Research Square

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A novel chromosomally encoded OXA-12-like β-lactamase variant (bla OXA-1433) in a new Aeromonas veronii sequence type (ST3591) from ready-to-eat oysters

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Research Article

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Abstract

Antibiotic resistance, a natural feature of bacterial evolution, is not confined to clinical settings but actively circulates across aquatic and terrestrial ecosystems, food chains, and animal and human populations. Aquatic ecosystems serve both as reservoirs of resistance determinants and as sources of food production, making seafood a critical interface for human exposure to environmentally derived resistance genes. This study aimed to characterize a novel chromosomally encoded OXA-12-like β-lactamase variant ($bla_{OXA-1433}$) identified in a new *Aeromonas veronii* sequence type (ST3591) isolated from ready-to-eat oysters, and to investigate its genetic, structural, and phenotypic features. The isolate was subjected to hybrid whole-genome sequencing using short- and long-read technologies, followed by genome assembly, multilocus sequence typing, genomic context characterization, and antimicrobial susceptibility testing. The novel $bla_{OXA-1433}$ variant was further characterized through sequence and phenotypic analyses. MLST analysis revealed a novel sequence type (ST3591), while genome mining identified a previously undescribed OXA-12-like β-lactamase variant. OXA-1433 shared 95.08% amino acid identity with OXA-12 and differed by 13 amino acid substitutions while retaining the conserved motifs characteristic of class D β-lactamases. Genomic context analysis demonstrated that $bla_{OXA-1433}$ was chromosomally located within a conserved genomic region lacking detectable mobile genetic elements. Phenotypic assays demonstrated imipenem hydrolysis, consistent with carbapenemase activity. The identification of a novel *A. veronii* sequence type carrying a previously undescribed OXA-12-like β-lactamase variant expands current knowledge of the genetic diversity of this species in food-associated environments and highlights the value of genomic surveillance of seafood-associated bacteria for monitoring the evolution of antibiotic resistance determinants.

1. Introduction

The *Aeromonas* genus comprises a diverse group of aquatic Gram-negative bacteria frequently recovered from fish, shellfish, and coastal environments, highlighting their widespread distribution in aquatic ecosystems and their relevance to the food chain [1, 2]. Some species are recognized as opportunistic or emerging human pathogens and exhibit considerable genomic plasticity, characterized by mobile elements and recombinant regions that facilitate the acquisition and dissemination of resistance determinants [3]. Moreover, the occurrence of specific sequence types (STs) across distinct niches suggests transmission potential, making the emergence of new STs relevant to understand the link between intraspecific diversity and niche adaptation as well as novel evolutionary combinations of genetic determinants with potential public health implications [4].

The OXA-12-like β-lactamases are a subfamily of oxacillin-hydrolyzing class D OXA beta-lactamases derived from OXA-12, conferring resistance to β-lactams through the hydrolysis of its β-lactam ring. OXA-12-like enzymes have been associated with species of the *Aeromonas* genus, suggesting that this specific β-lactamase group may represent intrinsic resistance determinants within this taxon [5]. Furthermore, the frequently observed chromosomal location of OXA-12-like genes suggests stable maintenance of these β-lactamase-encoding determinants throughout the *Aeromonas* evolutionary

history, reinforcing the role of aquatic environments as natural reservoirs and diversification hubs for this enzyme family [6].

Despite the increasing number of antimicrobial resistance reports focusing on aquatic environments and *Aeromonas* species [7, 8], relatively few studies have concurrently characterized novel β-lactamase variants, identified emerging new clonal profiles, and investigated their occurrence in ready-to-eat foods. In the context of antimicrobial resistance monitoring in aquaculture systems, we recovered a new *Aeromonas veronii* sequence type from ready-to-eat oysters that exhibited resistance against carbapenems. Hybrid whole genome sequencing analyses enabled the detection and characterization of a novel OXA-12-like β-lactamase variant, including its genomic context, evolutionary relationships, structural characteristics, and associated phenotype.

2. Materials and Methods

2.1 Bacterial isolation and identification

In January 2023, ready-to-eat oysters were sampled from a commercial establishment in Florianópolis, Brazil. Dissected samples from 15 ready-to-eat oysters were weighed and transferred to bottles containing sterile 0.9% saline at a 1:1 (w/v) ratio, followed by homogenization using an ULTRA-TURRAX disperser. Aliquots of 50 mL of the homogenate were then transferred to enrichment bottles containing 450 mL of tryptic soy broth (TSB) and incubated at 37°C with agitation for 6 h. From the enrichment culture, 500 µL was inoculated into tubes containing 4.5 mL of TSB supplemented with ertapenem (0.5 µg/mL) and incubated overnight at 37°C. Subsequently, 10 µL aliquots were streaked onto MacConkey agar plates and incubated at 37°C for 24 h. A single isolated colony was selected for taxonomic identification using the MALDI-TOF VITEK® MS system (bioMérieux).

2.2 Phenotypic antimicrobial susceptibility testing

The antimicrobial resistance profile of the isolate was assessed on Mueller-Hinton agar plates using the agar disk diffusion method, according to the European Committee on Antimicrobial Susceptibility Testing guidelines [9]. Antimicrobial susceptibility testing was performed using commercial antimicrobial disks (Oxoid) containing aztreonam (30 µg), cefepime (30 µg), ceftazidime (30 µg), ciprofloxacin (5 µg), ertapenem (10 µg), imipenem (10 µg), and meropenem (10 µg). Results were interpreted according to the clinical breakpoints for *Aeromonas* spp. defined in the same EUCAST version.

2.3 Genomic DNA extraction and whole genome sequencing

Genomic DNA was extracted from bacterial cultures grown overnight in TSB at 37°C using an in-house protocol based on enzymatic and chemical cell lysis, followed by organic extraction and alcohol precipitation. Briefly, bacterial pellets were resuspended in buffer (75 mM NaCl, 25 mM EDTA, 20 mM

Tris-HCl, pH 7.0) and lysed with SDS at a final concentration of 1% (w/v), lysozyme (0,25 mg/mL), and proteinase K (0,1 mg/mL), with incubation at 37°C for 1 h. Subsequently, NaCl was added to a final concentration of 1.2 M, followed by extraction with chloroform:isoamyl alcohol (24:1, v/v), added at a 1:1 (v/v) ratio to the cell lysate, and centrifugation. The aqueous phase was recovered, and genomic DNA was precipitated with cold isopropanol, washed with 70% ethanol, air-dried, and resuspended in sterile ultrapure water for downstream analyses.

Whole-genome sequencing was performed using both short- and long-read platforms. Illumina sequencing was carried out in Novaseq 6000 by Eurofins Genomics (Germany) in paired-end mode (2 × 150 bp). Long-read sequencing was performed in-house on an Oxford Nanopore Technologies (ONT) MinION Mk1B using an R10 (v10) flow cell, with libraries prepared using the ONT Rapid Barcoding Kit 96. All procedures followed the manufacturers' protocols.

2.4 Read processing, genome assembly, and quality assessment

Raw read quality was assessed using FASTQC (0.74 + galaxy1). Read trimming and filtering were performed with FASTP (1.0.1 + galaxy3). Long-read assemblies were generated using Flye (2.9.6 + galaxy0). Hybrid genome assemblies were constructed by aligning short reads to the long-read assemblies using BWA-MEM2 (2.3 + galaxy0), followed by polishing with Pilon (1.20 + galaxy0). The quality and completeness of the final assemblies were evaluated using CheckM2 (1.1.0 + galaxy0). Kraken2 (2.17.1 + galaxy0) and the k-mer approach confirmed taxonomy classification, which was further validated by Average Nucleotide Identity (ANI) analysis using FastANI (1.3 + galaxy0) against the *Aeromonas veronii* type strain genome deposited under accession GCF_000820285.1.

2.5 Antimicrobial resistance gene identification and genomic characterization

Antimicrobial resistance genes were retrieved using AMRFinderPlus (3.12.8 + galaxy0). Gene organization, GC content, and genomic features were analyzed using the Proksee online platform (https://proksee.ca, accessed in January 2026).

2.6 Identification, alignment, and phylogeny of the novel *bla*$_{\text{OXA–12–like}}$ variant

The *bla*$_{\text{OXA–12–like}}$ gene identified in the assembled genome was submitted to the NCBI BankIt platform for nomenclature assignment and confirmation of its status as a putative novel variant. All available OXA-12-like variants (n = 13) were retrieved from the Beta-Lactamase Database (http://www.bldb.eu/, accessed January 2026) (Supplementary Table S1). Amino acid sequences were aligned using MAFFT (7.526 + galaxy2), and phylogenetic relationships were inferred using maximum likelihood in IQ-TREE (2.4.0 + galaxy1). The best-fit substitution model was selected using ModelFinder according to the Akaike Information Criterion (AIC), which identified JTTDCMut + I as the optimal model. Branch support

was evaluated using 1,000 bootstrap replicates. Tree visualization and editing were performed using phylo.io.

2.7 Genomic context analysis of *bla*$_{OXA-1433}$

The genomic context harboring the *bla*$_{OXA-12-Like}$ gene was investigated within a 20 kb region comprising 10 kb upstream and 10 kb downstream of the target locus. These regions were extracted from selected *Aeromonas* genomes carrying OXA-12-like β-lactamase variants (Supplementary Table S2), and comparative synteny analysis was performed using clinker (https://github.com/gamcil/clinker). Genome annotation was conducted with Prokka (1.14.6 + galaxy1). Mobile genetic elements were investigated using Integron Finder (2.0.5 + galaxy1) and ISEScan (1.7.3 + galaxy0), while plasmid-associated sequences were identified using PlasmidFinder (2.1.6 + galaxy2).

Three-dimensional structure prediction of bla$_{OXA-1433}$

The three-dimensional protein structure of *bla*$_{OXA-1433}$ was predicted via the SWISS-MODEL server (https://swissmodel.expasy.org, accessed in January 2026) using the crystal structure of OXA-427 (PDB accession code 6HUH, chain B) as reference. Validation with AlphaFold 3 (https://alphafoldserver.com/, accessed in February 2026) confirmed structural consistency.

2.8 Phenotypic detection of carbapenemase activity (Blue-Carba test)

The ability to hydrolyze imipenem was evaluated using the Blue-Carba test (BCT). The reagent consisted of an aqueous solution containing 0.04% bromothymol blue, 0.4 mM zinc sulfate, 5 mg/mL imipenem, and 5 mg/mL cilastatin (pH 7.0). For the inhibition tests with EDTA, 10 mM EDTA was added to the solution. A loopful of an overnight culture grown at 37°C on tryptic soy agar was suspended in 100 µL of reagent and incubated at 37°C for 2 h. As bromothymol blue was used as the pH indicator, a color change from blue to yellow indicated imipenem hydrolysis and carbapenemase activity. A *bla*$_{NDM}$-producing metallo-β-lactamase isolate and a *bla*$_{KPC}$-producing serine carbapenemase isolate from the biobank of the Applied Molecular Microbiology Laboratory, Federal University of Santa Catarina, with resistance genes confirmed by DNA sequencing, were used as positive controls. Reagents without imipenem served as blanks.

3. Results and Discussion

3.1 Hybrid whole-genome sequencing revealed a novel *Aeromonas veronii* sequence type

In the context of antimicrobial-resistance surveillance in ready-to-eat oysters, the isolation of an *Aeromonas veronii* strain exhibiting resistance to carbapenems prompted further investigation of its genetic determinants. The *A. veronii* isolate' genome was sequenced using a hybrid short- and long-read

approach. Species identification was supported by Average Nucleotide Identity (ANI) analysis against the *A. veronii* type strain genome (accession GCF_000820285.1), yielding an ANI value of 96.3033%. Hybrid assembly generated a high-quality, closed genome, allowing comprehensive genomic characterization and accurate contextual analysis of antimicrobial resistance determinants.

The assembly resulted in two contigs corresponding to a circular chromosome and a plasmid. The chromosome was 4,628,862 bp in length, comprising 4,272 annotated genes and exhibiting an overall GC content of 58%, consistent with previously reported *A. veronii* genomes [10]. The plasmid was 7,699 bp in length and showed 100% query coverage and 99.97% nucleotide identity with the *Aeromonas salmonicida* plasmid pAsaXII (accession number MF621618), a small cryptic plasmid previously associated with bacterial adaptation and persistence [11]. Genome analysis indicated 100% completeness.

In silico multilocus sequence typing analysis revealed that the isolate belonged to a previously unreported sequence type. Analysis of the individual MLST *loci* demonstrated that the *gltA*, *gyrB*, *metG*, and *ppsA* alleles were identical to sequences previously deposited in the database, whereas the *groL* and *recA* loci exhibited previously undescribed nucleotide variations. These novel alleles were deposited in the PubMLST database as *groL*-1339 and *recA*-1641, respectively. Based on this allelic profile, the isolate was assigned a new sequence type, ST3591, which was subsequently deposited in the PubMLST database.

3.2 OXA-1433 is a novel, conserved member of the OXA-12-like β-lactamase family

Screening for antimicrobial resistance genes identified two chromosomally encoded β-lactamase genes, comprising an OXA-type β-lactamase belonging to the OXA-12-like group and the metallo-β-lactamase gene *cphA4*. Sequence analysis confirmed that the OXA-type gene represents a novel variant, designated $bla_{\text{OXA-1433}}$, following submission and curation in the NCBI database.

Phylogenetic analysis of the OXA-12-like variants placed $bla_{\text{OXA-1433}}$ within the established OXA-12-like clade, forming a distinct branch that supports its classification as a novel variant rather than a divergent allele of an existing enzyme (Fig. 1).

[Figure_1_here]

Comparative analysis with the thirteen previously described OXA-12-like variants (n = 14) revealed a high degree of sequence conservation. OXA-1433 shared 95.08% amino acid identity with the reference OXA-12 sequence and differed by 13 amino acid substitutions distributed throughout the protein sequence (Supplementary Figure S1). Importantly, all canonical motifs characteristic of class D β-lactamases were preserved, including the S-T-F-K, Y-G-N, K-T-G, Q-X-X-X-L, and S-X-V motifs previously described for this enzyme family [6, 12]. The substitutions distinguishing OXA-1433 from OXA-12 were predominantly

located outside these conserved regions, including residues involved in active-site architecture and substrate binding.

Three-dimensional structural modeling of the OXA-12-like protein from *A. veronii* ST3591 revealed a conserved class D β-lactamase fold, characterized by a predominantly α/β architecture typical of OXA-type enzymes. The predicted structure displayed the canonical active-site serine motif and a well-defined catalytic pocket compatible with carbapenem binding [13] (Supplementary Fig. 2).

The preservation of these motifs suggests maintenance of the core catalytic framework typical of OXA-12-like enzymes. Likewise, sequence variability among OXA-12-like β-lactamases is frequently concentrated in peripheral regions and may contribute to subtle differences in substrate affinity or enzyme stability without compromising β-lactamase activity [6, 13].

3.3 Genomic context supports *bla*$_{OXA-1433}$ as an intrinsic resistance determinant

Detailed analysis of the *bla*$_{OXA-1433}$ genomic region revealed that the gene is embedded within a chromosomal locus characterized by conserved flanking genes, forming a well-defined genomic context that is conserved across multiple members of the *Aeromonas* genus, including strains belonging to different species within the genus (Fig. 2). No insertion sequences, transposases, integrons, or plasmid-associated replication genes were detected in the immediate vicinity of *bla*$_{OXA-1433}$, indicating a low likelihood of recent mobilization [6].

[Figure_2_here]

In addition, *bla*$_{OXA-1433}$ was located in close proximity to essential housekeeping genes, including *glmS* and *glmU*, which encode key enzymes involved in peptidoglycan biosynthesis [14], as well as multiple subunits of the ATP synthase operon (*atpA* to *atpH*). These genes are highly conserved and subject to strong purifying selection [15]. The presence of *bla*$_{OXA-1433}$ within such a genomic context is consistent with a high degree of chromosomal stability and suggests that this resistance determinant is tightly integrated into the core genome rather than associated with recently acquired mobile elements.

This genomic stability contrasts with the behavior of clinically relevant OXA enzymes found in *Enterobacterales* and *Acinetobacter* spp., which are frequently associated with mobile genetic elements and horizontal gene transfer events [6, 16, 17]. These findings reinforce the concept that OXA-12-like enzymes in *Aeromonas* serve primarily as intrinsic resistance determinants rather than drivers of rapidly disseminating resistance. Nevertheless, their presence in food-associated bacteria warrants attention, as environmental and foodborne reservoirs may contribute to the long-term evolution of resistance genes.

3.4 Phenotypic carbapenem hydrolysis by the *A. veronii* ST3591 strain is consistent with OXA-type β-lactamase

activity

The *A. veronii* ST3591 was susceptible to aztreonam, cefepime, ceftazidime, and ciprofloxacin. For carbapenems, although no official breakpoints are defined by EUCAST, the isolate displayed reduced inhibition zones for imipenem and ertapenem, while for meropenem it exhibited larger zones.

Phenotypic analysis using the Blue-Carba test demonstrated imipenem hydrolysis even in the presence of EDTA, indicating that the responsible carbapenemase is metal ion-independent, consistent with the expression of OXA-12 enzymes (Fig. 3). Although the *cphA4* gene, encoding a metallo-β-lactamase dependent on metal ions, was present, the imipenem hydrolysis profile also observed in the presence of EDTA is incompatible with this enzyme activity profile, indicating that apparently it did not contribute significantly to the resistance phenotype exhibited by this isolate in the BCT.

[Figure_3_here]

The OXA-12 enzyme, a class D serine β-lactamase, has been previously reported in *Aeromonas* species with variable hydrolytic activity, showing either limited activity against both carbapenems and cephalosporins or preferential activity toward carbapenem hydrolysis [6, 18]. This profile is consistent with the observed susceptibility to cefepime, ceftazidime, and aztreonam in the isolate reported in this study, suggesting that subtle sequence variations within OXA-12-like enzymes may modulate substrate specificity, resulting in carbapenem hydrolysis without concurrent cephalosporin degradation.

Aeromonas spp. frequently harbor multiple intrinsic β-lactamases, including AmpC cephalosporinases, OXA-12-like enzymes, and CphA metallo-β-lactamases, whose combined expression may influence the overall β-lactam susceptibility profile (19). In the present isolate, however, only $bla_{\text{OXA–1433}}$ and *cphA4* were identified among β-lactamase genes. The persistence of imipenem hydrolysis in the presence of EDTA argues against a predominant contribution of the metal-dependent CphA4 enzyme and supports the involvement of OXA-1433 in the observed phenotype.

3.5 Food-associated *Aeromonas veronii* as a reservoir of intrinsic β-lactamase diversity

The identification of a novel $bla_{\text{OXA–12–like}}$ variant in a new *A. veronii* sequence type (ST-3591) isolated from ready-to-eat oysters highlights the dynamic genetic landscape of *Aeromonas*, which is particularly relevant in food-associated environments. Aquatic environments and seafood products can serve as reservoirs for diverse β-lactamase genes, facilitating their dissemination across bacterial populations [20]. Although OXA-12-like enzymes are not typically associated with clinically significant resistance, their evolutionary diversification and presence in multiple *Aeromonas* lineages [6] suggest that these genes may act as a hidden reservoir for resistance determinants that could be mobilized under selective pressure.

4. Conclusion

This study describes the genomic characterization of a novel *Aeromonas veronii* sequence type, ST3591, isolated from ready-to-eat oysters, harboring a previously undescribed chromosomal OXA-12-like β-lactamase variant, $bla_{OXA-1433}$. This new variant differs from the classical OXA-12 enzyme by 13 amino acid substitutions, while retaining the canonical conserved motifs essential for class D β-lactamase function, consistent with its experimentally confirmed carbapenemase activity. Importantly, $bla_{OXA-1433}$ is located within a stable and conserved chromosomal region, lacking associated mobile genetic elements, supporting its genomic stability and suggesting a limited potential for horizontal dissemination. The identification of both a novel sequence type and a distinct chromosomally encoded β-lactamase highlights the underexplored genetic diversity and adaptive eco-evolutionary potential of *A. veronii* in food-associated niches.

Statements and Declarations

Ethical Approval

Approval by an ethics committee was not required since this study does not involve experiments with animal or human subjects.

Competing interest

The authors declare that there are no competing interests. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Data availability

The data underlying this article are available within the article and its online supplementary material. The whole-genome sequence generated in this study has been deposited in the National Center for Biotechnology Information (NCBI) under BioProject accession number PRJNA1426971. The genome assembly is available under BioSample accession number SAMN56300468. The amino acid sequence of the novel OXA variant has been deposited in GenBank under accession number PX843018.

CRediT authorship contribution

Gustavo Rocha: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Visualization, Writing – original draft. **Mateus R. Ribas**: Conceptualization, Methodology, Writing – review and editing. **Jussara K. Palmeiro**: Funding acquisition, Supervision, Writing – review and editing. **Thais C. M. Sincero**: Conceptualization, Funding acquisition, Supervision, Writing – review and editing. **Mónica V. Cunha**: Conceptualization, Funding acquisition, Investigation, Methodology, Validation, Supervision, Writing – review and editing.

AI Declaration

The authors declare that no generative AI or AI-assisted technologies were used during the preparation of this manuscript.

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Figures

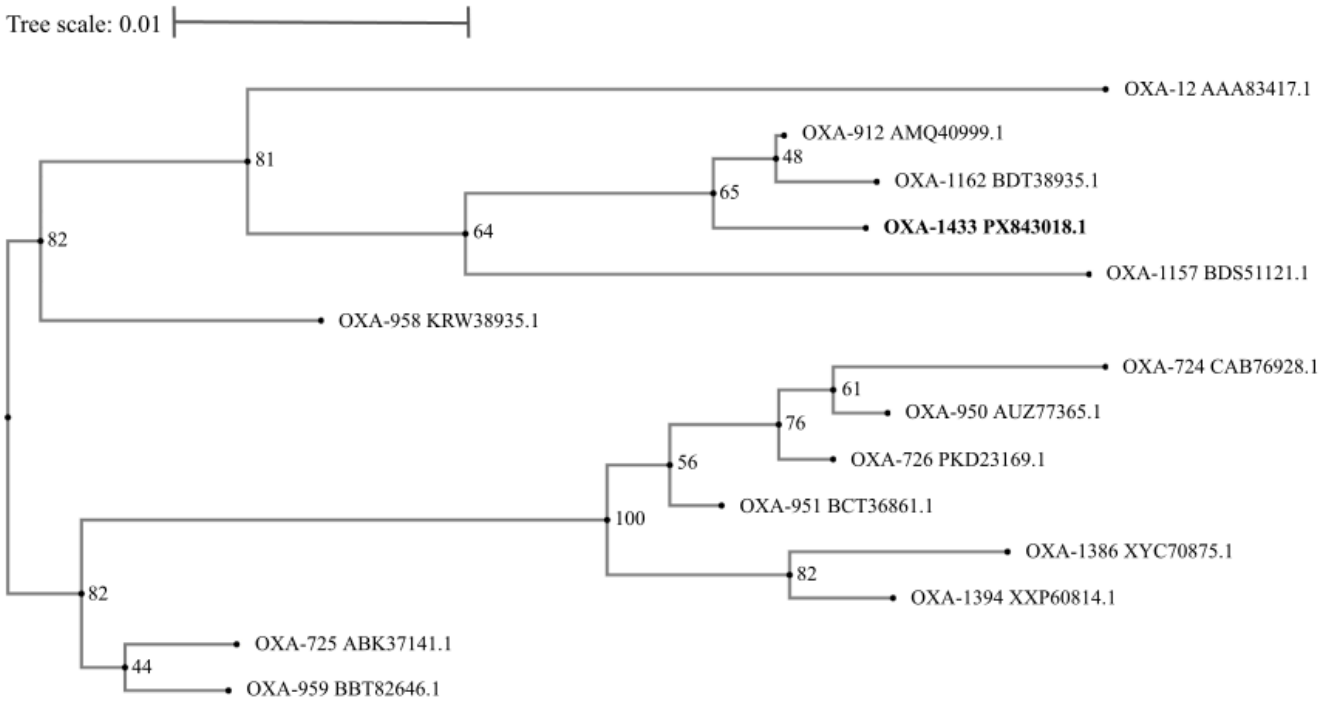


Figure 1

Phylogeny of OXA-12-like β-lactamase variants. Phylogenetic tree of OXA-12-like β-lactamase amino acid sequences, including OXA-1433. Bootstrap support values (1,000 replicates) are shown at the nodes.

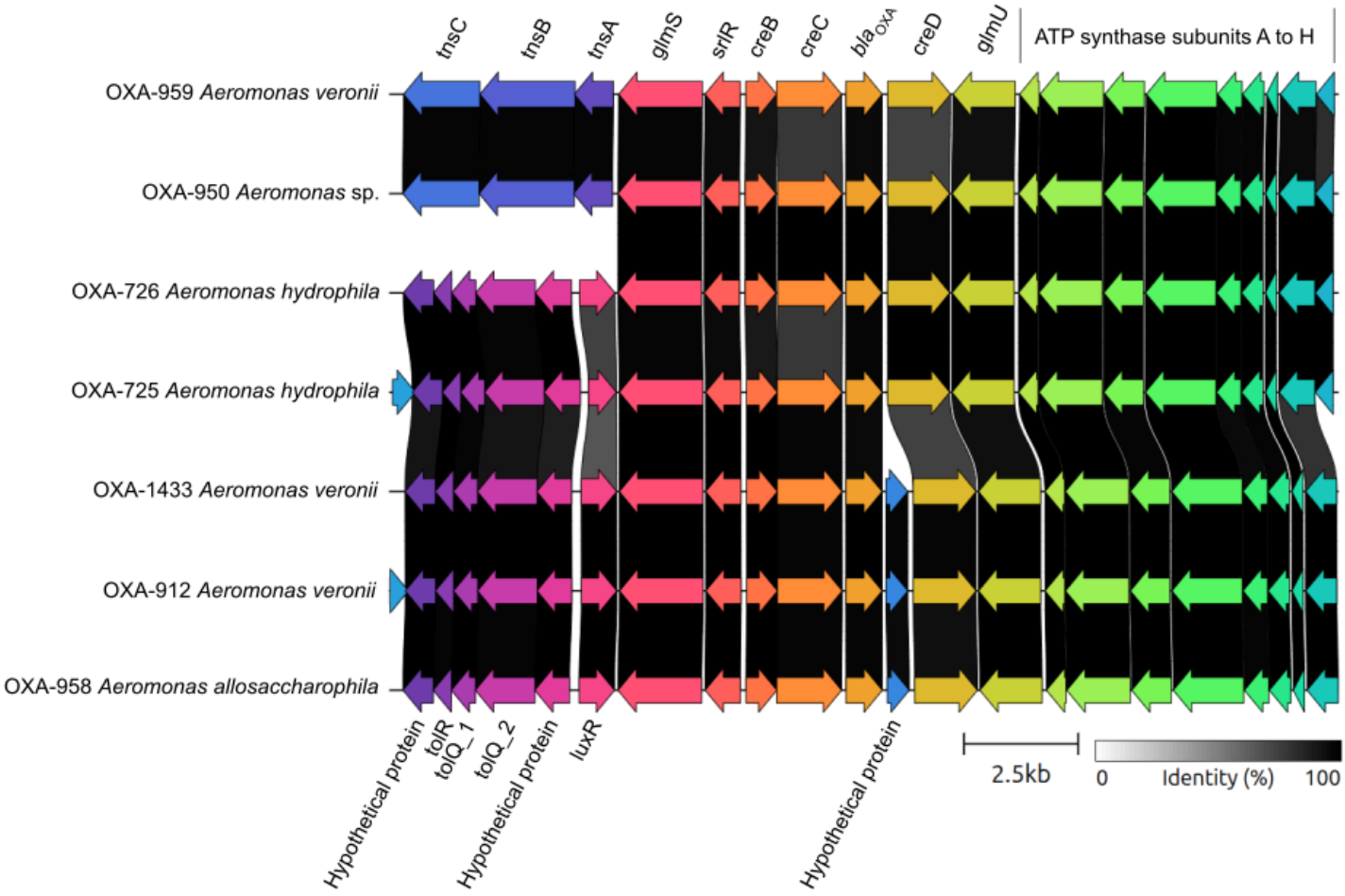


Figure 2

Schematic representation of the chromosomal genomic context surrounding the *bla*$_{OXA}$ gene. The locus is characterized by a conserved gene organization and synteny across multiple members of the *Aeromonas* genus harboring OXA-12-like β-lactamase variants, including strains from different species (more information in Supplementary Table S2).

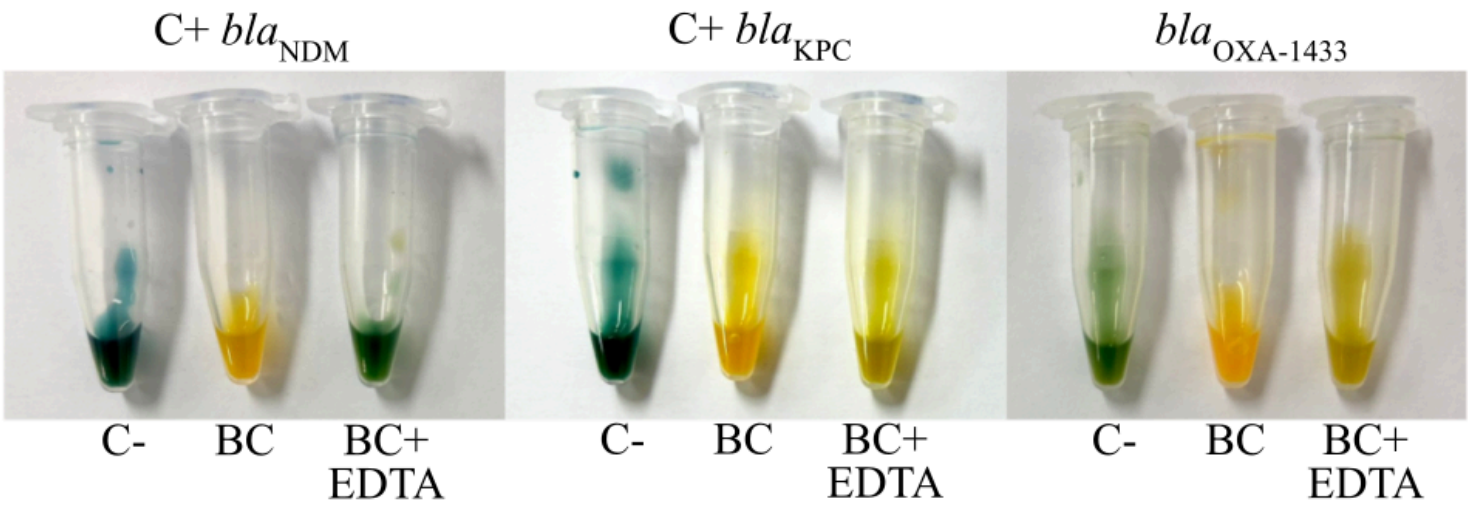


Figure 3

Phenotypic detection of carbapenemase activity by the Blue-Carba assay in *Aeromonas veronii* ST3591 and control strains. The Blue Carba assay demonstrates imipenem hydrolysis through a color change from blue to yellow. The bla_{NDM}-positive control, the bla_{KPC}-positive control and the tested *A. veronii* ST3591 isolate are depicted. For each strain, results from the three conditions are shown: a blank control without imipenem (C-), the Blue-Carba test (BC), and the Blue Carba test with EDTA (BC + EDTA). The *A. veronii* ST3591 isolate exhibited a positive result, indicated by a color change from blue to yellow in both BC and BC + EDTA conditions, confirming imipenem hydrolysis and indicating metal-independent carbapenemase activity, consistent with OXA-type enzymes.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryTable2.xlsx
- SupplementaryTable1.xlsx
- SupplementaryFigure2.svg
- SupplementaryFigure1.svg
- Supplementarymaterial.docx