun, J.S., Aswani, R., Sebastain, K.S., Radhakrishnan, E.K., Mathew, J., 2021. Genome sequencing and annotation of multi-virulent *Aeromonas veronii* XhG1.2 isolated from diseased *Xiphophorus hellerii*. Genomics 113, 991–998. https://doi.org/10.1016/j.ygeno.2020.10.034.

De Silva, B.C.J., Hossain, S., Dahanayake, P.S., Heo, G.J., 2018. Frozen white-leg shrimp (*Litopenaeus vannamei*) in Korean markets as a source of *Aeromonas spp*. harboring antibiotic and heavy metal resistance genes. Microb. Drug Resist. 24, 1587–1598. https://doi.org/10.1089/mdr.2018.0035.

Dobiasova, H., Videnska, P., Dolejska, M., 2016. Complete sequences of IncU plasmids harboring quinolone resistance genes qnrS2 and aac(6')-Ib-cr in *Aeromonas spp*. from ornamental fish. Antimicrob Agents Chemother 60, 653–657. https://doi.org/10.1128/AAC.01773-15.

Doster, E., Lakin, S.M., Dean, C.J., Wolfe, C., Young, J.G., Boucher, C., Belk, K.E., Noyes, N.R., Morley, P.S., 2020. MEGARes 2.0: A database for classification of antimicrobial drug, biocide and metal resistance determinants in metagenomic sequence data. Nucleic Acids Res 48, D561–D569. https://doi.org/10.1093/nar/gkz1010.

Elarabi, N.I., Halema, A.A., Abdelhadi, A.A., Henawy, A.R., Samir, O., Abdelhaleem, H.A. R., 2023. Draft genome of *Raoultella planticola*, a high lead resistance bacterium from industrial wastewater. AMB Express 13. https://doi.org/10.1186/s13568-023-01519-w.

El-Beltagi, H.S., Halema, A.A., Almutairi, Z.M., Almutairi, H.H., Elarabi, N.I., Abdelhadi, A.A., Henawy, A.R., Abdelhaleem, H.A.R., 2023. Draft genome analysis for *Enterobacter kobei*, a promising lead bioremediation bacterium. Front Bioeng Biotechnol 11. https://doi.org/10.3389/fbioe.2023.1335854.

Fernández-Bravo, A., Figueras, M.J., 2020. An update on the genus *Aeromonas*: Taxonomy, epidemiology, and pathogenicity. Microorganisms. https://doi.org/10.3390/microorganisms8010129.

Flores-Kim, J., Darwin, A.J., 2016. Interactions between the Cytoplasmic Domains of PspB and PspC Silence the *Yersinia enterocolitica* Phage Shock Protein Response. J Bacteriol 198, 3367–3378. https://doi.org/10.1128/JB.00655-16.

Frey, J., Origgi, F.C., 2016. Type III secretion system of *Aeromonas salmonicida* undermining the host's immune response. Front Mar Sci. https://doi.org/10.3389/fmars.2016.00130.

Galardini, M., Biondi, E.G., Bazzicalupo, M., Mengoni, A., 2008. CONTIGuator : a bacterial genomes assembler for structural insights on draft genomes Abstract : Genome 2–6.

Gupta, S.K., Padmanabhan, B.R., Diene, S.M., Lopez-Rojas, R., Kempf, M., Landraud, L., Rolain, J.M., 2014. ARG-annot, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58, 212–220. https://doi.org/10.1128/AAC.01310-13.

Halema, A.A., El-Beltagi, H.S., Al-Dossary, O., Alsubaie, B., Henawy, A.R., Rezk, A.A., Almutairi, H.H., Mohamed, A.A., Elarabi, N.I., Abdelhadi, A.A., 2024. Omics technology draws a comprehensive heavy metal resistance strategy in bacteria. World J Microbiol Biotechnol. https://doi.org/10.1007/s11274-024-04005-y.

Hatrongjit, R., Kerdsin, A., Takeuchi, D., Wongsurawat, T., Jenjaroenpun, P., Chopjitt, P., Boueroy, P., Akeda, Y., Hamada, S., 2020. Genomic analysis of *Aeromonas veronii* C198, a novel Mcr-3.41-harboring isolate from a patient with septicemia in Thailand. Pathogens 9, 1–13. https://doi.org/10.3390/pathogens9121031.

Hu, X., Xiao, Z., Li, B., Xue, M., Jiang, N., Fan, Y., Chen, P., Qi, F., Kong, X., Zhou, Y., 2023. Isolation, Identification, and Characterization of *Aeromonas veronii* from Chinese Soft-Shelled Turtle (*Trionyx sinensis*). Microorganisms 11. https://doi.org/10.3390/microorganisms11051304.

Janda, J.M., Abbott, S.L., 2010. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. Clin Microbiol Rev 23, 35–73. https://doi.org/10.1128/CMR.00039-09.

Kamruzzaman, M., Wu, A.Y., Iredell, J.R., 2021. Biological functions of type ii toxin-antitoxin systems in bacteria. Microorganisms. https://doi.org/10.3390/microorganisms9061276.

McArthur, A.G., Waglechner, N., Nizam, F., Yan, A., Azad, M.A., Baylay, A.J., et al., 2013. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother 57, 3348–3357. https://doi.org/10.1128/AAC.00419-13.

Nawaz, M., Sung, K., Khan, S.A., Khan, A.A., Steele, R., 2006. Biochemical and molecular characterization of tetracycline-resistant *Aeromonas veronii* isolates from catfish. Appl Environ Microbiol 72, 6461–6466. https://doi.org/10.1128/AEM.00271-06.

Pastorello, I., Rossi Paccani, S., Rosini, R., Mattera, R., Ferrer Navarro, M., Urosev, D., et al., 2013. EsiB, a Novel Pathogenic *Escherichia Coli* Secretory Immunoglobulin A-Binding Protein Impairing Neutrophil Activation. mBio 4, 1–11. https://doi.org/10.1128/mBio.00206-13.

Pérez-Oseguera, Á., Cevallos, M.A., 2013. RepA and RepB exert plasmid incompatibility repressing the transcription of the *repABC* operon. Plasmid 70, 362–376. https://doi.org/10.1016/j.plasmid.2013.08.001.

Piotrowska, M., Popowska, M., 2015. Insight into the mobilome of *Aeromonas* strains. Front Microbiol 6. https://doi.org/10.3389/fmicb.2015.00494.

Sanchez-Garrido, J., Ruano-Gallego, D., Choudhary, J.S., Frankel, G., 2022. The type III secretion system effector network hypothesis. Trends Microbiol 30, 524–533. https://doi.org/10.1016/j.tim.2021.10.007.

Silver, A.C., Kikuchi, Y., Fadl, A.A., Sha, J., Chopra, A.K., Graf, J., 2007. Interaction between innate immune cells and a bacterial type III secretion system in mutualistic and pathogenic associations. Proc Natl Acad Sci U S A 104, 9481–9486. https://doi.org/10.1073/pnas.0700286104.

Skwor, T., Shinko, J., Augustyniak, A., Gee, C., Andraso, G., 2014. *Aeromonas hydrophila* and *Aeromonas veronii* predominate among potentially pathogenic ciprofloxacin- and tetracycline-resistant *Aeromonas* isolates from Lake Erie. Appl Environ Microbiol 80, 841–848. https://doi.org/10.1128/AEM.03645-13.

Sun, Y., Zhao, Y., Xu, W., Fang, R., Wu, Q., He, H., et al., 2021. Taxonomy, virulence determinants and antimicrobial susceptibility of *Aeromonas spp*. isolated from bacteremia in southeastern China. Antimicrob Resist Infect Control 10, 1–9. https://doi.org/10.1186/s13756-021-00911-0.

Tataje-Lavanda, L., Ormeño-Vásquez, P., Altamirano-Díaz, R., Espinoza-Salazar, L., Zimic, M., Fernández-Sánchez, M., et al., 2019. Draft Genome Sequence of Heavy Metal-Resistant *Aeromonas veronii* CTe-01, Isolated from a Peruvian Wastewater Treatment Plant. Microbiol Resour Announc 8, 1–3. https://doi.org/10.1128/mra.01147-19.

Walczak, N., Puk, K., Guz, L., 2017. Bacterial flora associated with diseased freshwater ornamental fish. Journal of Veterinary Research (poland) 61, 445–449. https://doi.org/10.1515/jvetres-2017-0070.

Zankari, E., Hasman, H., Cosentino, S., Vestergaard, M., Rasmussen, S., Lund, O., Aarestrup, F.M., Larsen, M.V., 2012. Identification of acquired antimicrobial resistance genes. J. Antimicrob. Chemother. 67, 2640–2644. https://doi.org/10.1093/jac/dks261.

Zhou, Y., Yu, L., Nan, Z., Zhang, P., Kan, B., Yan, D., Su, J., 2019. Taxonomy, virulence genes and antimicrobial resistance of *Aeromonas* isolated from extra-intestinal and intestinal infections. BMC Infect. Dis. 19, 1–9. https://doi.org/10.1186/s12879-019-3766-0.